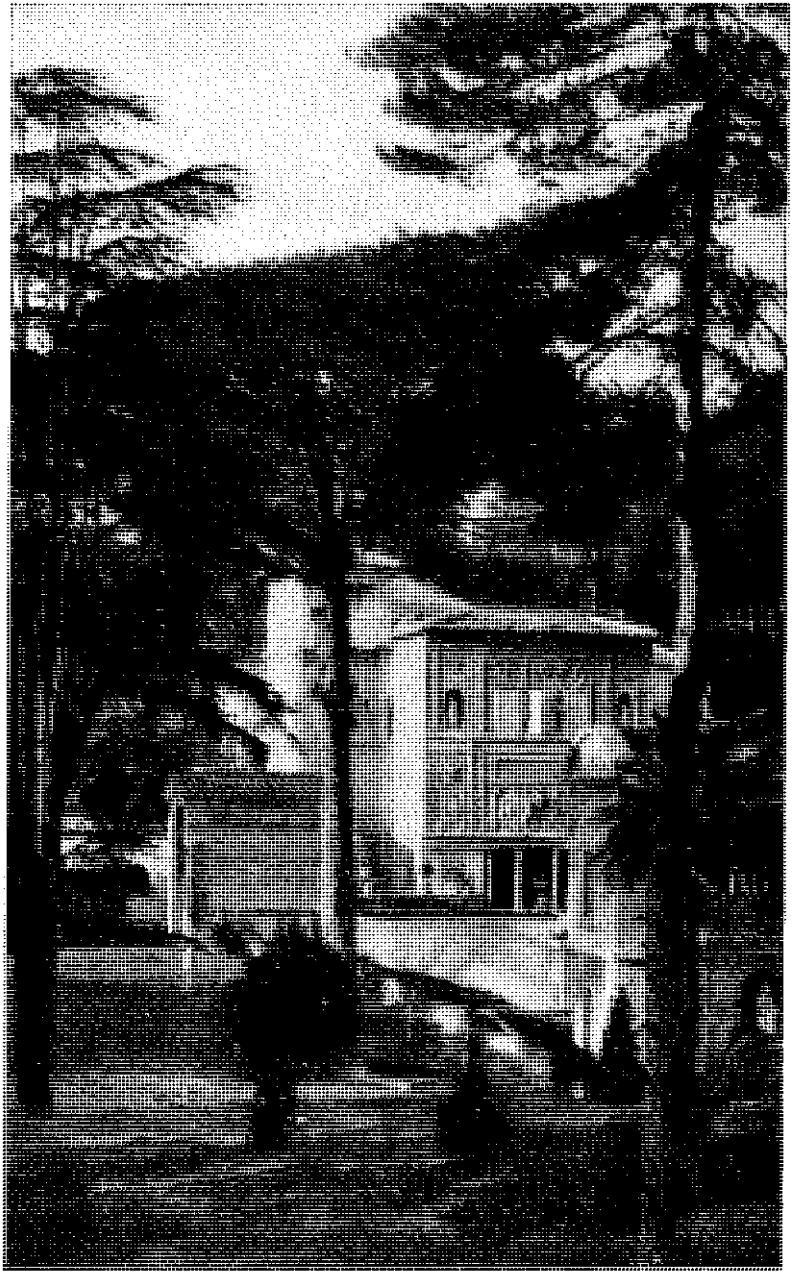

PONTIFICIAE ACADEMIAE SCIENTIARVM SCRIPTA VARIA

41

STUDY WEEK

ON

NATURAL PRODUCTS AND THE PROTECTION OF PLANTS

October 18-23, 1976

EDITED BY G.B. MARINI-BETTÒLO

PONTIFICIA
ACADEMIA
SCIENTIARVM

EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

MCMLXXVII

PONTIFICIAE ACADEMIAE SCIENTIARVM SCRIPTA VARIA

41

SEMAINE D'ETUDE

SUR LE THÈME

PRODUITS NATURELS ET LA PROTECTION DES PLANTES

18-23 octobre 1976

ÉDITÉ PAR G. B. MARINI-BETTÒLO

PONTIFICIA
ACADEMIA
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MCMLXXVII

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PREFACE

Le présent volume contient les communications et les discussions tenues à l'Académie Pontificale des Sciences lors de la Semaine d'Etude du 18-23 octobre 1976 sur le thème « Produits Naturels et Protection des Plantes ».

La Réunion a été jugée à l'unanimité particulièrement intéressante et fructueuse et ses objectifs, clairement illustrés par le Prof. G.B. Marini-Bettòlo lors de son discours d'inauguration, ont été pleinement satisfaits. Nous espérons que ce présent volume sera de grande utilité à tous ceux qui sont déjà engagés dans un domaine aussi extraordinaire et attractif, de même qu'à tous ceux qui désirent commencer leur activité sur ce sujet. Je suis convaincu qu'il s'agit d'un domaine scientifique très promettant, où des résultats significatifs peuvent et doivent être obtenus rapidement, et où la recherche de base et la science appliquée doivent progresser ensemble.

En présentant ces Actes de la 14ème Semaine d'Etude de l'Académie Pontificale des Sciences, je désire remercier chaleureusement tous les Participants à la Semaine d'Etude qui ont brillamment contribué par leurs communications et interventions au succès obtenu. A ces remerciements, je désire ajouter l'expression de ma vive gratitude au Prof. G.B. Marini-Bettòlo pour la façon exquise avec laquelle il a accompli la tâche d'organisateur et de Président de la Semaine d'Etude.

Je désire également exprimer ma profonde appréciation au Rév. Père Enrico di Rovasenda, Directeur de la Chancellerie de

l'Académie, et à Mme. Michèle Porcelli-Studer et Mr. Filippo Colelli pour l'impeccable organisation de la Réunion et pour leur infatigable zèle et assistance lors de son déroulement.

Finalement, je désire remercier également Mme. Nerina Anglesio Cibrario, responsable du Secrétariat technique.

CARLOS CHAGAS
Président de l'Académie Pontificale des Sciences

FOREWORD

In this volume are published the papers presented and the discussions held at the Pontifical Academy of Sciences during the Study Week on « Natural Products and the Protection of Plants » convened from October 18-23, 1976.

It was an interesting meeting and in general consensus a fruitful one, whose objectives well presented by Prof. G.B. Marini-Bettòlo in his initial discourse were more than largely fulfilled. We hope that the present publication may become of great usefullness to all those already engaged in such an extraordinary attractive field, as much as to those who wish to initiate their work in its realm. It is my belief that this one is a field of scientific endeavours of great prospectives where significant progress can and should be achieved rapidly. It is an engaging field in which basic research and applied science have to move in nearness.

In forewarding this volume, as the Proceedings of the 14th « Study Week » held at the Pontifical Academy of Sciences, I wish to present my deepest thanks to all the participants of the Week, who so brillantly contributed by their papers and interventions to the success obtained. To these thanks I wish to add the expression of my gratitude to Prof. G.B. Marini-Bettòlo for the superb way in which he has completed the task of organizer of the Study Week and performed as its chairman.

I wish also to express my deepest appreciation to Father Enrico di Rovasenda, Director of the Chancellery of the Academy and to his assistants Mrs. Michèle Porcelli and Mr. Filippo Colelli for

the flawless organisation of the meeting and their constant zeal and help during its development.

Finally I wish to thank also Dr. Nerina Anglesio Cibrario and her assistants, responsible for the technical Secretariat.

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Participants à la Semaine d'Etude

AUDIENCE DU SAINT-PERE

Le 23 octobre à 12 heures le Saint-Père accorda dans la Salle du Consistoire du Palais Vatican une Audience solennelle aux Membres de l'Académie Pontificale des Sciences, aux Participants à la Semaine d'Etude et à la Médaille d'or Pie XI, Dr. LUCIO LUZZATTO.

Le Groupe était guidé par le Président de l'Académie Prof. CARLOS CHAGAS, le Président émérite Rév. P. DANIEL JOSEPH KELLY O'CONNELL et le Directeur de la Chancellerie Rév. P. ENRICO DI ROVASENDA.

A l'Audience étaient présents, outre les Epouses des Académiciens et des Participants à la Semaine d'Etude, les Membres de la Chancellerie de l'Académie et du Secrétariat de la Semaine d'Etude.

L'Assemblée était honorée par la participation de dix Eminentissimes Cardinaux, de Leurs Excellences Mons. GIOVANNI BENELLI, Substitut à la Secrétairerie d'Etat et Mons. AGOSTINO CASAROLI, Secrétaire du Conseil pour les Affaires Publiques de l'Eglise, et du Corps Diplomatique accrédité près le Saint-Siège.

Après avoir gagné le trône, le Saint-Père donna son assentiment au Président de l'Académie le Prof. CARLOS CHAGAS, qui s'adressa au Souverain Pontife en ces termes:

Sainteté,

Permettez-moi de Vous exprimer vivement, Très Saint-Père, au nom de Votre Académie Pontificale des Sciences, notre profonde gratitude et notre joie pour la généreuse bienveillance avec laquelle Vous accompagnez nos travaux, et pour l'appui et l'encouragement que Vous donnez à nos initiatives, montrant combien au milieu des immenses préoccupations Vous trouvez encore le temps de réfléchir sur les sources qui peuvent pourvoir à l'existence temporelle de l'homme.

Je dois joindre à mes remerciements ceux des Participants à la Semaine d'Etude qui vient de se dérouler. Les grands spécialistes qu'ils sont tous, se sentent heureux d'avoir pu, en toute liberté, réfléchir et discuter pendant six jours du problème que pose la protection des plantes dans un monde où on s'effraye de plus en plus de la rupture des écosystèmes et des apports indésirables à la nutrition humaine, dus à l'emploi démesuré des pesticides indispensables à la conservation des aliments.

La nouvelle voie de protection offerte par l'utilisation des « produits naturels » ainsi classés, certaines substances dérivées de l'activité métabolique des animaux, et entre eux les insectes eux-mêmes, et des végétaux, semble pouvoir présenter une importance majeure dans la préservation du monde végétal et de ses récoltes.

En abordant du point de vue scientifique ce sujet, Votre Académie démontre une fois de plus son désir de poursuivre les voies où mieux s'identifie l'action favorable que la science peut et se doit d'avoir sur le processus social, en même temps qu'elle désire faire avancer les fondements scientifiques de notre connaissance sur laquelle repose la grande aventure intellectuelle et humaine, qui est la science de nos jours.

Sainteté! notre Réunion cette année a une signification spéciale. Nous fêtons le 40ème anniversaire de la transformation par le Souverain Pontife Pie XI de vénérée mémoire de l'Académie des « Nuovi Lincei » en Académie Pontificale des Sciences, unique par son caractère international et oecuménique.

Sous la présidence de mes éminents prédécesseurs, auxquels je rends hommage, les regrettés Père Agostino Gemelli et Monseigneur Georges Lemaître, et de mon prédécesseur heureusement ici présent, le Père Daniel O'Connell, source constante de conseils pour moi, l'Académie a essayé d'accompagner l'évolution scientifique. Ils ont été aidés dans leur tâche par le zèle laborieux du Prof. Pietro Salviucci, de même que j'ai l'aide inestimable du Père di Rovasenda. L'activité de l'Académie est témoignée par ses réunions, par ses innombrables publications, parmi lesquelles les Volumes où s'insèrent les Actes des Semaines d'Etude ont obtenu une répercussion universelle.

Toutefois, la période que nous vivons apporte à l'Académie de nouvelles perspectives et obligations conséquentes à la transformation qui s'opère dans le panorama de la science moderne. C'est que les

années qui ont suivi le dénouement de la seconde guerre mondiale ont connu un développement scientifique et technique extraordinaire, dont beaucoup d'êtres humains n'ont pas pu se rendre compte, bien qu'ils en vivent les bonnes ou mauvaises applications. A côté de la poussée technique, qui est allée jusqu'à permettre l'exploration des spaces sidéraux, un essor prodigieux, dirais-je, s'est produit dans presque toutes les disciplines scientifiques, dont je cite seulement l'astrophysique, la géotectonique, la chimie des polymères, les mathématiques opérationnelles, la physique de l'état solide et la biologie moléculaire. Cet essor a été facilité par un immense progrès des méthodes de recherches, dont les ordinateurs en sont un exemple. La science, pendant cette période de 40 ans, a ainsi acquis seigneuriage dans le processus de l'évolution sociale.

Toutefois, à l'euphorie qui a accompagné les résultats spectaculaires des applications techniques de la science, dans l'après-guerre, et qui a gagné les pays en voie de développement après leur indépendance, a succédé fréquemment une certaine appréhension sur l'excès de l'usage des nouvelles techniques, sur l'orientation qui leur a été donnée, et on est même arrivé à attribuer au développement scientifique les injustices et difficultés créées par la société de consommation. On peut concevoir que la Science parce qu'elle a été mal utilisée a donné lieu à la création d'un système, lequel, destiné à servir l'homme, l'a par contre assujetti et a créé dans l'esprit de celui-ci le mythe d'un Progrès conçu comme l'accroissement illimité des biens matériaux et de la jouissance, pouvant conduire à l'affluence qui inhibe la poursuite de la recherche désintéressée. Les signes d'inquiétude sont trop évidents pour qu'on les cache. Ils ont conduit à des exagérations qui peuvent nuire au progrès scientifique. En conséquence, la communauté scientifique, convaincue que la destinée de l'homme sur terre dépend de l'apport qui lui sera donné par le progrès scientifique, s'effraye, parce que les positions prises peuvent éveiller un anti-scientifisme déjà latent et s'émeut face à ces mouvements de perplexité et d'anxiété, voire de colère.

La communauté scientifique sait que la société n'accepte plus passivement le développement, mais qu'elle exige qu'une réflexion se fasse sur les avantages et les risques que l'usage des découvertes scientifiques apporte. L'humanité aujourd'hui met en cause la signification même du développement technologique et les modèles de

civilisation industrielle qui en déroulent et sent aussi combien la sagesse en a été éloignée. Ceci, toutefois, ne touche pas à la vraie signification de la Science, sinon pour réaffirmer qu'elle est une partie intégrante de la culture.

L'Académie Pontificale des Sciences, consciente de ses grandes responsabilités morales et éthiques, se doit aussi de se pencher sur ces aspects de l'évolution de la pensée et a essayé d'y être attentive. A côté des Commémorations, Communications et Conférences habituelles, une journée de la Séance Plénière qui vient de s'achever a été dédiée à un Colloque sur « la science et le monde contemporain ». Il a donné lieu à une ample discussion sur « le rôle de la science », « la formation du scientifique et du technologue » et « la responsabilité du scientifique ». Ces sujets de réflexion ont mérité un traitement en profondeur. La publication des présentations et des débats qui ont suivi portera certainement des fruits.

D'autres études de caractère épistémologique vont être poursuivies en accord avec les finalités que déterminent les nouveaux Statuts que Votre Sainteté a bien voulu promulguer récemment. Pour répondre aux anxiétés dont j'ai parlé, les Semaines d'Etude plus récentes ont traîté des sujets qui intéressent le développement humain. C'est le cas de la réunion sur « les membranes biologiques et artificielles et la désalinisation de l'eau », déjà publiée, et de celle qui vient d'avoir lieu. L'année prochaine une Semaine extraordinaire présentera les nouvelles données concernant la préservation et le traitement du cancer, ce qui se joint à Vos préoccupations.

Votre Académie, Très Saint-Père, se tourne aussi vers d'autres sujets de grande importance, dont l'intérêt prospectif est certain. Des mises-au-point sur « les mutations dirigées chez l'homme » et sur « le danger des radiations nucléaires » ont été faites par des Groupes de Travail composés d'Académiciens et de spécialistes. Ainsi, l'activité de l'Académie s'accroît. Cet accroissement s'opère afin de poursuivre l'avancement de nos connaissances sur les lois de la Nature et la juste adaptation du progrès scientifique pour l'amélioration matérielle de la condition humaine.

Je vous prie, Sainteté, d'accepter mes remerciements pour la concession de la médaille d'or Pie XI au Dr. Lucio Luzzatto: ses travaux sur la génétique moléculaire, la génétique humaine et la génétique des populations sont de valeur exceptionnelle et le rendent

digne de recevoir cette distinction. Chez Lucio Luzzatto nous trouvons l'esprit d'union entre la science fondamentale et la science appliquée, essentiel sans doute, mais qui devient rare à cause de la spécialisation à outrance.

L'Académie Pontificale des Sciences a proposé à Votre décision souveraine le nom du Dr. Lucio Luzzatto exclusivement pour la valeur de ses travaux. Elle désire, néanmoins, à cet instant, souligner l'idéal humanitaire qui a animé sa vie et l'a ennoblie et qu'elle admire dans le dévouement qu'il porte à la cause humaine.

Sainteté, en rentrant chez nous, où nous attendent nos besognes quotidiennes, nos espérances et nos soucis, nos joies et nos souffrances, nos réussites et nos déboires, nous, les Académiciens Pontificaux et les Participants à la Semaine d'Etude, porterons avec nous le grain d'espérance que Votre accueil et Votre bonté déposent dans nos coeurs: il y poussera comme la bonne semence.

Le Saint-Père daigna répondre par le Discours suivant:

Excellences,

Nous sommes heureux de vous accueillir en audience spéciale au terme de votre Semaine d'Etude, dont le thème central était d'un intérêt tout particulier: « Les substances naturelles et la protection des plantes ». Nous vous saluons tous très cordialement et tenons à vous assurer que Nous apprécions sincèrement l'oeuvre précieuse que vous accomplissez, avec dévouement et esprit de sacrifice, au bénéfice du progrès scientifique. Notre estime est d'autant plus vive que votre préoccupation fondamentale, Nous le savons, est de servir l'homme, et c'est bien là aussi le but final de votre recherche. Vous sentez profondément en vous la solidarité qui vous lie à l'humanité d'aujourd'hui et de demain, et c'est pourquoi vous adoptez une attitude qui est celle de tout scientifique sérieux, l'attitude de celui qui — comme Nous avons eu l'occasion de le souligner lors de notre rencontre de l'an dernier — « doit se poser loyalement la question de l'avenir terrestre de l'humanité et, en homme responsable, concourir à le préparer, à le préserver, à éliminer les risques » (AAS 67, 1975, p. 268).

Le thème choisi pour la présente Semaine reflète d'une manière évidente cette sollicitude: face aux agents nocifs qui menacent les plantes, dont les fruits constituent directement ou indirectement la source principale de subsistance

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pour l'être humain, la protection se réalise surtout aujourd'hui grâce aux produits chimiques de synthèse; mais ces derniers suscitent des préoccupations de plus en plus graves, en raison de leurs possibles effets toxiques à long terme sur l'homme, en raison aussi des modifications qu'ils apportent dans le milieu naturel, avec pour conséquences des perturbations dans l'équilibre écologique. C'est ce qui détermine le savant à intervenir pour étudier la possibilité d'utiliser, pour une telle oeuvre de protection, des substances naturelles, qui se trouvent déjà dans le milieu et ne devraient donc pas provoquer de dommages écologiques. Tel est précisément le thème de votre Semaine.

Nous espérons que cette possibilité de confronter et de discuter les résultats de vos recherches en ce domaine aura contribué efficacement à faire progresser la connaissance scientifique des moyens de défense mis à la disposition de l'homme. Puisse-t-elle aussi favoriser la mise en oeuvre de formes de sauvegarde qui ne soient pas nocives à la santé! Stimuler le progrès des sciences pour le service de l'homme représente la fin institutionnelle de cette Académie Pontificale des Sciences.

Il Nous plaît de le rappeler en cette circonstance puisque nous célébrons cette année le quarantième anniversaire de sa fondation par notre prédécesseur Pie XI. Le Motu Proprio *qui instituait ce nouvel organisme en définissait ainsi les buts: « Notre voeu et notre désir est que les* Academici Pontifici, *grâce à leur et à notre Institut, favorisent toujours plus et toujours mieux le progrès des sciences, et Nous ne leur demandons pas autre chose, puisque c'est ce noble but et cette tâche élevée qui constituent le*

service que Nous attendons de ces hommes attachés à la vérité » (*cf.* AAS 28, 1936, p. 442).

Ces quarante années d'activité n'ont pas déçu cette attente: à travers les Semaines d'Etude, les groupes de travail, les publications scientifiques et les autres initiatives des décennies passées, l'Académie Pontificale, Nous le disons en Nous faisant l'interprète de votre légitime fierté, a apporté une contribution de valeur non seulement au progrès des connaissances scientifiques, mais aussi à la cause de la collaboration et de la compréhension entre les hommes.

La composition même de l'Académie, qui accueille des hommes de science sans distinction de nationalité, de religion, ou de croyance, souligne efficacement cette universalité de la science qui est un élément premier de rencontre et d'entente entre les peuples. La science tend par nature à dépasser les limites que les hommes se sont données en dressant des frontières entre eux: elle recherche une vérité qui n'admet, comme telle, aucune coloration politique, et elle se livre à cette recherche des méthodes rationnelles qui ne peuvent qu'être les mêmes pour tous les scientifiques, quelle que soit leur origine. Elle favorise donc une mentalité qui permet un dialogue confiant, sincère et respectueux avec tous ceux qui se trouvent engagés dans le destin commun de l'humanité. On voit alors à l'évidence quel instrument de compréhension réciproque et de paix peut représenter une recherche scientifique sérieuse, et quelle aide l'Assemblée que vous constituez peut apporter de ce point de vue pour favoriser une vie plus solidaire et pacifique entre les nations.

L'Eglise a toujours salué, et d'une manière particuliè-

rement vigoureuse au terme du Concile, les chercheurs de vérité que sont les hommes de science dont les sentiers ne sont pas étrangers aux siens (*cf.* Message aux hommes de la pensée et de la science). *Non seulement elle reconnaît la légitime autonomie méthodologique de la science moderne* (*cf.* Const. Gaudium et Spes n. 36), *mais elle salue, dans la mutation que cette dernière entraîne dans le mode de penser et de vivre, des valeurs positives qui ne sont pas sans rapport avec l'oeuvre du salut dont elle a reçu la charge. C'est pourquoi l'Eglise a besoin de vous, de votre sens exigeant de la recherche et de votre amour de la vérité.*

Nous vous encourageons donc à poursuivre généreusement votre chemin de chercheurs consciencieux, tendus vers la conquête de nouvelles possibilités pour le progrès humain. En reprenant encore une parole du grand Pontife Pie XI, Nous exprimons le souhait que « cette Académie devienne une source toujours plus riche de cette charité bénéfique qu'est la Vérité » (*cf.* Discours pour la séance du 27 décembre 1925 de l'Académie pontificale des Sciences, Nuovi Lincei). *Et ce souhait, Nous l'accompagnons de notre prière, en demandant au Dieu Tout-Puissant, Source de la vie et de l'esprit humain, de vous assister dans votre recherche au service de l'humanité et de vous bénir personnellement, ainsi que tous ceux qui vous sont chers.*

Translation of the speech to the Holy Father by the President of the Academy:

Your Holiness:

Allow me, most Holy Father, to express warmly in the name of Your Pontifical Academy of Sciences our deep gratitude and our joy for the great kindness with which you follow our work and for the support and encouragement which you give to our initiatives. In the midst of Your Holiness' many concerns you still find time to think about sources which may provide for the temporal support of mankind.

I want to add to my thanks those of the eminent scientists and specialists — for they all are such — who took part in the "Study Week" which has just reached its end. They feel happy to have been able in complete freedom to discuss and consider during six days the problems involved in the protection of the plants in a world where one is in constantly greater fear of a rupture of the eco-systems and of the undesirable consequences for human nutrition arising from the excessive use of pesticides in the protection of food.

The new sources of protection offered by the use of the so-called "natural products" which are substances derived from the metabolic activity of animals, including the insects, and of the plants, seem to be of major importance in the preservation of the vegetable world and its crops.

In considering these subjects from a scientific angle, your Academy shows once more its desire to follow the courses where science can best contribute and should contribute to social development at the same time that it endeavours to advance the scientific foundations of our knowledge on which rests the great intellectual and human adventure which is science in our days.

Your Holiness, our gathering this year has a special meaning. We celebrate the 40th anniversary of the transformation by His Holiness Pius XI — of venerable memory — of the Academy of

"Nuovi Lincei" into the Pontifical Academy of Sciences which is unique in its international and ecumenic character.

Under the Presidency of my eminent predecessors, to whom I rend tribute, the lamented Father Agostino Gemelli and Monsignor Georges Lemaître and of Father Daniel O'Connell, who is fortunately here today, and is my constant source of advice, the Academy has endeavoured to accompany the advance of science.

They have been assisted in their efforts by the constant zeal of Professor Pietro Salviucci in the same way in which I have the incomparable help of Father di Rovasenda. The activity of the Academy is proven by its meetings and its numerous publications, among which the volumes containing the minutes of the "Study Weeks" have received worldwide attention.

However, the times in which we live, bring to the Academy new prospects and obligations arising from the transformation which is taking place in the panorama of modern science. The years which followed the closing of the second World War have seen an extraordinary scientific and technical development which so many people do not realize, even though they are living through its good or bad consequences.

Along with the technical drive which has gone as far as permitting tbe exploration of space, a prodigious effort has taken place in practically all scientific activities among which I will mention only astrophysics, geotectonics, operational mathematics, solid state physics and molecular biology. This progress has been assisted by an immense development in the methods of research, of which the computer is an example. Science has thus, in this period of 40 years, mastered a leading position in the process of social evolution.

However, after the enthusiasm which followed the spectacular results of the technical appliance of science since the war and which reached the countries in course of development after their independence, a certain concern has followed regarding excessive use of new technology and the course to which they are directed, and scientific development has even been held responsible for the social injustices and difficulties arising from a consumer society.

One can understand that science if badly used may have given place to the creation of a system which tbough intended to serve

men has nevertheless enslaved him and created in his mind the myth of a progress conceived as an unlimited growth of physical property and the enjoyment of affluence, which obstruct the pursuit of disinterested research.

The signs of this unrest are too evident to be ignored. They have led to exaggerations which may be detrimental to scientific progress. Consequently, the scientific community being convinced that the destiny of men on earth depends upon the support which he may receive from scientific progress, becomes afraid, because of positions taken which may awake an already latent anti-scientific feeling, which may be released by these movements of perplexity, anxiety and even hate.

The scientific community knows that society no longer accepts development in a passive attitude but that it demands that the advantages and risks of the use of such development be the object of reflection. Humanity today places in doubt the very meaning of technical development and of the models of industrial civilisation which arise therefrom and feels how far they may be from wisdom. This, however, does not reach the true meaning of science, except to reaffirm the thought that science is an integral part of culture.

The Pontifical Academy of Sciences, faced with its great moral and ethical responsibility, must also consider these aspects of evolution and thought and has tried to devote its attention thereto.

Besides the usual commemorations, communications and conferences, a day of the Plenary Session which has just finished was devoted to the discussion of "Science in the Contemporary World".

It gave occasion for an ample discussion regarding "the role of science", "the formation of the scientist and the technicians" and "the responsibility of the scientist". These subjects of reflection deserve to be treated in depth. The publication of the presentations and discussions which followed will certainly bear fruit.

Other studies of an epistemological nature are to be followed in accordance with our proclaimed aims recently promulgated by Your Holiness. In response to the anxieties which I mentioned, the most recent "Study Weeks" have dealt with subjects which interest human development. One of these was the meeting on "biological and artificial membranes and desalinization of water"

which has already been published and the same holds for the "Study Week" which has just taken place. Next year a special meeting will provide new data concerning the role of non-specific immunity in the prevention and treatment of cancer for which Your Holiness has also shown interest and concern.

Your Academy, most Holy Father, turns its attention also to other subjects of great importance and promise. The discussion on the "directed mutations in man" and on "the danger of nuclear radiation" have already been brought to a head by task-forces composed of members of the Academy and of specialists. In this way the activity of the Academy is widened. This widening takes place in order to pursue the advancement of our knowledge regarding the laws of nature and the just adaptation of scientific progress to the material improvement of man's condition.

I beg Your Holiness to accept my thanks for giving the Pius XI Gold Medal to Dr. Lucio Luzzatto: his work on molecular genetics, human genetics and population genetics are of exceptional importance and single him out for this honour. In Lucio Luzzatto we find the spirit of union between fundamental and applied science, no doubt essential, but which is rare because of the present trend for extreme specialization.

The Pontifical Academy of Sciences has proposed to Your Holiness' supreme decision the name of Dr. Lucio Luzzatto exclusively on account of the value of his work. The Academy desires nevertheless, at this moment, to underscore the humanitarian ideal which has filled and enobled his life and which the Academy admires in the devotion which he has given to the cause of humanity.

Upon returning to our homes where our daily work, our hopes and our concerns, our joys and our sufferings, our successes and our failures await us, we, the Members of the Pontifical Academy and those who participated in the "Study Week", take with us the grain of hope which Your Holiness' welcome and kindness has sown in our hearts: It will grow as a good seed on favourable soil.

The Holy Father answered with the following Discourse:

Your Excellencies,

We are happy to receive you in special audience at the end of your Study Week, the central subject of which was of quite particular interest: "Natural substances and plant protection". We greet you all very cordially and are anxious to assure you that we sincerely appreciate the valuable work you are carrying out, with dedication and spirit of sacrifice, for the benefit of scientific progress. Our esteem is all the deeper in that your fundamental concern, as we know, is to serve man, and that is also the final aim of your research. You feel deeply within you the solidarity that binds you to mankind today and in the future, and that is why you adopt an attitude which is that of ever serious scientist, the attitude of one who — as we had the opportunity to stress during our meeting last year — "must honestly pose himself the question of the earthly future of mankind and, as a responsible man, contribute to prepare it, preserve it and eliminate risks" (*AAS* 67, 1975, p. 268).

The subject chosen for the present Week reflects this concern in an evident way. With regard to the harmful agents which threaten plants, the fruits of which constitute directly or indirectly the main source of subsistence for the human being, protection is carried out today thanks above all to synthetic chemical products. But the latter are causing more and more serious concern, owing to their possible long-term toxic effects on man, and owing, too, to the changes they bring to the natural environment, with the consequent disturbances of the ecological balance. This is what prompts the scientist to intervene to study the possibility of using, for this work of protection, natural substances, which are already found in the environment and

should not therefore cause ecological damage. This is precisely the subject of your Week.

We hope that this possibility of comparing and discussing the results of your researches in this field, will have contributed effectively to furthering the progress of scientific knowledge of the means of defence put at man's disposal. May it also encourage the use of forms of safeguard which are not harmful to health! To stimulate the progress of science for the service of man represents the institutional purpose of this Pontifical Academy of Sciences.

We are happy to recall it on this occasion since we are celebrating this year the fortieth anniversary of its foundation by our predecessor Pius XI. The *Motu Proprio* which instituted this new organism defined its aims as follows: "Our wish and our desire is that the *Academici Pontifici*, thanks to their and our Institute, will promote the progress of science more and more and better and better, and we ask nothing else of them, since it is this noble aim and this high task which constitute the service we expect of these men attached to truth" (cf. *AAS* 28, 1936, p. 424).

These forty years of activity have not disappointed this expectation: through Study Weeks, working groups, scientific publications and the other initiatives of the past decades, the Pontifical Academy, we say so expressing your legitimate pride, has made a valuable contribution not only to the progress of scientific knowledge, but also to the cause of collaboration and understanding among men.

The very composition of the Academy, which gathers men of science regardless of nationality, religion or belief, effectively emphasizes this universality of science which is a first element of meeting and understanding among peoples. Science tends by its very nature to go beyond the limits that men have given themselves by setting up frontiers between them. It seeks a truth which does not admit, as such, any political colouring. It engages in this research with rational methods which cannot but be the same for all scientists, whatever their origin may be. So it fosters a mentality which permits

a trusting, sincere and respectful dialogue with all those involved in the common destiny of mankind. It can clearly be seen, then, what an instrument of mutual understanding and peace serious scientific research can represent, and what a contribution the Assembly which you constitute can make from this point of view to promote a more united and peaceful life among the nations.

The Church has always greeted, and in a particularly forceful way at the conclusion of the Council, the seekers of truth that scientists are, whose paths are not alien to her own (cf. *Message to men of thought and science*). Not only does she recognize the legitimate methodological autonomy of modern science (cf. *Gaudium et Spes*, n. 36), but she greets, in the change that the latter brings into the way of thinking and living, positive values which are not unrelated to the work of salvation with which she is charged. That is why the Church needs you, your demanding sense of research and your love of truth.

We encourage you therefore to continue generously on your way as conscientious seekers, aiming at the conquest of new possibilities for human progress. Quoting once more some words of the great Pontiff Pius XI, we express the wish that "this Academy will become an increasingly rich source of his beneficial charity which Truth is" (cf. Address for the meeting on 27 December 1925 of the Pontifical Academy of Sciences, Nuovi Lincei). And we accompany this wish with our prayer, asking Almighty God, the Source of life and of the human spirit, to assist you in your research in the service of mankind and to bless you personally, as well as all those who are dear to you.

Achevé son Discours et reçu l'hommage des Eminentissimes Cardinaux, le Pape s'entretint ensuite avec le Président de l'Académie, le Président Emérite et le Directeur de la Chancellerie, avec les Académiciens et les Participants à la Semaine d'Etude et la Médaille d'or Pie XI, le Dr. LUCIO LUZZATTO.

Avant de quitter Rome, les Participants à la Semaine d'Etude envoyèrent le télégramme suivant pour exprimer au Saint-Père leur profonde gratitude:

Tous les savants réunis dans la Cité du Vatican qui ont participé à la Semaine d'Etude sur l'utilisation des substances naturelles dans la protection des plantes expriment a Votre Sainteté les sentiments de leur profond dévouement et remerciement pour avoir eu le privilège de participer a cette Réunion organisée par l'Académie Pontificale des Sciences afin d'échanger leurs expériences et leurs idées dans un climat d'entière liberté et sérénité au but de poursuivre leur chemin de chercheurs consciencieux tendus vers la conquête de nouvelles possibilités pour le progrès de l'humanité stop Votre chaleureux accueil et vos paroles d'encouragement représentent la plus haute récompense à nos efforts et à notre engagement dans un domaine scientifique de si grande importance et actualité stop Daignez agréer Sainteté nos plus chaleureux remerciements pour l'Audience solennelle et l'enseignement précieux que nous portons avec nous

CHAGAS, ABOKHATWA, ALVES, BALLIO, BELL, BERNAYS, BOWERS, BRADER, BUYCKX, CANONICA, CARDANI, CHAPMAN, CRUICKSHANK, DORN, ELLIOTT, GILBERT, GONZALEZ, GRANITI, HEIMPEL, JACOBSON, KARLSON, KNÜSLI, MARINI-BETTÒLO, NAKANISHI, QUIJANO-RICO, SCHILDKNECHT, SHOREY, SIDDALL, SOMERVILLE, STAAL, WAIN, WIGGLESWORTH, WILLIAMS, ZANINI.

TRAVAUX SCIENTIFIQUES
ET
DISCUSSIONS

I

INTRODUCTION

MODERN TRENDS IN THE USE OF NATURAL PRODUCTS FOR CONTROLLING PESTS AND PLANT DISEASES

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Introduction

Man's survival depends on the availability of food, and plants, directly or indirectly, supply that food. To ensure adequate food production man must fight daily against pests, such as insects, fungi molds, rodents and nematodes which would otherwise destroy the greater part of his crops.

In spite of the use of all available means for plant protection about one-third of the yearly harvest of the world (worth about 75 billion dollars) is destroyed by these pests [1].

In addition to the food plants like the cereals, fruits and legumes other plants of commercial and industrial importance like cotton, coffee, sugar cane, sugar beet, bemp and the trees wbich provide timber and cellulose are also at risk.

Tropical countries, because of their temperature and their particular environments suffer the most severe losses from pests. These losses may lead to serious famine in large areas of the world, many of which are densely populated.

Until 1945 the weapons used against the pests were mainly based on metallic salts (frequently copper salts), a few synthetic

substances, and a limited number of natural products like nicotine, rotenone, rianodine, quassin and the pyrethrins.

Not all these substances were particularly efficient in protecting cultivated plants from the attack of insects and fungi however.

Therefore the finding in the early 1940's of the extraordinary properties of such substances as DDT and later BHC (Benzenhexachloride) and the subsequent development of the chlorinated cyclodienes, of organophosphates and of carbamates marked a major advance in the field of plant protection [2].

The use of these substances in the last twenty-five years has contributed greatly to the increase of food production but it has also raised a number of ecological and medical problems. In addition the repeated and continuous use of chemicals, some of which are very persistent, has led to the development of resistant strains which are unaffected by compounds which were previously very efficient in controlling these particular pests. The phenomenon of resistance is rather complex and generally is based on an induced biochemical modification which allows the pest to metabolize the pesticide. As a consequence, in the past, higher and higher quantities of the pesticide were frequently used, and ultimately new and more powerful pesticides had to be introduced for the treatment of the plants. It must also be remembered that insects very often develop a resistance not merely to a single compound but rather to a group of related compounds [3].

The use of insecticides may also lead to ecological imbalance, the destruction of species which prey upon the harmful insects as well as the harmful insects themselves and the destruction also of pollinating insects.

Some of the pesticides, e.g. organochlorine derivatives, are degraded very slowly by atmospheric and biological factors and thus they may last in the environment for years. This fact leads to the development of resistant strains among the pests on the one hand to the contamination of the environment and the food chain on the other.

Considerable effort has been made all over the world to study the fate of the persistent organochlorine pesticides which accumulate

in human and animal bodies and now pollute the food chain even in remote areas like the arctic region.

The presence of organochlorine in the human bodies and the associated danger of long-term toxicity has resulted in national and international legislation to limit and even to ban the use of some compounds of this group. High toxicity is also a problem with some organophosphate compounds as well, and this problem taken together with the development of resistant pest strains makes the ongoing search for control agents which are less toxic to man and more readily degradable a matter of urgency [4].

The search for such compounds is based both on the random screening of as great a range of known organic products as possible and on the testing of new synthetic compounds which have been "tailor made" according to the general rules which relate structure to biological activity. By these means a number of new products come into use every year and help in the struggle to keep losses due to pests within limits [5].

In addition to the efforts of the chemists, biologists have developed biological weapons for pest control. Such methods of biological control are mostly in the early stages of development however.

Clearly an integrated approach using biological and chemical means separately or together as is most appropriate must be the ultimate objective. A number of examples of this approach such as the work at the Cañete Valley in Peru have shown how efficient it can be, even in very difficult conditions [6].

Basic research over more than thirty years on the biology and biochemistry of insects and plants has made it possible to envisage not only how new pesticides may be synthesised but also a completely new approach for the protection of plants using secondary plant products which may be toxic to a specific pest species yet harmless to man.

I should like to emphasize at this meeting that even though fundamental research is considered by many people and even by some administrators and governments as an academic exercise and even a luxury, it is only the results of many years of fundamental research which allow new practical solutions for extremely difficult

problems to be reached. This is particularly true of plant protection where the new solutions envisaged are based on the combined knowledge arising from fundamental research in biology, biochemistry, chemistry, plant and insect physiology.

The purpose of this study week is to examine the present state of the basic research on natural products which may afford protection to plants and assess their possible use in agriculture.

In considering natural products we shall also consider those which can be more readily synthesised than extracted and also close synthetic analogues which are likely to be readily biodegradable like the natural products themselves.

In this meeting natural products which are not only those extracted from plants or insects but also their synthetic analogues provide the common denominator for our work. We shall consider and examine not only their application in the control of insects, using our knowledge of insect secretions, such as pheromones, molting hormones, repellents, attractants, antifeeding compounds, etc. but also our knowledge of other natural substances such as those obtained from plants, bacteria and the viruses.

A second matter that we shall consider is the biochemical basis of plant resistance. The finding of particular substances only in resistant plants, and the recognition that other substances may be formed under the stimulating action of insects or molds, illustrates how fundamental research is bringing us to a better understanding of plant resistance. We shall consider how this knowledge may be applied in the near future to the practical problems of crop protection.

* * *

In introducing this meeting I do not intend to summarize the results so far obtained in the two main areas described as we have here some of the most distinguished researchers in these fields. These researchers themselves will present up-to-date critical reports of the present knowledge in these different areas and these reports will form the basis of our discussions. My task will therefore be limited to tracing the guidelines for the discussions and outlining the aims and the spirit of the present "Study Week".

Biochemistry of insects

Early research on the biochemistry and physiology of insects led to the discovery not only of insect hormones but also of a number of other substances which condition insect behaviour. Knowledge of compounds like pheromones, repellants, attractants and antifeeding substances have been integrated by recent research which has led to the isolation and identification of a great number of natural products from insects which show interesting biological properties [7]. For example, more than 400 repellant substances have been isolated and characterised from 700 species of arthropods, and we have structural information on compounds affecting the physiology or behaviour of a number of different insects [8].

Most of the natural substances obtained from insects have rather simple structures like Juvenile Hormones (JH), which are alicyclic terpenes with several functional groups. Owing to the extremely low concentration of these substances in the insects, however, the isolation work constitutes one of the most interesting features of the chemistry of natural products [9]. More complicated structures have been found in the Molting Hormones (MH), like the ecdysones, and in some particular substances like pederin and dendrolasin. The fact however that the majority of these substances possess simple molecular structures allows their synthesis and the synthesis of analogues for biological assay. Some compounds like the ecdysones are not available in large quantities and this makes a practical application more difficult although new important sources of these hormones have been found in plants [10].

According to the phytotherapeutical rules slight modification of the structure of the natural product may modify or enhance its activity. Such modification may affect the absorption of the substance and also render it more resistant to degradation by light, atmospheric oxidation or geochemical agents. Industry has supported much work in this area recently, both in the laboratory and in the field.

In the last year a discovery of particular importance was the finding of precocenes in plants. These substances of rather simple structure belonging to the chromene group, are able to modify the

role of juvenile hormones and under their influence dwarf insects are produced [11]. The use of precocenes thus opens another avenue to the chemical control of insects.

These findings of insect biochemistry have permitted field experiments to be carried out both in temperate and in tropical areas. When the results of these experiments have been fully assessed it may be possible to see more clearly how such products can be used most effectively in the future. Their evaluation should not be limited to their efficacy in controlling plant pests however, but must take into account their impact on the environment and their eventual toxicity to man even over very prolonged periods. Their cost and the difficulties of producing them on an industrial scale must also be considered.

All these points which will be decisive in determining whether a natural product will find a practical use in agriculture should be thoroughly discussed in order to establish if we can seriously consider such a substance as providing a new approach to the chemical control of pests.

Insecticides from plants, bacteria and viruses

We have considered the products which constitute the chemical agents of biochemical regulation and behaviour in insects. We have now to examine another application of natural products chemistry, that is the use of products obtained from plants, bacteria and viruses, as new weapons against insects.

Rotenone, ryanodine and, to a large extent, nicotine, have been abandoned for major field use for several reasons; the pyrethrins, obtained from *Chrysanthemum cinerafolium* are still widely used and the plant is cultivated over large areas in the highlands of Africa and South America. At present the main use of pyrethrin is for domestic insecticides because it is non-toxic in man and mammals and is highly sensitive to light.

Much work has been carried out on the synthesis and biological evaluation of pyrethrin analogues and these have given encouraging results in field trials.

[1] I - *Marini-Bettòlo* - p. 6

Bacterial toxins have also been shown to be effective weapons against many insects, but only toxins of *Bacillus thuringiensis* have given positive results in the field against lepidoptera. It is certain that the use of toxins specific for certain insects could, with the help of modern microbiological fermentation techniques, be of great importance in the future strategy of plant protection [12].

The use of viruses for the control of insects and disease vectors has been studied in the last years and much information has been obtained in the use of some specific viruses, like those of the nuclear polyhedrosis. These methods could represent a new important approach in the integrated pest control although the risks will have to be carefully evaluated before introducing them in agriculture [13].

Plant resistance

The second approach to the protection of plants from attack by insects or fungi, is through the biochemistry of plant resistance.

The introduction of resistant strains of plants after severe outbreaks of disease, for example of maize, have solved some extremely difficult situations in the past few years. It will also be remembered that in the last century European vineyards were saved by using American vines resistant to *Phyloxera.*

Resistant varieties are not always of good quality nor give high yields. Nevertheless plant geneticists have a very important responsibility to identify resistant varieties of the most important industrial and economically valuable annual plants in order that modified strains may be available in case of need.

Work carried out over many years primarily in the United Kingdom has given us much information on the chemical basis of the plant resistance. On the one hand resistance may be due to the presence of some particular substance or substances which are accumulated in all plants of the resistant strain, on the other hand the substances that confer resistance may only be produced by the plants under attack by the insect or the molds; these are the so-called "stress products". Many of these substances have been isolated and their structure determined. They are generally

substances of particular structures like the phytoalexins derived from furan or from pterocarpans [14].

There are also some rather uncommon stress substances formed in the plant under the stimulus of mold attack like the mansonones in elms infected by *Ceratostomella ulmi,* or lubimin and related germacrolides which are synthesised by *Datura stramonium* when infected by *Monilina fructicola.* Recent literature reports a number of these substances with unfamiliar structures and much work on their biological activity remains to be done [15]. The mechanism which induces the formation of the substances which protect the plants against further attack is also far from being understood. The first recognised example of this type of protection was provided by *Quercus* (oak) which produces gallotanic acid in response to the attack of the insect *Cynips.* The function of special substances present in the pest which induce resistance may give a reply to this point.

In a recent report it was stated that fungi like *Phytophtora megasperma* produce polyglucans, called *elicitors* which have the property of inducing the formation of defence products in plants. This finding may give a new lead to the control of pests especially if it can be confirmed that elicitors can be produced also from simple fungi like common yeasts (*Saccharomyces cerevisiae*) and that the eliciting action is due not to the whole molecule of polyglucan but to a low molecular product with 6 glycoside residues, easily soluble in water and thus absorbed by the plant [16].

Another approach to the problem is the study of the mechanism of action of pathogenic fungi on plants.

It has been known for many years that some strains of pathogenic fungi produce metabolites of low molecular weight which are apparently the chemical agents of the plant diseases. These substances which belong to several chemical groups were named *phytotoxins*: one of the first of these to be studied was lycomarasmin which causes the wilt of Tomato [17].

Recent research on the role of phytotoxins in plant diseases has provided results which make it possible to consider new means for the protection of plants [18].

[1] I - *Marini-Bettòlo* - p. 8

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I must apologize if, even though very rapidly, I have summarised facts that you all know well, but the aim of this meeting is not only to review critically all the chemical and biological data we have on the issue of this pressing problem, but also to try to foresee ways in which natural products may be used to protect plants and thus crops, thereby increasing the availability of food for the growing world population, which is largely concentrated in tropical areas and in the developing countries where for climatical conditions pests are much more diffuse [19].

The presence here at this Study Week of scientists from all over the world representing different fields of Science, together with the representatives of the Food and Agriculture Organisation of the United Nations and the researchers from agriculture and from industry, provides a unique opportunity to consider ways to establish new practical and economical systems for the defence of crops and for the protection of the natural environment. Such ways could be a great contribution to the wealth and health of mankind.

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II

PRODUCTS ACTIVE ON PLANT PESTS

Products from arthropods - Products from plants
Products from microorganisms

CHEMISTRY AND PHYSIOLOGY OF INSECT HORMONES AND INSECT PHEROMONES

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Control of insect pests is a necessity. On the other hand, there is much concern about the ecological side-effects of the present day insecticides. Covering whole areas with tons of toxic material is certainly not the method of choice; what we would need in the future are highly specific agents that eliminate pest insects but do no harm to other species and to higher vertebrates including man. Products of this kind can only be developed on the basis of special features of insect physiology with which the specific agents can intervene. One possible rationale is the use of insect hormones or insect pheromones or of their analogues.

In this introductory lecture I will review our basic knowledge of the chemistry and physiology of insect pheromones, leaving the special aspects of their use in insect control to other speakers in this symposium.

Chemistry of Insect Hormones

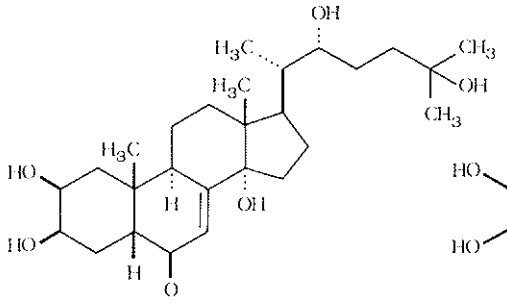
According to their chemistry, insect hormones can be grouped into three classes: Peptide hormones, steroid hormones and isoprenoid hormones. For a detailed discussion, the reader is referred to recent reviews [1, 2, 3].

Peptide hormones are often products of neurosecretory cells. Though many types of neurosecretory cells have been described, advances in the physiology and especially in the chemistry of neurosecretory hormones of invertebrates have been slow. Only few neurosecretory hormones are well characterized: The diapause hormone of *Bombyx mori* [4, 5], the "brain hormone" (prothoracotropic hormone, neurohormone D) [6, 7] and Bursicon [8]. Some of these peptide hormones have been obtained in highly purified form and analyzed for their amino acid composition, but their chemical structure (the amino acid sequence) has not yet been elucidated. Moreover, from the point of view of plant protection, they can be largely omitted from our discussion.

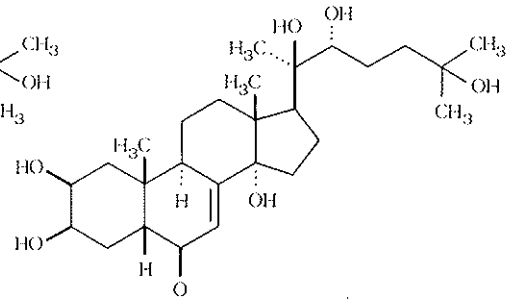
The *steroid hormones* are represented by ecdysone, ecdysterone, and other ecdysteroids. They and some related compounds are often called " ecdysones ". However, it is undesirable that the term ecdysone is used at the same time as a generic term and as the trivial name for 2β, 3β, 14α, 22, 25-pentahydroxy-cholest-7-en-6-one. Therefore, the term "ecdysteroids" has been proposed [9, 10] as a generic term for steroids with moulting hormone activity. The ecdysteroids are biochemically derived from cholesterol, still containing its C_{27} carbon skeleton. Fig. 1 gives the chemical structures of the ecdysteroids isolated from insects. Ecdysone (I) is the product of the prothoracic gland, ecdysterone (II) seems to be the active entity; the other compounds are presumably intermediates in biosynthesis or metabolic inactivation.

It was certainly a great surprise when Australian [11] and Japanese [12] workers first reported that certain plant steroids have the biological activity of ecdysone. These compounds are often called "phytoecdysones"; a better term would be "phyto-ecdysteroids". More than 30 compounds of this class have been isolated [for reviews, see 13 and 3].

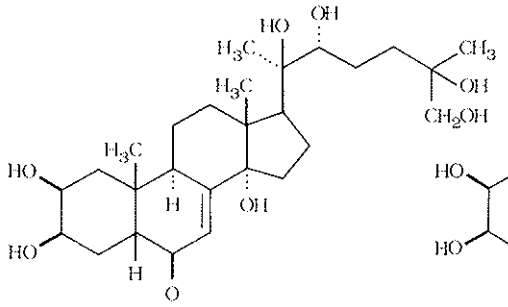
A survey of the structures of insect ecdysteroids and phytoecdysteroids show that they all contain the α,β-unsaturated carbonyl groups in ring B. The greatest structural variation is in the side chain. The biological activity varies; some of the phytoecdysteroids are nearly as active as ecdysterone, others show an activity of one to two orders of magnitude less. The possibility that these phyto-



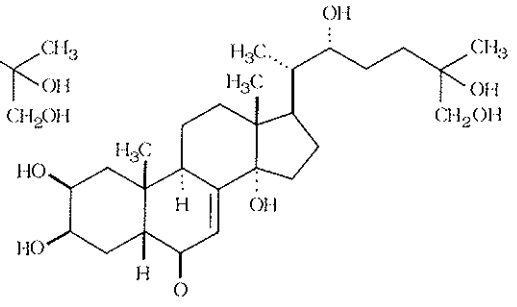
Ecdyson (I)



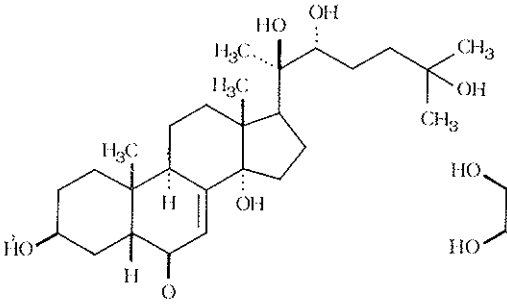
Ecdysteron (II)



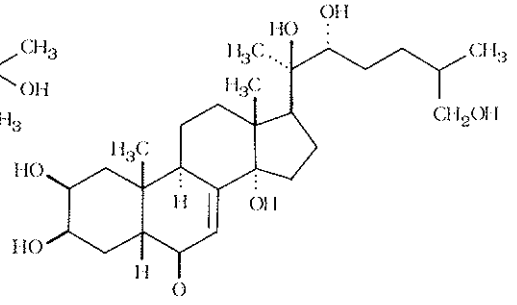
20,26-Dihydroxyecdyson (III)



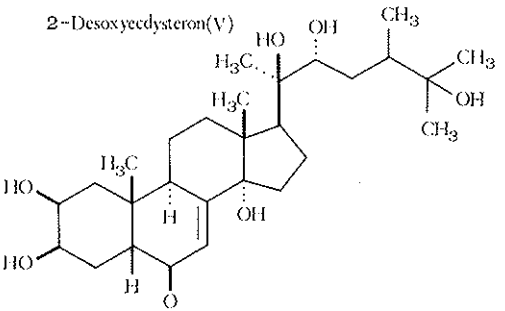
26-Hydroxyecdyson (IV)



2-Desoxyecdysteron (V)



26-Hydroxy-25-desoxyecdysteron (VI)



24-Methylecdysteron (VII)

FIG. 1 — Chemical structures of ecdysteroids isolated from insects.

ecdysteroids are essentially inactive but are converted to active compounds in the insect body should be kept in mind.

The juvenile hormones — While the ring system of sterols also serves as the basis of mammalian hormones, and peptide hormones are known all over the animal kingdom, the basic structure of the juvenile hormone is unique to the insects, at least as far as hormones

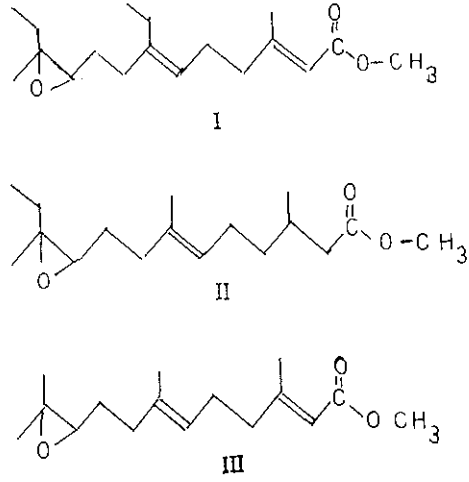


FIG. 2 — Chemical structures of juvenile hormones: I, Juvenile hormone I; II, Juvenile hormone II; III, Juvenile hormone III (= methyl juvenoate).

are concerned. The juvenile hormones [14] can be regarded as isoprenoids, though they are apparently derived not only from mevalonate but also from homomevalonate. Three different compounds have been isolated from insects; their chemical structure is given in fig. 2. However, the structural specificity is even less pronounced than with the ecdysteroids, and a rather large number of natural and synthetic compounds are active in bioassays for juvenile hormones [2]. The most prominent example is presumably juvabione, the "paper factor" of Slama.

Physiology of insect hormones

The *general principles* of hormonal control of the post-embryonic development of insects are outlined in figure 3. Moulting is initiated

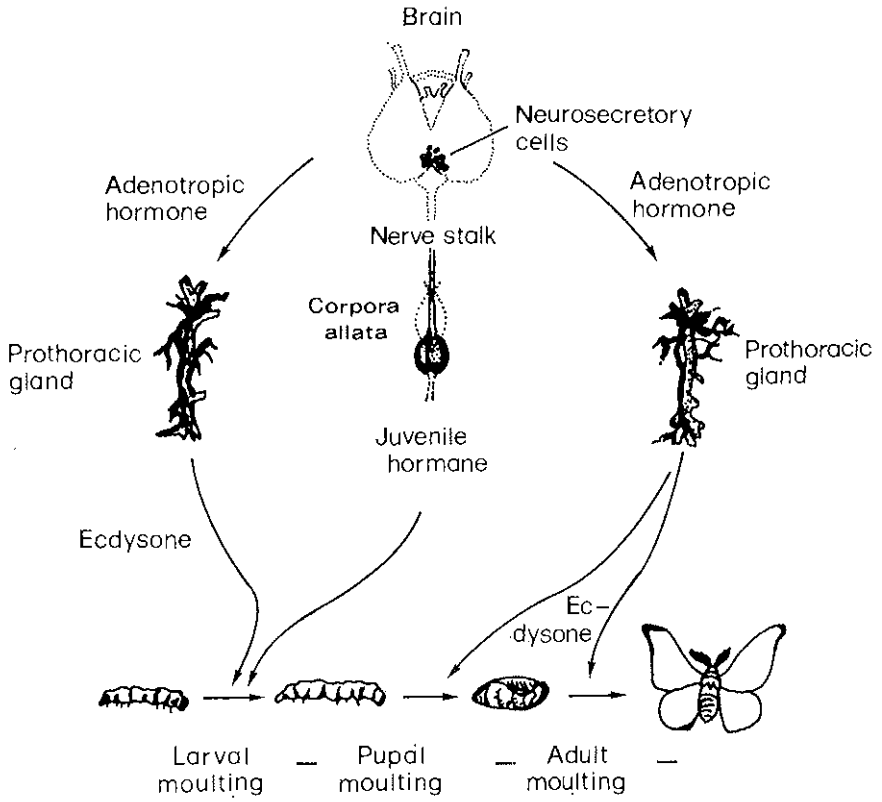


FIG. 3 — Action of insect hormones. In the upper part, the hormone-producing glands are represented. Below, the moults initiated by the hormones are shown.

by a peptide hormone released from the neurosecretory cells of the brain that activates the prothoracic glands. The long debate about the endocrine role of the prothoracic glands has now been settled: these glands produce ecdysone which is converted in the peripheral tissues (e.g. the fat body) to 20-hydroxy-ecdysone (= ecdysterone). Ecdysterone acts on the target tissues inducing moulting.

The character of the moult is determined by the activity of the corpora allata. If the corpora allata secrete juvenile hormone be-

fore or during the secretion of ecdysone, the moult will be a larval one; if only small amounts of juvenile hormone are present, a pupal (or nymphal) moult results. No juvenile hormone is needed for an imaginal moult.

In the adult female, juvenile hormone again has an important function in stimulating the production and incorporation of yolk into the eggs.

Hormone production and elimination — From the foregoing discussion it is evident that the titer of ecdysterone and of juvenile hormone is of special physiological importance. Generally, hormone titers depend on the rate of hormone synthesis and secretion and the rate of inactivation and elimination. As far as ecdysterone is concerned, the rate of conversion of ecdysone to ecdysterone may also play a role.

Very little is known about the control of the rate of secretion of juvenile hormone. Since the corpora allata have nerve connections with the corpora cardiaca and with the brain, neural and/or neuroendocrine control was postulated; there is some experimental evidence of neural inhibition of juvenile hormone secretion, and neuroendocrine mechanisms may play a role in stimulation of biosynthesis or release of juvenile hormone from the corpora allata [16].

Inactivation of juvenile hormone is brought about by specific esterases; in addition, the oxiran ring is enzymatically hydrolysed. The biological half-life is rather short.

As far as ecdysone is concerned, the most important factor regulating its secretion is presumably the prothoracotropic hormone, as already mentioned; moreover, juvenile hormone is also stimulatory. The mechanism by which prothoracotropic hormone stimulates ecdysone formation is not known. The control seems to be rather efficient; determinations of ecdysone titers in larvae of the last instar reveal a rather steep rise at the critical period [17, 18]. But not only the rate of secretion of ecdysone, but also its conversion to ecdysterone seems to be controlled. At the beginning of the last larval instar of *Locusta*, only ecdysone is present; at the time where the titer rises, only ecdysterone is found (Fig. 4).

Inactivation of ecdysterone occurs in part by oxidation to 3-dehydroecdysterone, in part by conjugation with sulphuric acid

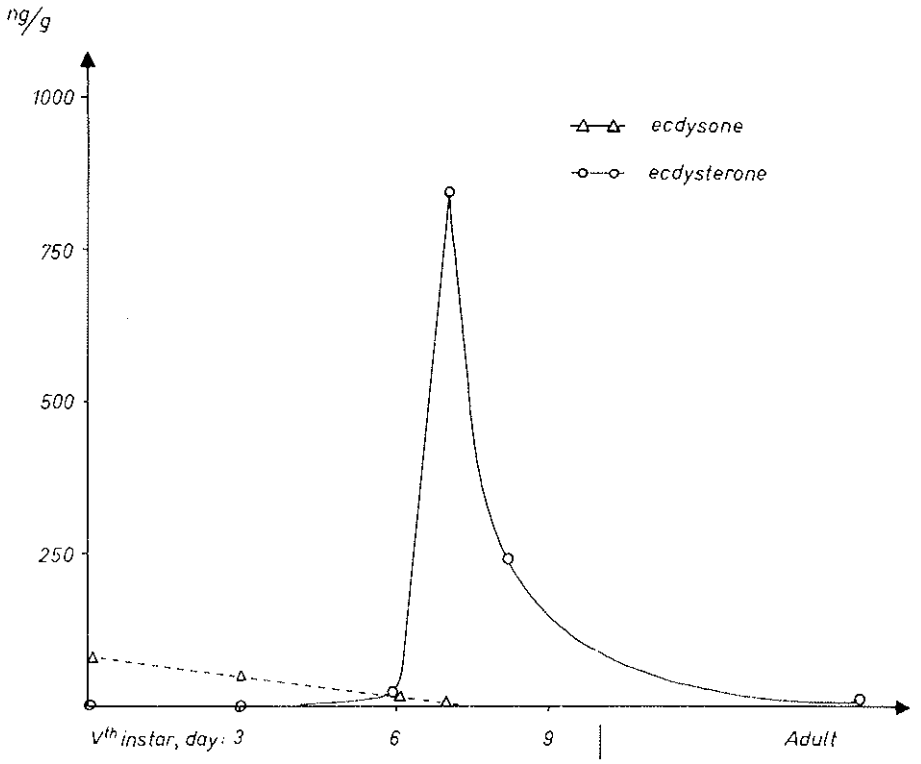


FIG. 4 — Titers of ecdysone and ecdysterone in the Vth instar of *Locusta migratoria*. With the rise of ecdysterone, ecdysone virtually disappears.

[19]. These conjugates are excreted with the feces. The rate of conjugation and excretion seems also to be controlled [18, 20].

Mechanism of action — Ecdysone is known to induce specific puffs in salivary gland chromosomes, to stimulate RNA synthesis and to induce dopa decarboxylase. From these and other findings, it can be inferred that ecdysone acts at the level of the cell nucleus, controlling transcription at specific genes. There are indications that the same might be true for juvenile hormone. A detailed discussion would be beyond the scope of this article; the reader is referred to recent reviews [21, 22].

Pheromones

Pheromones are signal substances between individuals of the same species. The classical example is the sex attractant of the commercial silk moth, *Bombyx mori,* isolated by BUTENANDT *et al.* in 1959 [23]. While BUTENANDT *et al.* had to collect altogether nearly 1 million abdomen tips, modern methods of separation and structure determination make it possible to determine pheromone structures with about one thousand scent glands. It was thus possible to isolate and analyze a large number of pheromenes [Reviews: 24, 25, 26].

Chemical structure — Though there is a large variety of chemical structures serving the purpose of pheromones, there are certain classes which can be grouped together: (i) unsaturated aliphatic alcohols (mainly with a chain length of C_{10} to C_{20}) or their acetates (Table 1), (ii) terpenoids or sesquiterpenoids as hydrocarbons, alcohols, ketones etc., (iii) small, volatile molecules like isopentenylacetate, hexenol, hexanoic acid and others; they serve mainly as trail or alarm pheromones. There are some pheromones outside these classes; a case of special interest is a heterocyclic ketone, danaidone, which is derived from a product of the plant on which the male moths feed. It is stored and transferred to the hair-pencil of the males where it serves as an aphrodisiac for the females.

Within related species, e.g. within the same genus or family, the sex pheromones are often chemically closely related. Cases are known where the pheromone effect is due to a mixture of two components, the relation being 10:90 in one species and 50:50 or 60:40 in other species. The evolutionary aspects of these findings are discussed by KARLSON and SCHNEIDER [27].

Physiology of pheromones — All the pheromones relevant in context with plant protection are releaser pheromones, i.e. they elicit a behavioural response. Of greatest importance are the sex attractants, followed by trail pheromones and alarm substances and the general attractants ("gathering pheromones").

Pheromones are "smelled" i.e. they are perceived through chemoreception. The receptor cells are often very specific for the pheromone; related molecules (homologues, stereoisomers) are not perceived or they have a much higher threshold concentration. More-

TABLE 1 - *Sexual pheromones with aliphatic chains.*

Substance	Species a	Family
Z-5-Decenyl-isovaleriate	*Nudaurelia cytherea*	Saturniidae
n-Undecanal	*Galleria mellonella*	Pyralidae
	Achroea grisella b	
E,E-8,10-Dodecadienol	*Carpocapsa pomonella*	Tortricidae
Z-7-Dodecenyl-acetate	*Trichoplusia ni*	Noctuidae
E-7-Dodecenyl-acetate	*Argyroploce leucotreta*	Tortricidae
Z-8-Dodecenyl-acetate	*Grapholita molestra*	Tortricidae
Z-9-Dodecenyl-acetate	*Paralobesia viteana*	Tortricidae
Z-9-Tetradecenyl-acetate	*Spodoptera frugiperda*	Noctuidae
	Prodenia eridania b	Noctuidae
	Adoxophyes orana b	Tortricidae
	Adoxophyes fasciata b	Tortricidae
Z-11-Tetradecenyl-acetate	*Adoxophyes orana* b	Tortricidae
	Adoxophyes fasciata b	Tortricidae
	Argyrotaenia velutinana	
	Choristoneura rosaceana	
	Ostrinia nubilabis	Pyralidae
Z,E-9,12-Tetradecadienyl-acetate	*Plodia interpunctella*	Pyralidae
	Cadra cautella b	Pyralidae
	Anagasta kühniella	Pyralidae
	Prodenia eridania b	Noctuidae
E-11-Tetradecenal	*Choristoneura fumiferana*	Tortricidae
E,Z-3,5-Tetradecadienoicacid	*Attagenus megatoma*	Dermestidae (Coleopt.)
E-2,4,5-Tetradecatrienoic acid-methyl ester	*Acanthoscelides obtectus*	Bruchidae (Coleopt.)
E,Z-10,12-Hexadecadienol (Bombykol)	*Bombyx mori*	Bombycidae
Z-14-Methyl-hexadec-8-en-1-ol	*Trogoderma inclusum*	Dermestidae (Coleopt.)
Z-14-Methyl-hexadec-8-enoicacid-methylester	*Trogoderma inclusum*	Dermestidae (Coleopt.)
2-Methyl-heptadecan	*Holomelina* spp.	Arctiidae
	Pyrrharctia isabella	Arctiidae
Z-11-Octadecenal	*Achroea grisella* b	Pyralidae
Z-7,8-Epoxy-2-methyl-octadecan	*Lymantria dispar*	Lymantriidae
Tricosen	*Musca domestica*	Muscidae (Dipt.)

a The substance is produced by the female unless stated otherwise.

b In this species, the substance given is only one component of the sexual phenoma which is a mixture.

over, the receptor cells are highly sensitive; SCHNEIDER *et al.* have calculated that possibly one molecule may suffice to elicit a positive response, i.e. a change in the receptor cell potential that is transmitted as a series of spikes (nerve impulses).

The threshold concentration of a pheromone in air may be very low. For *Bombyx,* about 10^4 molecules of bombykol per cm^3 air are sufficient, and the gypsy moth *Lymantria dispar* responds to similar concentrations of disparlure. In other species, the threshold may be higher by several orders of magnitude, but even then, minute amounts suffice for a positive reaction.

The fact that sex pheromones are relatively simple molecules whose synthesis on a larger scale seems feasible, that they are active in small amounts, and that they are highly species-specific would in principle allow a very fine control of pest insects. As we will undoubtedly hear, the practical difficulties are great, but it is at least a hopeful approach. Insect hormones are not specific for certain species, but they are essentially non-toxic to vertebrates, and their practical application seems to be easier. Thus, the outlook seems to be not bad; but we will hear more about this from more competent speakers during this week.

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DISCUSSION

MARINI-BETTÒLO

I thank you Prof. Karlson, for your very brilliant exposition on the chemistry and physiology of insect bormones and insect pheromones.

NAKANISHI

It is about your proposal of ecdysteroids. I would just like to get a general feeling. My feeling is that to propose a name at this stage may be too late. What you said of ecdysteroids really makes sense; but the name alpha-ecdysone is so widespread. On the other hand it would be nice for people to give some thought to this because besides crustecdysone there must be 3 or 4 other ways of calling beta-ecdysone. It would be very nice if we could come to some sort of general agreement provided it does not lead to more confusion if we change the nomenclature.

KARLSON

I do not think that the term "Ecdysteroids" must lead to confusion. And if I can convince you and a few other very active people in this field, and we write together a paper in Science in which we propose this name, then this term will be accepted world-wide very soon.

MARINI-BETTÒLO

Thank you Prof. Karlson, for this proposal. I think this must be, it should be rather important because the ecdysones are widespreading and I think that a general name like ecdysteroids could be very useful like those of corticosteroids as it was said before. I should like to have the opinion also of Prof. Canonica who has been working in this field.

CANONICA

I agree completely with the proposal of Prof. Karlson. Yesterday when I was preparing my lecture I noticed a lot of mistakes because one name was "ecdysone-like" compounds, another time it was ecdysoids and so on. So I think it is useful to accept this name of *ecdysteroids* proposed by Prof. Karlson.

MARINI-BETTÒLO

Now are there other topics for discussion besides the nomenclature?

BOWERS

I would like to point out that one of the insect peptide hormones has been isolated and sequenced. This is the adipokinetic hormone and Professor Mordue of Imperial College has just reported on it in Washington. There is considerable interest in insect peptide hormones in several other laboratories as well. We need much more information about the peptide hormones; they regulate many of the other hormones including the steroid hormones and the juvenile hormone. I know Professor Karlson has said he does not believe that there is much room for insect control with the peptide hormones, but I would like to suspend judgment for awhile until we know more about them.

KARLSON

I am glad to learn that studies of insect peptide hormones have come to the stage where sequences are being studied. Thank you for this information.

WILLIAMS

The sequence was published in a recent number of "Nature", about a month ago.

ABO-KHATWA

Would Professor Karlson comment on some of the recent findings regarding the presence of relatively high titers of ecdysones in adult

insects, particularly in the ovaries of silkmoths and termites despite the fact that the prothoracic glands, the source of ecdysones, are degenerated; and what are their physiological functions?

KARLSON

I do not know what the function is. We have found, to the surprise of many insect physiologists already in 1956, insect hormones in the abdomen of females: in female moths of Bombyx (*). The titer is much lower than in the pupae, it is about a tenth or so. The question: Where does the ecdysone come from? is really a very important one, but I have no answer. Ecdysone is converted into inactivation products very rapidly, and the latter are excreted. There might be a possibility that some conjugates of ecdysone may be stored in the insect tissue and released later. Phosphates might be good candidates for this type of conjugation.

NAKANISHI

Prof. Monot has published a paper based on radio-immunoassay in which he showed the existence of ecdysones in queen termite ovaries. On the other hand, getting some samples from Africa, we have recently restudied the ovaries, in order to identify ecdysone, by high resolution liquid chromatography. The sensitivity level is several nanograms.

We have extracted the entire ovary of a big Macrotermes queen and at least under those conditions we cannot find the ecdysones. I therefore think that this is still a question to be solved. In other words, the radio-immunoassays will respond to ecdysones, but on the other hand, we don't know about the structure specificity.

So I think the presence of ecdysones in termite ovaries, as far as chemistry is concerned, is still a matter of future proof.

BRADER

I would just like to ask a practical question. You started your talk

(*) KARLSON P. and STAMM-MENENDEZ M. D.: *Hoppe Seyler's Z.*, Physiol. Chem., *306*, 109-111 (1956).

by saying that the present problems with pesticides are partly their side effects. How strong a claim can you make that the insect hormones will not have side effects?

KARLSON

This is of course a very important point. The rationale is that a compound, which interferes with a biochemical or a physiological process typical for arthropodes, e. g. moulting, would be harmless to vertebrates and therefore harmless to man. Now, as far as ecdysone is concerned, this is true. Ecdysone is very rapidly excreted by the mouse. Ecdysone was tested in the laboratories of the Schering AG Berlin, and they could not find any pharmacological effects of synthetic ecdysone, even with high doses, i. e. with doses that will not occur as contaminations or as residues. I believe that the same can be said for juvenile hormone although Dr. Siddal may be the better expert to answer this question. And as far as pheromones are concerned, I think these might be even safer since they are not sprayed over the country but only exposed to the air at appropriate places, so that contamination and the problem of residue is very much less.

WILLIAMS

I would be interested in your views as to whether insects contain and are putting to good use such polypeptide hormones as insulin, glucagon, and gastrin. I am referring in particular to the publications of Seecof and Dewhurst (*).

KARLSON

I have not seen that paper, I must confess, and I can not give a comment.

BELL

Prof. Karlson you have told us that the pheromones are highly

(*) « Cell Differentiation », *3*, 63 (1974); TAGER *et al*, « Biochem. J. », *156*, 515 (1976), and KRAMER *et al*, « Gen. Comp. Endo », in press.

specific, but not totally specific. Where you have cross-reaction is this due to identical molecules or to closely related molecules in different species?

KARLSON

Both are possible, both happen. There is cross-reaction in part due to the same molecules. In that case the behaviour of the insects is different, e. g. they may fly at different times of the night, one species early in the dawn, maybe from 8 to 10, and the other only later. That separates the species attracted by the same molecule. In cases where the pheromone is only similar and shows overlapping activity, you generally need a higher dose. The concentration in the air must be higher by two or three orders of magnitude to get the electrophysiological response with the wrong pheromone.

MARINI-BETTÒLO

I think that here a very interesting question has been raised: the relationship between toxicity and specificity. Specificity avoids overuse of these substances, and toxicity is not in the question because you use very low quantities of these substances. Is there somebody who has some data about specificity and toxicity of pheromones?

SIDDAL

There are now some published data on the toxicity of analogs of juvenile hormones and also on several natural pheromones. The mountain of information which is available on toxicological effects of juvenile hormone analogs which are used in practical commercial sense for insect control is very large. I think it is not possible to make a generalisation. One should expect that each chemical substance may have unexpected toxicological properties, but the findings in the case of a small number of analogs which have been investigated extensively for the purpose of government registration for pest control, are very simple: the analogs are virtually devoid of side effect in mammalian systems. This is probably attributable in large part to the rationale which Prof. Karlson iterated, that the specific function of the juvenile hormone in insects and not in

mammals is fairly clear by now and well documented, and that the close resemblance of chemical structure of those few hormone analogs which have been used commercially preserves the relatively low mammalian toxicity which is present in the natural hormones.

MARINI-BETTÒLO

May I ask Prof. Karlson if these chlorinated analogs have also the same low toxicity and if they are used?

KARLSON

Well, I think Dr. Siddal would be the better person to answer this question. I think that chlorinated analogs are not used, are they?

MARINI-BETTÒLO

I was just asking this question because before there has been shown a formula with the chlorinated derivatives of Farnesoic acids. I do not know if it is used in the field.

SIDDAL

The chlorinated analogs have been replaced in more recent times by other analogs. The chlorinated analogs are not used in practical pest control; as far as I know there is no toxicological data on these compounds, so one cannot say whether they have toxic properties or not.

CANONICA

It is interesting to discuss which compound has to be considered the true molting hormone. Today is it is generally assumed to be the ecdysterone, but in nature some compounds have been found which have a larger molting hormone activity. For example, muristerone A which has been discovered by us a few years ago in the seeds of an Indian Ipomea and which is a 5β, 11α-hydroxyderivative of ponasterone; it shows on the silkworm a molting hormone activity thirty times larger than that of ecdysterone. The fact that the activity of a hormone can be

increased by the introduction of certain substituents in certain positions is well known in other steroid hormone families, for example in the cortisone family. In this case the activity is always associated with the same structure of the side-chain, while in the molting hormones it is not so. For some active ecdysteroids it is possible to imagine an easy conversion into ecdysterone in the insects: for example through a 25-hydroxylation in the case of ponasterone, through a degradation of the 24-methyl group in the case of makisterone and so on. For other active phytoecdysteroids, for example for cyasterone, it is difficult to assume they can be transformed into ecdysterone.

A possible explanation seems to be: all these compounds and ecdysterone itself are not true hormones but only prohormones: everyone of them can be degraded through a 20,22-fission which leads to the same product, to the pregnane derivative named poststerone. This degradation, as we could observe in our researches on the microbiological transformation of ecdysteroids, appears as a general process of the living matter, independent, at least in some cases, from the structure of the side-chain. It is the same degradation pattern which is utilized by the higher organisms in order to transform cholesterol in adrenocortical and sex hormones. If this degradation really takes place in the insects, it cancels the structure differences due to different substitutions in the side-chain.

In this bypothesis the true hormone had to be poststerone or one of its transformation products. Nevertheless, poststerone is inactive if it is injected in the usual test insects. But, one can imagine that poststerone when injected appears to be inactive because it is not vehiculated to the proper active centers, whereas the active ecdysteroids are. I would like to know your opinion about this.

KARLSON

The question was if ecdysone or ecdysterone would be transformed by cleavage at this position bere (between C-20 and C-22) and thereby give rise to a compound called poststerone which is active at the target tissue. First of all, you mentioned already that poststerone has very little activity. I must also say that although this cleavage of the side chain has been observed in Calliphora and also in Bombyx mori, the amount of poststerone produced from ecdysterone is very, very low.

Moreover, we have done some experiments on the metabolism of ecdysone with a sample labeled with tritium in the side chain (at C-23 and C-24), and we find that after injection of ecdysone this is very rapidly converted into ecdysterone; both are in part metabolized to 3-dehydro-compounds. But the side chain is not broken down. What we find as excretion products is a mixture of conjugates of ecdysone, ecdysterone, their 3-dehydroproducts and some other products in which the side chain is still present. There is very little radioactive label lost, so that at least not a large extent of injected ecdysone can be converted to the C-21 compounds.

JUVENILE HORMONES AND THEIR ANALOGS

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Although the elucidation of structure and the synthesis of natural products have already made a major contribution to the science of organic chemistry, there is no obvious reason why such research should not continue to provide both important and interesting contributions to chemistry. In the course of such work, there has been assembled a remarkably large collection of structures of the known natural products, but in contrast the number of natural products and their structural analogs which have found practical uses in plant protection appears relatively small. It is anticipated that this Study Week Conference will explore new strategies and chemicals for plant protection and may raise the question whether the majority of known natural products are inappropriate for plant protection, or whether the majority have simply not been examined for this purpose.

The three natural products which are known to be insect juvenile hormones (Figure 1) have been examined in detail and modified structurally to provide an insecticide (Figure 2) and a plant protectant (Figure 3). Since these hormones were discovered and modified only recently, it would seem appropriate to try to look ahead into some areas of insect chemistry which remain to be explored. In so doing, it will be necessary to reexamine continually whether such areas of research on chemical pest control will lead to selectivity on the one hand for a limited number of insect families, or on the other hand for all insects as a class with safety to higher

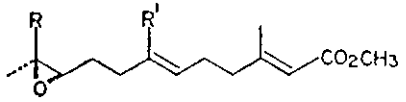
animals. Because the hormonal regulation of insect development is so fundamentally different from that of higher animals, the latter kind of selectivity has been inherent in chemical pesticides which interfere with this regulation, and it would seem wise to continue the search for class selective pesticides of this type.

Among such avenues which are relatively unexplored, are the control of molting hormone synthesis and secretion and the pathway of molting hormone biosynthesis. The mechanism of C-20 hydroxylation of α-ecdysone and the involvement of cofactors remain unknown, even though this is a crucial step in the genesis of the active hormone β-ecdysone. What is almost certain is that larval development without ecdysones would be impossible. It is likely that the higher centers controlling the timing and the rate of synthesis of the known hormones will exert their action through small peptide neurohormones associated with complex protein carriers, in addition to electrical control through direct innervation. Even the direct nervous control of endocrine glands by electrical means will probably involve conversion of nervous impulses into chemical transmitters which inhibit glandular activity. Although the accumulation for isolation and structure elucidation of such neurohormones and transmitters is a formidable task, the potential for the use of such knowledge in pest control is surely no less formidable. With the future in mind it is this writer's hope to gain some perspectives of insect development and its hormonal control as a guide to future research, by reviewing a brief selection of events which have punctuated the spectacular development of hormonal pesticides as insect growth regulators.

Hormone Isolation

Between the discovery of insect juvenile hormone (JH) some forty years ago [1] and the beginning of work on chemical structure elucidation, over twenty years elapsed for the major reason that there was simply no usahle source of the hormone until WILLIAMS discovered a rich depot in the abdomens of male silkmoths [2, 3] in which about 3 micrograms was apparently stored in 10 grams of tissue. Without careful experimentation [3] it was generally assumed that the unstable hormone was protected in a large quantity

of oily lipids present in the abdomens. However, in 1976 it was discovered that the accessory sex glands of the male Cecropia moths have the exclusive ability to sequester JH [4]. Quite apart from the implications for the study of insect sexuality, the possession of such knowledge of accessory gland storage in 1956 would almost certainly have revolutionized the tedious process of JH isolation and purification which was not accomplished until 1966 [5]. In connection with the future isolation of the rare neurohormones of insects, one may usefully recall this localization of JH. No doubt the surgical isolation of accessory sex glands would have been much simpler as a purification scheme than the numerous column and gas chromatographic procedures employed. Closely related to these events in its implications was the discovery that JH could be isolated, albeit in minute quantities, from *in vitro* cultures of the endocrine organs [6]. Because culture medium is relatively free of extractable organic impurities, the higher state of purity of hormones obtained through organ culture by solvent extraction of the medium more than compensates for the smaller quantities obtainable. These simple considerations led to the discovery [7] of JH III (Figure 1) by culture of organs from *Manduca sexta* and to the elucidation of important elements of hormone biosynthesis from propionate, acetate, and mevalonate [8]. It seems likely therefore that the



[1] R = R' = Et, JH I

[2] R = Et, R' = Me, JH II

[3] R = R' = Me, JH III

FIGURE 1

techniques of organ and tissue culture will play a major role in the isolation of workable quantities of insect neurohormones. The original isolation of molting hormones from insects and crayfish was certainly no less laborious than work on JHs, and recent advances

in organ culture of prothoracic glands have been reported [9, 10]. These advances not only verify the original hypothesis that α-ecdysone is secreted by prothoracic glands, but also provide an invaluable tool for the future investigation of ecdysone biosynthesis. To date, tbe definitive conversion of cholesterol to α-ecdysone by these glands *in vitro* has not yet been reported, and without such evidence neither the detailed study of ecdysone biosynthesis nor its inhibition by chemicals as potential pesticides can be expected to progress rapidly.

During the events leading to the isolation of JH I, a notable paper of W. S. BOWERS and co-workers [11] predicted most accurately all the structural features of the now known JHs (Figure 1) with the sole exception of the unprecedented ethyl branches on the terpenoid chains of JH I and II. On examining the basis of this prediction, it seems that Bowers carefully pieced together small items of information from the literature and the laboratory bench even though none of these taken alone would have sufficed to elucidate the structure of natural JH I, which was accomplished two years later in 1967 by RÖLLER and co-workers [5]. Both of these publications of discrete chemical structures with hormone activity undoubtedly opened the door to the numerous chemists whose skills lay in synthesis and structure optimization for maximum biological activity. From these events one may conclude that the long and hard labor of hormone isolation was clearly worthwhile, and that the skillful application of new techniques of the past decade could shorten considerably the isolation of new insect hormones and physiologically active substances for research in pest control.

Juvenile Hormone Analogs as Insecticides

The possibility of using insect hormones as insecticides arose as a by-product of studies of insect physiology in 1956 and the concept is attributed to WILLIAMS [2, 12]. In chemical terms the discovery of JH activity in farnesol and farnesal from feces of meal worms by the late P. SCHMIALEK, could be regarded as the beginning of JH analog chemistry [13]. Although it soon became clear that farnesol was not identical with natural JH, the important fact of its possession of demonstrable JH activity probably formed the

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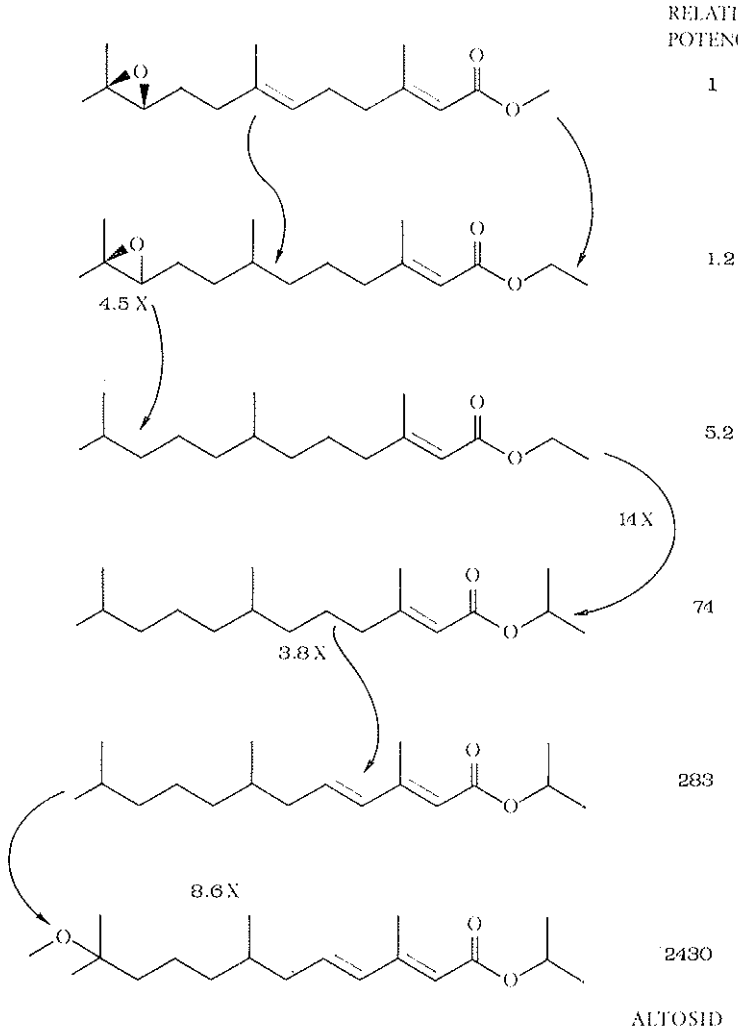

FIGURE 2

basis for BOWERS' and co-workers' [11] elaboration of (E, E)-10,11--epoxymethylfarneosate. From this latter compound there has emerged a large class of potent analogs, mostly esters, which are based on the 15 carbon skeleton of farnesane, and these have been reviewed in detail by STAAL [14]. Since this class of compound contains the only two chemicals which have so far received government approval for use as insect growth regulators (Altosid or methoprene, Figure 2, and kinoprene, Figure 3), their discovery will be examined in more detail. The hypothetical evolution of Altosid from epoxymethylfarneosate is illustrated schematically in Figure 2,

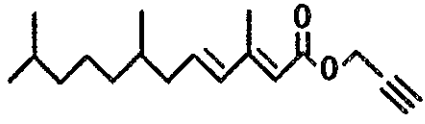

FIGURE 3 — Kinoprene.

where curved arrows indicate molecular structural changes and the nearby notations such as 4.5X and 14X denote the increases in biological potency associated with each change. By late 1971 these changes had been reported [15] as leading to an increase in relative potency of 1900 fold compared with JH III (Figure 2, top), in laboratory mosquito bioassay. More recent assay data indicate an increase of 2,430 times, resulting in laboratory activity sufficient to prevent emergence of adult mosquitoes with 0.1 parts per billion in water. Of the six molecular changes in Figure 2 perhaps the most important are replacement of the 10, 11 epoxide by a tertiary methoxyl group and the introduction of a conjugated dienoic ester system, both of which contribute markedly to increased stability in the field. Clearly, several hundred changes were explored during this process of structure optimization and several of these have been reported in detail [14, 16]. The chemical and biological properties of the geometrical isomers of a related ethyl ester have been reported [17] and a general rule for this class is that the 2E, 4E isomer (all *trans*) is the most biologically active of the four possible. Several approaches to their synthesis have been explored [16, 17, 18] but

the method of choice is a stereoselective synthesis [19] involving the condensation of dialkyl 3-methylglutaconates with 7-methoxy-citronellal, in turn manufactured from the pinenes present in oil of terpentine.

At this point the history of the concept of hormonal control of insects should be recalled, since the major reasons for the selection of JH as a rational lead for pesticide design were the beliefs that JH occurred only in insects and not in other animals. The implication was that JH would therefore be selectively active in insects with no significant effects on other forms of life. In the case of JH analogs of the farnesane skeleton, extensive studies of comparative toxicology have largely verified these beliefs. Toxicological results have been reviewed in detail [20] and a comprehensive study of the environmental fate and metabolism of methoprene has been completed [21].

In moving to other classes of JH analogs, major departures from the farnesane skeleton have been reported in the form of phenyl ethers [22, 23, 24], cyclohexenes such as juvabione [25], and small peptides [26] as an extreme case of completely selective action on one family of bugs. The latter compounds are most remarkable for the pronounced differential activity of their optical enantiomers, in which one antipode is several thousand times more active biologically than the other [27]. In connection with the peptides, it should be noted that there is no formal proof that these compounds exert their action as true mimics of juvenile hormones at the target tissue level. One may well ask whether these peptides act directly on the corpora allata glands as allatotropins.

Biosynthesis of Juvenile Hormones

Studies of the biosynthesis of the unique ethyl branched JH are important not merely for the sake of gaining knowledge, but for the major reason that a detailed knowledge of the pathway should assist in the design of irreversible inhibitors as new insect control agents. Despite the major difficulties of work with nanogram quantities of materials produced by organs of fluctuating synthetic capacity, considerable progress has been made since the introduction

of organ culture technique as a tool. In 1970 this author wrote that "advances in organ culture technique may later simplify such work and presently provide an avenue for fruitful research" [28]. By 1973 the use of *in vitro* cultures led to the elucidation by SCHOOLEY and co-workers [8] of the role of propionate as a precursor of the ethyl branches, and current work in several laboratories is divided between whole organ culture systems and homogenate systems summarized most recently in a comprehensive book entitled "The Juvenile Hormones" [29]. At the present time the candidacy of homomevalonic acid as a precursor of JH I and JH II is still attractive even though this compound has never been isolated from any living system.

In looking ahead to future methods of insect control based on biosynthetic inhibition, it would seem that a few years of hard work will be necessary to elucidate the individual steps of the pathways, as a basis for synthesis of substrate analog inhibitors. These inhibitors would be classified as anti-juvenile hormones and could be expected to show selective action on insects as a class. Such analogs are by no means just around the corner since at least two important properties that they should possess may be difficult to build into small organic molecules suitable for pest control. These properties are the ability to withstand general metabolic inactivation while retaining the ability to inhibit irreversibly the target enzymes of the corpus allatum and the property to accumulate selectively in corpora allata, a physically small target, so as to offset dilution in the general hody cavity.

Anti-juvenile Hormones

Despite the testing of several thousand JH analogs in many laboratories between 1961 and 1975, no confirmed report of JH antagonism appeared. Part of the reason for this may well have been the use of inappropriate bioassays, such as the classical *Tenebrio* test or *Galleria* wax wound assay; however, several laboratories maintained lengthier bioassays using early larval instars in which JH-antagonistic activity would most likely be detected. The expected symptoms of anti-JH activity in a test chemical would be

similar to those caused by surgical removal (allatectomy) of the corpora allata glands, which leads to premature metamorphosis of early stage larvae into pupae. Although the expression of premature metamorphosis at the time of a molt may lag behind allatectomy by several days or by an intervening larval instar, the surgery usually shortens the feeding stages of the larvae of moths and beetles, wbich are the damaging stages of the major pests of crops.

The search for defined chemical structures which will duplicate the effects of surgical allatectomy will most likely continue and intensify in the next few years, but in order for the search to be of any practical value it must focus upon the holometabolous insects which are the major pests of agriculture and the major insect vectors of disease. From the practical view-point, the recent discovery by W. S. BOWERS [30] that the bedding plant *Ageratum houstonianum* contains two chemicals which possess anti-JH activity on milkweed bugs but not on larvae of moths or beetles, is both intriguing and disappointing. Both chemicals showed a very narrow spectrum of activity on larvae, and the more potent named precocene-2 (Figure 4) produced effects which could be counteracted by JH III [31].

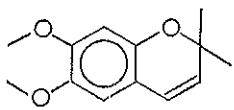

FIGURE 4 — Precocene-2

Consequently, it will be important for future research to examine at least two aspects of this work; to elucidate the mechanism of action of precocenes on larvae of bugs, and to find whether the reported sterilization of adult female insects and the reported induction of diapause in Colorado potato beetles [31] involve a similar mechanism of action.

In contrast with the present limited range of applications for insect growth regulators with JH activity, BOWERS states that "a hormone antagonist or anti-hormone would be a more efficacious insecticide" [31]. This statement is based on the idea that JH is necessary throughout most stages of insect life, and the expectation

that an anti-JH which tends to reduce the insect's JH level would be able to act on the insect throughout most stages of its life with the result of disruption of development. These oversimplified ideas would lead to the bright prospect of an anti-JH insecticide which could be used to control most larval stages of insects. However, these ideas overlook two vitally important factors. The developmental stage which will emerge at the molt from a given larval instar is decided only during a brief critical period in the early part of that instar, and the decision or determination for what will emerge as the next stage depends on whether the biologically effective titer of JH is above or below a critical level during the brief critical period. Thus for normal development involving five larval instars there would be five critical periods, four of which could be influenced by chemical reduction of JH titer to disrupt development. It is not yet understood why three JHs are present during certain stages of larval development, nor is it known whether the ratios of the hormones are important in the determination of the next molt. The very presence of three hormones having different morphogenetic potencies suggests a buffer system which stabilizes the biologically effective level of JH. The effective level of JH may prove to be only the portion which is bound to hypothetical target tissue receptors and not the portions bound to carrier protein or in free circulation, though each level will influence the others and all will contribute to whole body titers measured by recently available techniques [32, 33, 34].

There emerges a very complex picture of three hormones synthesized and secreted at variable rates, competing for carrier binding proteins, presumed receptor proteins, epoxide hydratase and carboxyl esterase enzymes [35, 36]. It is possible experimentally to measure the timing of critical periods for larval determination and to measure total levels of JH at these critical periods although both measurements involve extreme difficulty. Approaches to this were described recently by G. B. STAAL [37] using third instar larvae of the tobacco hornworm moth, *Manduca sexta,* which were allatectomized and raised on JH impregnated diets as an experimentally reproducible method of JH therapy. One striking result of Staal's work was the very low morphogenetic potency of JH III

relative to JH I or II, measured as the ability to maintain normal larval-larval molting in the allatectomized insects.

Since the effects of precocene-2 can be abolished by addition of JH III [31], it may turn out that the precocenes are selective antagonists for JH III but not for the more potent JH I and II. If so, the narrow spectrum of activity of precocene-2 may be further *limited to those insects which lack the ability to biosynthesize JH I or JH II.* The presence of JH I has been reported in larval cockroaches which are very primitive insects [38]. Work in this laboratory to be reported in detail elsewhere [39] failed to detect any activity whatsoever of precocene-2 on nymphs of the cockroach, *Blattella germanica*, or *Schistocerca vaga*, or larvae of *Aedes aegypti* mosquitoes, or of the bug *Pyrrhocoris apterus* which is most surprising in view of its closer relationship to the milkweed bug. Similarly no effects on larval development or on egg maturation in *Manduca sexta* could be found. However, precocene-2 was reported [31] to induce diapause behavior to adult Colorado potato beetles which were independently found [40] to contain JH III as the only hormone present (280 picogram/animal). Adult females of *Manduca sexta* however contain JH II [34, 40] and traces of JH I [34] and are insensitive to the action of precocene-2. Although corpora allata of the grasshopper *Schistocerca vaga* were found [41] to synthesize only JH III *in vitro*, the hormones present in larvae (which are insensitive to precocene-2) have not been investigated. The analytical measurement of which hormones occur at what levels in larvae of various families of insects assumes added importance even though it remains to be seen whether precocenes act by changing the circulating titer of JHs.

The negative implications for pest control by precocenes themselves are clear, but it remains to be seen whether the expansion of their spectrum of activity is limited merely by the chemical structural features of precocenes or, more problematically, by the hormonal mechanisms which control insect development. In either case the JH antagonist approach to the control of larval insect pests presents a major challenge to chemical and physiological research.

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DISCUSSION

MARINI-BETTÒLO

Thank you, Prof. Siddal, for your excellent presentation. I think you have stressed some very important points, that means all the relationships in the formation of these juvenile hormones which can be of great value in considering how to influence the formation or the inhibition of these hormones. Secondly you have underlined the importance of receptors and also the question of the metabolism of juvenile hormones and there I may recall that some importance should also be given to these epoxide groups which are present also in other biologically active compounds which have nothing to do even with insects. Now I shall open the discussion on the presentation of Dr. Siddal.

SHOREY

I should like to get back to the question of selectivity. I suppose this will often have quite an impact in this meeting and elsewhere. One question will be whether juvenile hormone analogs are often more selective than basic JH as is found in the insects themselves. In your opening comments you indicated that because juvenile hormone is restricted to insects as far as it is known, this may indicate a sufficient selectivity, implying that these materials can be used safely for pest control. And you further qualified this to indicate that you were talking about selectivity, largely, perhaps, of mammals versus insects. In the discussion of the previous paper most of the question of selectivity was mammalian versus insect selectivity. But I think the question is much larger than that. In De Back's recent book on biological control, he made a very strong point that no more than one out of every one-hundred insect species that we know, is a pest. That leaves 99 insect species for every pest species that are either neutral or beneficial. In fact De Back goes much further and indicates that there are many more beneficial

insect species than there are pest insect species in the world. Therefore of course, we still have to think that we have the same potential for upsetting agro-eco systems by using a material which is relatively non selective even within the insect world.

I was particularly interested in your schematic diagram showing a 2430 fold increase in potency modifying the structures of JH. And my main question is: am I sure that they are often tested on specific insect species, that is particular target species and in fact do they have a much lower effect upon certain non target species?

SIDDAL

You have raised an important point and you also partly answered the question. First of all the slide which showed the 2 000-odd increase in potency referred to inhibition of emergence of adult mosquitos from larvae. If we take the same compound in the muthagenetic assay of *Galleria* pupae no significant increase of the relative potency of the muthagenetic activity is observed or at least a variation of the order of the factor ten.

So not only has the chemical modification increased the potency to ordered chosen target organisms, but in some ways has accidentally increased the specificity of this compound enormously. This is not always desirable. From a purely industrial point of view it can be very undesirable because the cost of doing this is enormous in terms of research and development and registration, and yet the recuperation or return of these costs without even any dividend is extremely difficult to achieve with a very specific compound. There are therefore at least two aspects: the chemical modification of structure almost invariably increases this selectivity toward a chosen organism which is used in the bio-assay in the laboratory. That is to be recommended in the hope that the bio-assay organism is either identical with the field pest or very closely related to it, or representative of the field pest. But there is also evidence that the selectivity of activity between insects and non-insects, other orders, other members of other classes such as crustaceans and spider-mice is preserved because of what I feel is just a chemical resemblance in the skeleton of the analogs compared with the skeleton of the hormone. So the selectivity for the class is still there. You mentioned a third point which is how does one distinguisb between the 99 beneficial insects and

the one pest insect. There is a way in which this is achieved and it is more evident with the juvenile hormone analog pesticides than is possible with conventional pesticides, because of the very brief time in which JH analogs are active in the field. We accidentally take advantage of the out of phase development of a pest and its predators. For example one finds that the lepidopterous insect pest co-occurs with a wasp parasite, but the developmental stage of these two insects from different orders is not synchronized. So when the moth, when the larva of the moth is sensitive to juvenile hormone, the wasp is not sensitive, having already become an adult; the similar situation in general could be just the out-of-phase development of the predators with a pest.

KARLSON

In contrast with this it was often said that the insects would not develop resistance against their own hormones. Now have you worked with analogs? What would you expect about the mosquito's acquiring resistance to these analogs?

KNÜSLI

I cannot give you a direct answer, but I can quote. Prof. A.W.H. Brown made a statement at the meeting of the American Chemical Society in New York this spring, that so far for all chemicals, however they are conceived, a build-up of resistance has been observed.

BOWERS

I agree, I am sure, very much with the statement that all insects can become resistant to any chemical. I am sure that under the constant pressure of particular chemicals even human beings will find a mechanism of resistance to toxic chemicals. I think one thing that has been pointed out is that it is always a compromise to try to control insects by chemicals or any other means, and that as I first mentioned, there are probably many more beneficial insects than there are pestiferous ones. Our object should be not to dream of perfect methods of insect control because there are none and never will be. In our compromise for developing methods of pest control we should simply push those few pest insects

back in the same category enjoyed by the other 99% which are either innocuous or beneficial. But I think that by approaching the development of alternative methods of insect control whether it be chemical or non-chemical, we understand that there are no perfect methods of insect control. The insects will eventually develop similar resistance to everything. We should plan our strategy to take this into consideration, that the approach to insect control must always be a new one, understanding that the insects will overcome even our best efforts. This study on hormones and pheromones is an attempt to get to those biochemical innovations which are not shared between men or insects, high animals and insects. Resistance, when it does develop, will develop at a much slower rate that it would to the outright metabolic protoplasmic poison such as organo-phosphor compounds.

MARINI-BETTÒLO

I agree, Prof. Bowers, on this point, because we never know what happens in the impact on man of greater quantities of chemicals even if natural products. So we must take care of this point and I think we should also have an idea how the juvenile hormones or related compounds are metabolized. Is it a rapid metabolism, and are they easily degradated or not? Could you tell us something about this metabolism, Dr. Siddal? Have you any information about that?

SIDDAL

I can say only that metabolism of juvenile hormones themselves is apparently fairly simple in chemical terms, and the literature records that the epoxide ring is open to dialdehyde, the ester is hydrolized to an acid, and the dialdehyde may be conjugated and excreted. No one has described oxydative breakdown of the chain so far, although this would be a very simple reaction; no one has described the conversion of the central double bond in the hormone, the natural hormone, to any other derivative. In the analogs metabolism is specific for each compound which is studied separately. In our experience the metabolism of metoprene was, well, the best comment I can make is that it was much too rapid; we would have chosen a much slower breakdown if we had a choice. A compound was very readily degradated by oxydative cleavage, by hydrolysis of the

ester, and so on. So it is not possible to make a general statement that analogs of natural products will be metabolized in a more favorable way than phosphate or carbamates or other compounds. I think each compound has its own special properties.

MARINI-BETTÒLO

Thank you Dr. Siddal. I would like to ask you: have you any evidence that the epoxide breakdown is caused by other systems present in the environment or in the plants? In effect I have noticed that natural products containing epoxide groups all show some biological activity probably due to interference of this group with enzymatic systems of cells or microorganisms as it was reported in particular in the case of antitumor activity of natural products (*).

SIDDAL

I realize that very little work has been done on the metabolism of the natural hormones. The analogs which have been studied in great detail for registration purposes do not have epoxides; the epoxides which have been studied by Zoecon Corporation may have undergone some other transformation in addition to hydroxy-hydration. It is an interesting point that perhaps there is a transformation of juvenile hormone at the epoxide ring during the processes which occur in the target issues, but these have never been detected and it would be very difficult to detect such a process because of the extremely low concentration. We tend to study the major metabolites which are readily visible on radiochromatogram but not the minor components which may be, they may alone be the biologically significant metabolites.

JACOBSON

I just have a simple comment to make in connection with Prof. Shorey's original question concerning selectivity of this compound. The epoxide compound shown by Prof. Siddal in his presentation, to which he referred as being used in agriculture is almost completely specific

(*) G. B. MARINI-BETTÒLO: Rev. Inst. Antibioticos Recife, *14*, 51 (1974).

with regard to dipterous insects, the houseflies, hornflies, stableflies and so on. Now I said "almost" because, strangely enough, we find that this particular compound is very active in fire ants.

NAKANISHI

You once showed by tracer studies that Altoside is converted into cholesterol in steers. Do you think there is a possibility that juvenile hormone may eventually become ecdysones? Another question: what was the difficulty you encountered in the studies on the biosynthesis of the juvenile hormone in the case of the terminal propionate unit?

SIDDAL

The first question: I do not think that juvenile hormone would be metabolized into ecdysone. Surely it would go to acetic acid I believe. The second question I am not sure that I understood it completely. I don't think there is an error in the original work. Could you repeat the question?

NAKANISHI

Yes. I am not saying an error, but was there an experimental difficulty? I thought your original or early stage experiments indicated that a propionate did not go into JH-2.

SIDDAL

No, propionate did not incorporate into JH-2. In the incorporation of tritium JH-2 did acquire a label.

NAKANISHI

We can discuss this later, was it then that you showed that the methyl label of the propionate did get incorporated?

SIDDAL

The difficulty was that the efficiency of incorporation of label from

hormone was extremely low compared with propionate where the dilution is almost unnoticeable. In other words, regarding the efficiency of incorporation of propionate if you measure the specific activity of the end product compared with the propionate itself there is a dilution of only 1.2 or 1.3 fold whereas the dilution of radioactivity for hormone was 90, greater than 90 fold; this is the experimental difficulty. Is that what you had in mind?

ABO-KHATWA

Your proposal concerning the hydroxylation reaction that takes place in the conversion of α to β ecdysone as a basis for selectivity is rather interesting. Is it well established that this hydroxylation reaction takes place in the microsomes; mediated by the mixed-function oxidases? if so, is it possible to inhibit this reaction by some MFO inhibitors such as SKF 525A?

SIDDAL

I do not know the answer to your question, but I believe that the inhibitor SKF 525A does not inhibit this reaction. There may be some better information from other people in the audience as I have not worked on this reaction for many years.

PROTECTIVE SUBSTANCES OF ARTHROPODS AND PLANTS (*)

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Institute of Organic Chemistry
University of Heidelberg - Germany

Many insects resort to a system of chemical defense and produce and store chemicals with which to ward off or harass any attackers large or small. Because of the minuteness of the organs in which the defensive secretions are prepared and held, and because often only a small number of experimental animals are available, classical methods for the enrichment, separation, and identification of mixtures of substances usually have to be dispensed with modern microprocedures applied or new ones developed in order to extract and purify the tiny amounts of substances. In addition we need all the new methods for analyzing minute quantities of natural products, i.e. methods permitting comprehensive analysis.

The Defensive Chemistry of Some Land Beetles

Beetles employ unusual means for the acquisition and preservation of their habitat, for wherever they live, under stones or in

(*) Protective substances of Arthropods Part. LXIV; Part. LXIII, H. SCHILDKNECHT, Chemical Ecology - A Chapter of Modern Natural Products Chemistry, Angew. Chem. Int. Ed. Engl. *15*, 214 (1976). Protective substances of Plants, Part IX; Part VIII, H. SCHILDKNECHT and R. MAURER, Die Struktu des Mezereins aus der Frucht des Seidelbastes Daphne mezereum, Chemiker Zeitung *94*, 849 (1970).

the ground, in soil or dung, there are always enemies present. They must defend themselves against various kinds of microorganisms, other insects, as well as against larger enemies such as birds, mice and reptiles.

In the defense reaction of Carabidae [1], which is initiated by contraction of a collecting bladder (Fig. 1), the beetles of a basic group discharge fairly harmless acids; isobutyric and isovaleric acids, for example, which can be detected by electrophoresis and gas chromatography in six groups of carabids. In the second stage of development, we find, in differentiated glands strong formic acid and unsaturated carbonyl compounds as derived defensive secretions. In 24 of 25 species of beetles, we found that formic acid was accompanied primarily by n-undecane, and in some cases by n-decane and n-tridecane. Like *Carabus auratus*, some other ground beetles when touched, release a vapor of almost pure methacrylic acid. In half of the 16 species examined, C_{10}- C_{11}- and C_{13}- paraffins have also heen detected.

Regarding the evolution of the defensive compounds [1], one may hypothesize that in the origin of a species of beetles, selection of a characteristic chemical can also be advantageous for that particular species. In this light, the saturated fatty acids may be viewed as "original" and the unsaturated acids as "derived". An unsaturated carbonyl group can be antibiotically active only if adequately lipophilic, either by an aliphatic residue in the molecule itself, or by addition of an aliphatic solubilizing agent. We have observed both principles. The pygidial defensive glands of *Asaphidion flavipes* and *Bembidion quatriguttatum*, for example, contain n-valeric acid mixed with paraffins, as well as, salicylaldehyde. From *Idiochroma dorsalis* we isolated not only the more lipophilic methyl salicylate but also n-undecane as the main component, together with 12% of formic acid and small amounts of n-decane. The phenolic group, which is present in the salicyl residue, occurs quite often in water beetles. Very probably the use of the particularly effective disinfectant m-cresol also represented an advance for the three species of *Chlaenius*. The hydroquinones are less effective than m-cresol, since they lack the alkyl group. Therefore we find them only as precursors of the bactericidal and fungicidal p-benzoquinones, which are also the characteristic defensive substances of the *Tenebrionidae*

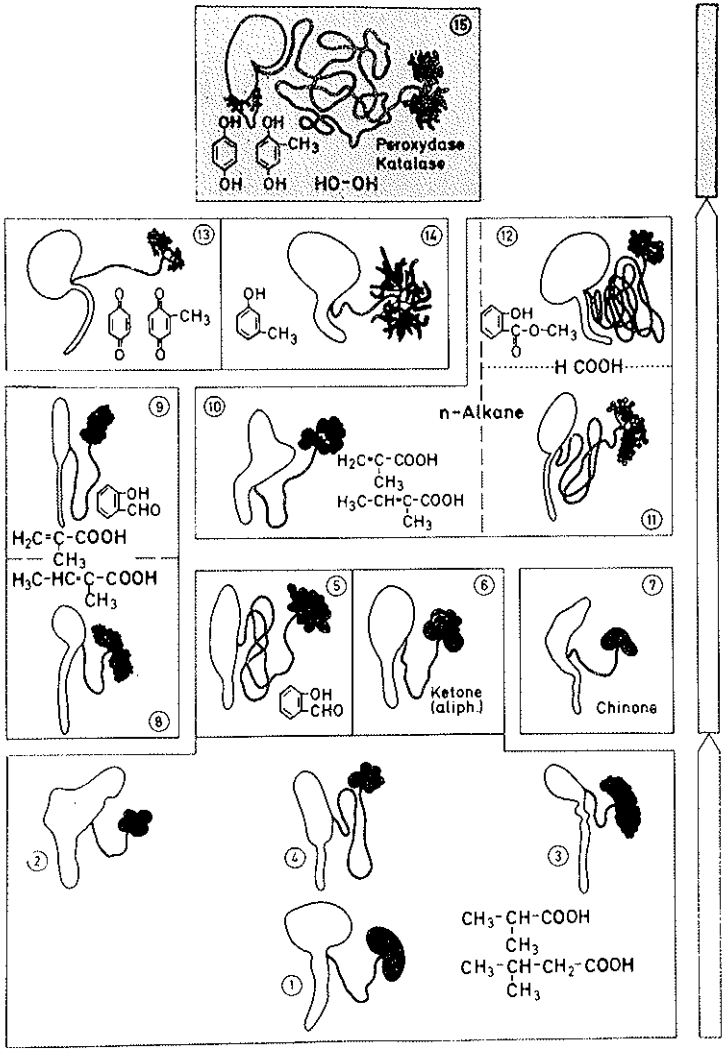


FIG. 1 — Defensive glands of the *Carabidae* and their secretions.

[2]. In an extensive gas chromatographic study, TSCHINKEL [3] showed that the defensive secretion of 147 species of tenebrionid beetles from 55 genera and 16 tribes contains toluquinone and ethylquinone; benzoquinone was relatively rare. In addition to quinones many species contained 1-alkenes. Also complementary to our 1968 postulated evolutionary lines for gland morphology, chemical composition, and function of secretion are the results published by MOORE and WALLBANK [4].

But neither the tenebrionid family nor other carabid genera can equal the brachinids in the effectiveness and elegance of their protection, where it has actually been shown experimentally that the secretion has defensive value [5]. Whether or not phylogenetically they should stand at the summit of a long evolutionary process is difficult to say; but from the chemical standpoint, they must do so. In 1957 we found that the precursors of the toxic gases shot against the predators — which consist of quinones and oxygen [6] — are hydroquinones and up to 25 per cent hydrogen peroxide. This explains in chemical terms why in 1796 Pastor Wilhelm compared the shot from the bombardier beetle to the report of a pistol with an apparent smell of powder and the liberation of slight heat. The biochemical basis of this defensive mechanism, has been explained when it was found that concentrated hydrogen peroxide is decomposed in the presence of particularly substrate-stable and donor-specified peroxidases. Both enzymes are more stable against H_2O_2 than most catalases and peroxidases investigated to date [7]. Such an explosion in a living organism would be inconceivable with any biochemical system other than that of the bombardier beetle. Only catalases produce an unlimited increase in activity with an increase in the substrate concentration. In addition, the "unnaturally" high reaction temperature of about 100°C produces a further increase in the reaction rate. The reaction can even be repeated, since not all of the enzyme mixture, which is only 1/100 to 1/10 µl of a protein solution responsible for ignition, is expelled with the shot: enough remains behind, suspended in a network of chitin bristles, which are shown in figure 2. We have detected only one type of cell in the lobular defensive glands of *Brachynus crepitans* and suggest, therefore, that the hydrogen peroxide and the hydroquinones are produced concurrently. The fine structure of these

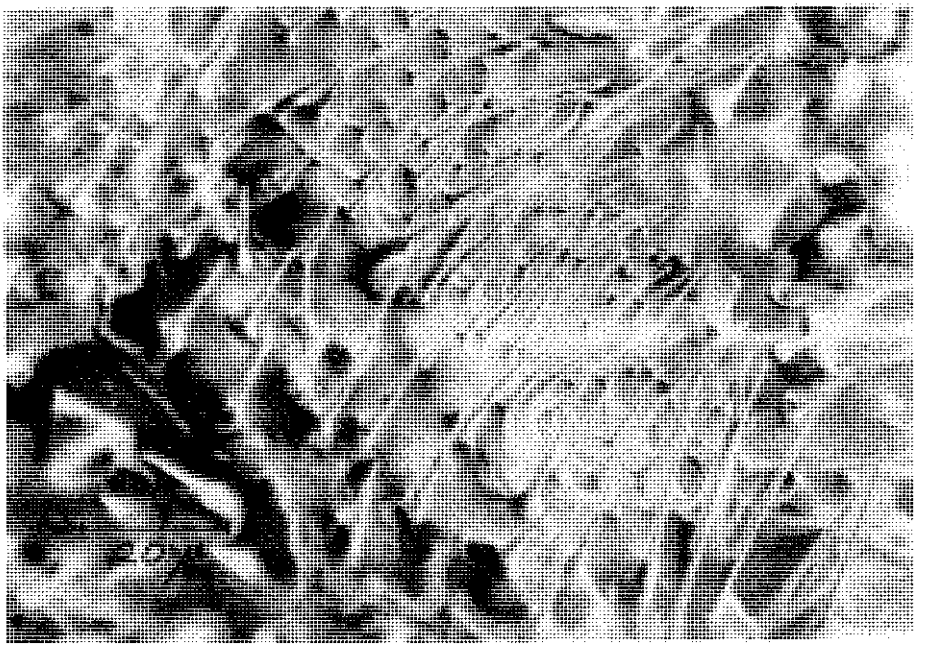


FIG. 2 — Chitin bristles of chamber wall of *Br. crepitans*; recorded with a scanning electron microscope (JSM-S1, Kontron GmbH).

cells is comparable to that of some other insect glandular cells (Fig. 3) despite their extraordinary products. Their cytoplasm contains a well developed endoplasmatic reticulum, some dictyosomes (D) and many lysosome-like organelles (L) of different kinds. The number of mitochondria is lower than might be expected [8].

The smallest bombardier beetle that we have studied [9] *Paussus favieri* (Fig. 4), is tolerated in the nest of the light brown ant *Pheidole pallidula Nyl.* It is 4 mm long and brownish in color like its hosts. When stimulated it secretes quinones in the same way as its relatives. As with the *Brachynidae*, we again found the defensive substances p-benzo- and p-toluquinone; however the *Paussidae* emit more toluquinone than benzoquinone. They accumulate the corresponding hydroquinones together with 15% hydrogen peroxide in pygidial defensive bladders measuring only 0,3 to 0,4 mm.

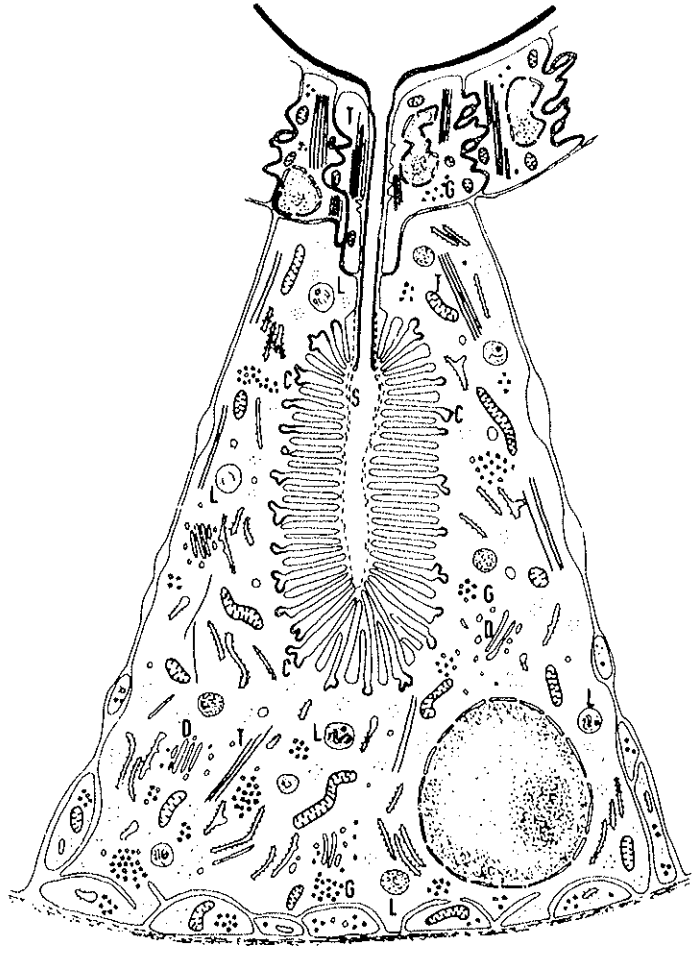


FIG. 3 — The gland cell of the lobular defensive glands of *Br. crepitans*.

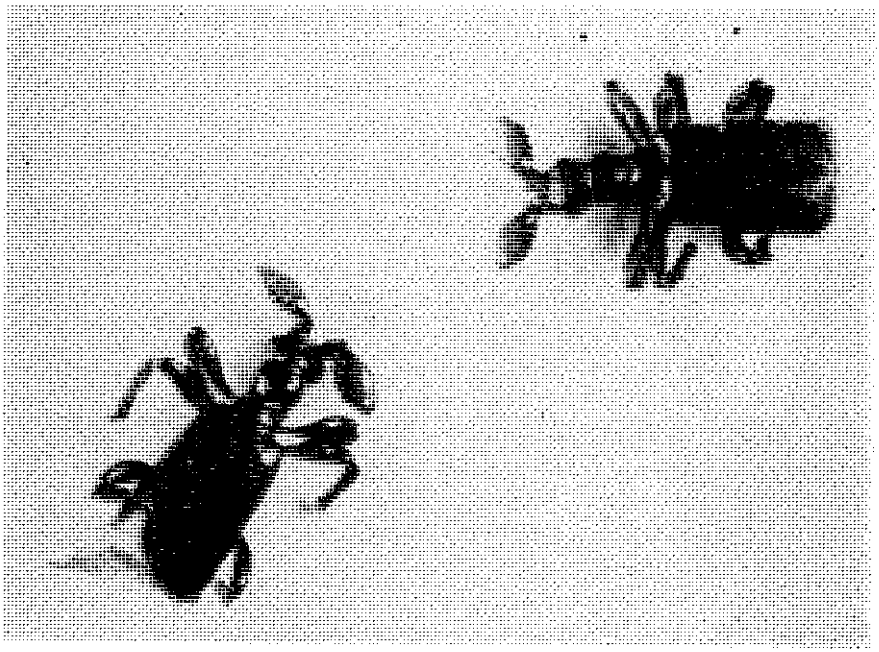


FIG. 4 — The Bombardier beetle *Paussus favieri.*

The myrmecophilic species *P. favieri* does not need to protect itself against external attack, that task being accomplished by the soldier caste of its hosts. Within the nest, it acquired respect by exploding quinone bombs and a certain degree of immunity lasting for a little while by virtue of the stubbornly disappearing quinone odor.

The Defensive Chemistry of some Water Beetles

So water is absolutely necessary for the life of animals which live at the banks of ponds and rivers but it can become a danger for their life if they cannot swim. While hunting for springtail on the banks the blue-green coloured staphylinid *Stenus comma* (Fig. 5), measuring 5 mm, sometimes loses its grip in a light breath of wind

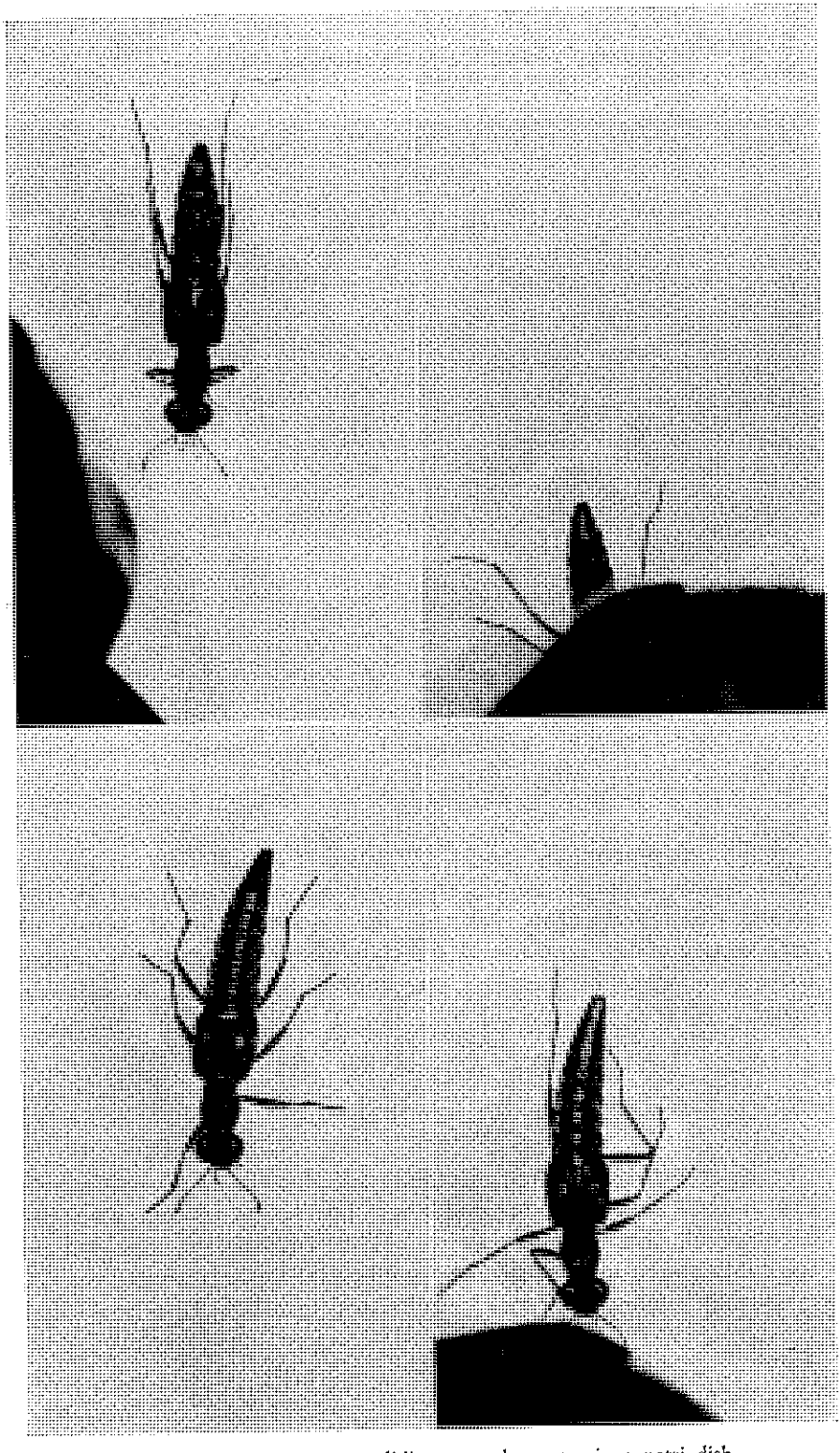


FIG. 5 — *Stenus comma*, gliding over the water in a petri dish.

— after all it only weighs 2,5 mg — and falls into the water. With a great effort it can keep itself above water by crawling and would reach the bank with great difficulty if it were not able to move over the surface of the water without using its legs. After briefly dipping the tip of its abdomen into the water it can dash over the surface at 40 to 75 cm/s, using its mobile abdomen as a rudder; like a water skier it does not sink at that speed.

More than 70 years ago, BILLARD and BRYANT [10] recognized that beetles of the genus *Stenus* are propelled forwards by a substance released from their anal end onto the surface of the water and compared its action with that of camphor and thymol. Indeed, the beetle secretion extracted from water with ether has an odor resembling that of eucalyptus oil, while also being amine-like. In order to perform detailed analysis we separated the secretion of the pygidial defensive glands into five fractions by gas chromatography (Fig. 6) [11]. We obtained three main and two further components. Comparison with the gas chromatogram of the secretion of the larger glands and the smaller glands revealed that the amine-like smelling fractions are the contents of the larger bladders and the refreshing smelling fractions the contents of the smaller bladders. Collection of fraction 1 yielded 0,8 mg, an amount sufficient to identify the compound as 1,8-cineole. Eucalyptol alone would explain the propelling action of the secretion because it spreads well on water. However, apart from the little pygidial defensive bladders in which *Stenus* stores the terpenoid compounds, the beetle also possesses a larger bladder from which two fractions having an unpleasant amine-type odor are excreted (Fig. 7). The substance present in the principal fraction, 6 mg from 1000 beetles, was called stenusin. The ^{1}H-NMR spectrum reveals a rather complicated pattern and was the basis for synthetic models. At this stage of our research work in the structure elucidation of stenusin a study of the metastable transitions in the mass spectrum of stenusin and in the model compounds by variation of the acceleration voltage and by application of the DADI technique favoured a N-ethyl-piperidine with only one further pentyl substituent in the 2 or 4 position. Finally the structure of the main component of the defensive substances of *St. comma* could be prooved as a N-ethyl-3-(2-methylbutyl) piperidine by electron pyrolysis (Fig. 8) [12].

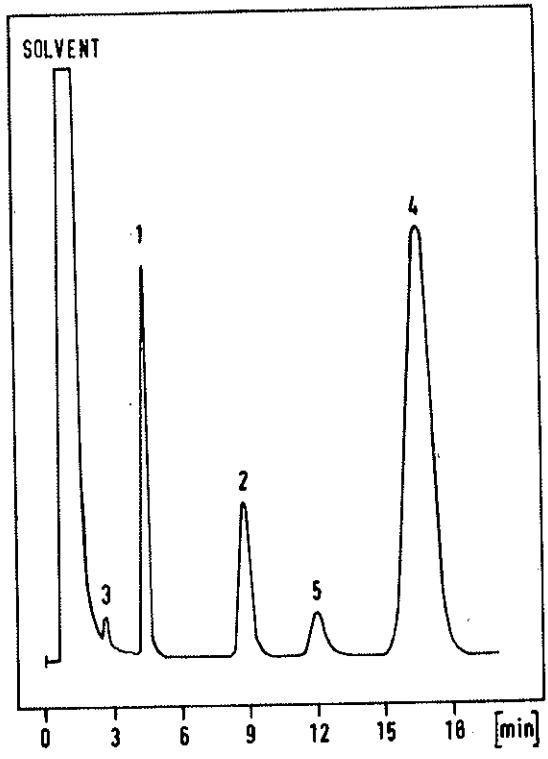


FIG. 6 — Components of the secretion of *Stenus comma*, separated by gas chromatography.

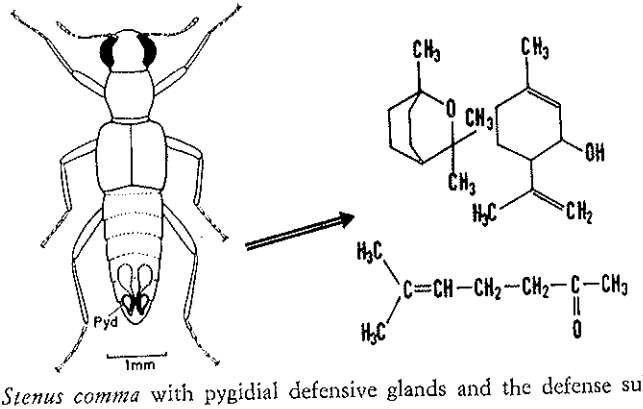


FIG. 7 — *Stenus comma* with pygidial defensive glands and the defense substances of the smaller bladders.

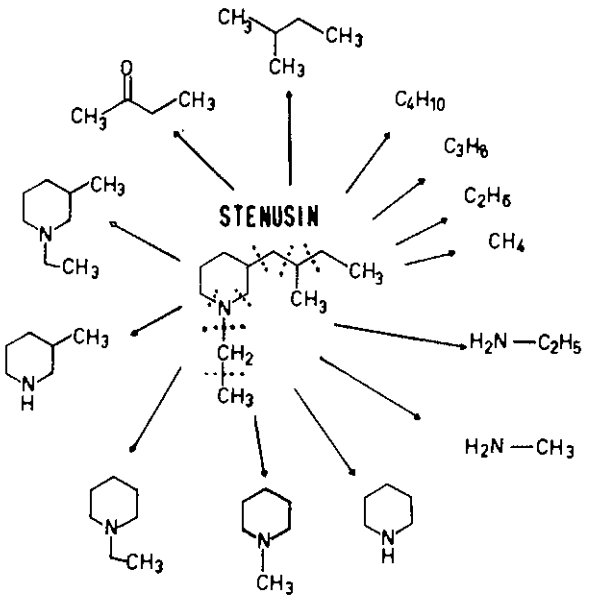


FIG. 8 — Scheme of electron pyrolysis of stenusin.

We have prepared stenusin by total synthesis. The specific rotation $[\alpha]_{365}^{20}$ of the synthetic product was 5,4 ± 0,3° (c = 3 mg/ml ethanol) and that of the natural product 5,8 ± 0,3° (c = 1,15 mg/ml ethanol). The absolute configuration of the insect alkaloid is currently being determined.

It is interesting that all Staphylinids which produce their defensive substances in pygidial glands seem to prefer terpenes or terpene – like substances (Table 1). *Staphylinus olens* excretes iridodial as the main component and in the pygidial gland exudates of *Bledius mandibularis* and *Bledius spectabilis* monoterpene aldehydes can be found, but the main component is dodeca-lactone besides smaller amounts of methyl-p-benzoquinone and 1-undecene. The above mentioned staphylinids are typical land beetles. *Stenus comma* is also a land beetle, but lives in the immediate neighbourhood of water and has to protect itself not only against microorganisms and animals, but also against the danger of drowning. Therefore it

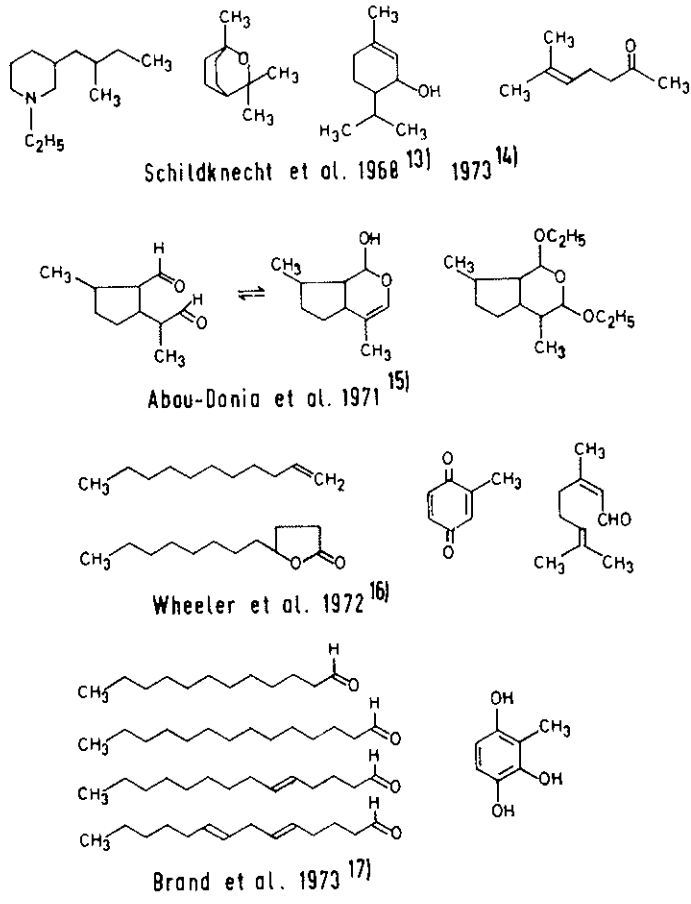


TABLE 1 — Defense substances of *Staphylinides*.

was interesting to find out how many of the compounds, excreted by *Stenus comma*, are spreading reagents.

The ratio of the main components of the secretion 1,8-cineole and stenusin amounts to 1 : 14. We may therefore assume that *Stenus comma* survives primarily owing to the properties of stenusin. However, the most striking property of stenusin is its extremely high spreading effect (Table 2). If a spreading pressure-time

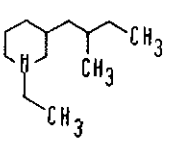
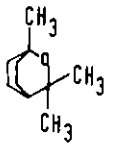
	 STENUSIN	 EUCALYPTOL
SURFACE TENSION	27,2 $\left[\frac{dyn}{cm}\right]$	28,6 $\left[\frac{dyn}{cm}\right]$
WATER SOLUBILITY	0,03 [weight %]	0,3 [weight %]
SPREADING PRESSURE	28,5 $\left[\frac{dyn}{cm}\right]$	2,2 $\left[\frac{dyn}{cm}\right]$
SPREADING VELOCITY	32,5 $\left[\frac{cm}{s}\right]$	18 $\left[\frac{cm}{s}\right]$

TABLE 2 — Surface activities of the *Stenus* secretion [11].

diagram is plotted for a constant spreading area with the aid of a film balance developed by Hans Kuhn then a spreading pressure of p = 28,5 dyn/cm is found for stenusin and only 2,2 dyn/cm for cineole. The large difference arises from the tenfold lower water solubility of stenusin compared to 1.8-cineole. From these values it may be assumed that stenusin is the actual spreading substance. Velocity-displacement curves that we have determined by means of a cine camera show a propagation rate of 32.5 cm/s for stenusin and only 18 cm/s for 1,8-cineole. Hence stenusin spreads much faster than 1,8-cineole and is therefore more valuable for the nonswimmer *Stenus comma*. Moreover stenusin renders the body of the beetle strongly hydrophilic and thus reduces drag by as much as 30%! The little staphylinid therefore skates over water, rather than swimming, on releasing its propellant.

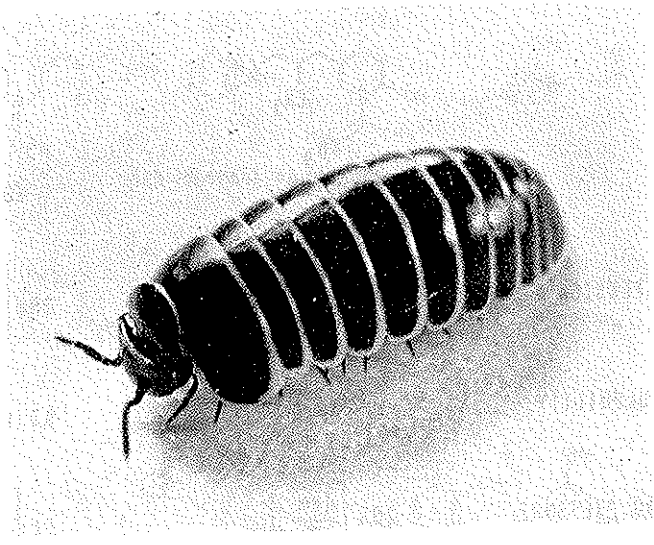
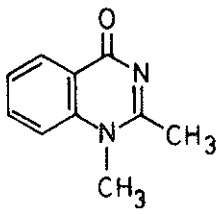


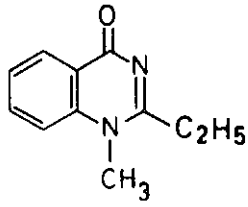
FIG. 9 — *Glomeris marginata.*

Both substances of the *Stenus comma* are terpenoid in character; in the course of evolution the oxygen heterocycle was transformed into the faster spreading nitrogen heterocycle — a terpenoid alkaloid. 1,8-cineole is active against various microbes. The presence of the original and the derived defense substance in the same organism constitutes the highly interesting finding of a comprehensive analysis of the defense substances of staphylinid.

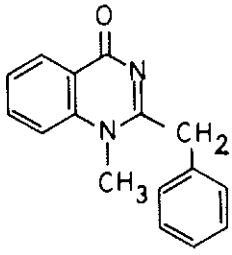
Stenusin is the third alkaloid we have found as defensive substances in arthropods. Glomerin, from the segmental glands of the milliped *Glomeris marginata* (Fig. 9), was identified as 1,2-dimethyl-4-quinazolone inter alia by electron pyrolysis; in addition the sticky colorless and odorless toxic secretion is found to contain the homologous 2-ethyl-1-methyl-4-quinazolone. Both alkaloids, which were not detected in natural substances so far, are closely related to the plant alkaloids (Table 3) arborine (1-methyl-2-benzyl-quinazolone-4), glycorine (1-methyl-quinazolone-4), glycosmine (2-benzyl-quinazolone-4) and peganine (2,3-(α-hydroxy-trimethylen)-quinazolone). It is known that peganine arises biogenetically from anthra-



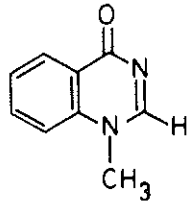
GLOMERINE



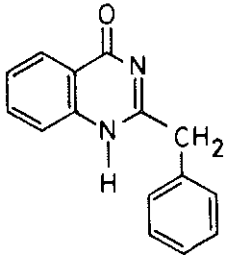
HOMOGLOMERINE



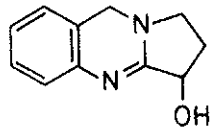
ARBORINE



GLYCORINE



GLYCOSMINE



PEGANINE

TABLE 3 — Arborine, glycorine, glycosmine, peganine, glomerine, and homoglomerine.

nilic acid and we were able to prove that anthranilic acid is also the precursor for glomerine and homoglomerine. A decision if kynurenine or tryptophan is the direct precursor for anthranilic acid could be reached by administering the labeled compounds to the animals by feeding or injection and measuring the incorporation rate [18].

Methyl-8-hydroxyquinolincarboxylate is the predominant toxin in the prothoracic defensive secretion of *Ilybius fenestratus* which leads an amphibious existence and has therefore developed toxins active against cold-blooded predators in and around the water and also against small mammals living on land. The toxin active against mammals is the alkaloid, while the steroids in the secretion of the prothoracic defensive glands are used in defense against predatory fish (Fig. 10). The alkaloid colours the secretion yellow; thus the blackish-brown water beetle displays the animal warning colours yellow/black only when in acute danger, e.g. when taken between thumb and index finger it presses two large drops of poison from two slits at the front edge of the prothorax (Fig. 11).

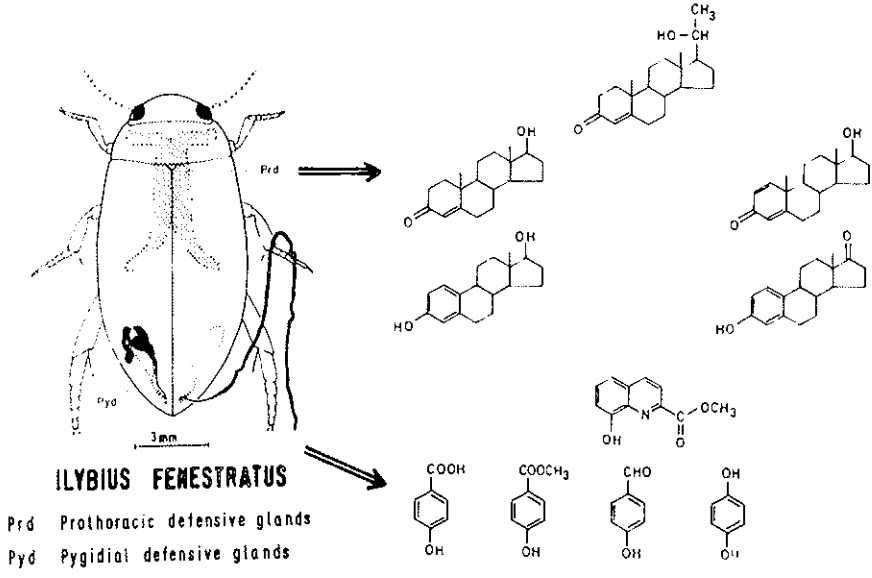


FIG. 10 — *I. fenestratus* and its defense substances.

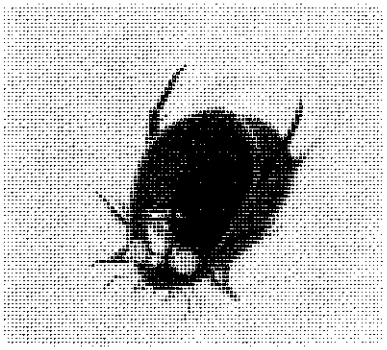


FIG. 11 — *I. fenestratus* in state of alarm.

All the three alkaloids exhibit the same effect when administered to mice: they produce clonic spasms. In the absence of comparative experiments, it cannot yet be said whether stenusin is also used as a defense substance against insectivores, as established so far only for glomerine and for the alkaloid from *I. fenestratus*.

All other substances in the prothoracic defensive secretion account well for the defense action of the secretions, but not for its odor. To explore the volatile components we subjected the secretion to analysis on a capillary GLC mass spectrometry direct coupling system [19].

The preliminary results could be confirmed by repetition of GCMS analysis of a methylene chloride extract of the secretion of 100 beetles. The extract was separated on a 150 m long glass capillary coated with the polar material Ucon HB 5100 using the MAT 111 Mass spectrometer as detector (Fig. 12).

This Figure shows the total ion current gas chromatogram. 28 compounds with MG 204 and 6 with MG 222 could be deduced. Shortly after the solvent a component with m/e 102 as highest mass number and the fragmentation pattern of methyl-isobutanoate was eluted.

Interpretation of the spectra of the anticipated sesquiterpenes was difficult, because of the lack of fragmentation directing groups and the large number of geometric and positional isomers in this class of compounds. Identification had to be done on the basis of

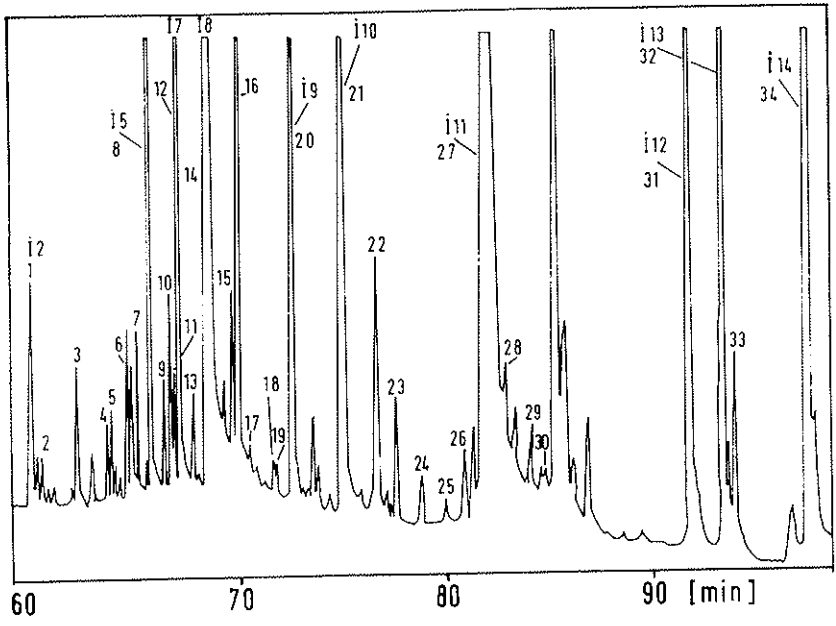


FIG. 12 — Partial total ion current gas chromatogram of the methylene chlorid extract of the *Ilybius* secretion (GCMS-System Varian MAT 111).

library search and comparison with reference compounds. Unfortunately both methods are limited. The success of library search depends on the number and quality of the spectra already recorded. The quality of spectra is critical. Due to the small amount of energy which is necessary to fragment the molecular ions of the terpenes in question the abundances of the molecular and fragmentation ions depend strongly on the temperature and other operational parameters under which the spectra are recorded. Furthermore one has to keep in mind that change of vapor pressure may occur during the record of a spectrum of a very sharp zone eluting from a capillary column. Comparision with spectra of reference compounds recorded under exactly the same conditions gives more security, but pure and well defined reference compounds are rare. In this situation Professor Norin at the Royal Institute of Technology in Stockholm helped us with a gift of crude α-Muurolene contaminated with

ε-Muurolene and δ-Cadinene. We analysed this sample and the methylene chloride extract of the secretion under identical conditions. This analysis allowed us to confirm the results of library search and the assignment of γ-Muurolene to compound I 5, α-Muurolene to compound I 7 and δ-Cadinene to compound I 8. Identification of compound I 3 as ε-Muurolene was possible by comparison of the GC-MS data of ε-Muurolene (a gift of Dragoco Company, Holzminden) with those of compound I 3. Structure proposals for 6 more compounds were possible by library search. (Table 4).

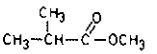
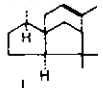
Compound Nr.	Proposed structure	
I 1		methyl-iso-butanoate
I 2		copaene
I 3		ε-muurolene
I 4		trans-β-farnesene
I 5		γ-muurolene
I 6		α-cedrene
I 7		α-muurolene
I 8		δ-cadinene
I 9		α-cubebene
I 10		γ-cadinene
I 11		γ-gurjunene
I 12		torreyol

TABLE 4 — Number of sesquiterpenes from *Ilybius fenestratus* [19].

We may say that a larger number of sesquiterpenes play a role in the scent composition of the prothoracic defense secretion of *Ilybius fenestratus*. The biological function of these compounds is not known yet. Terpenes are recognized as alarm and defensive substances of some insect families. Sometimes they have multifunctions, like in the family of the *Staphylinidae*, as already pointed out [20]. Terpenes are also found among the sex stimulating signal substances of insects [21]. *Arithonomus grandis* for example uses a mixture of two terpene alcohols and two terpene aldehydes to attract the females of his genera, but in the field the same compounds stimulate aggregation behaviour of male and female. *Ophris orchids* give off small amounts of sesquiterpene alcohols and hydrocarbons of cadinene and copaene type, which provoke the male copulatory behaviour of certain species of aculeate *Hymenoptera* [22]. The insect's movements are indispensable to pollination. It is not very probable that *Ilybius fenestratus* uses the same type of terpenes as sex stimulating pheromone, because the substances are released from the prothoracic glands together with the other compounds of the defense secretion in case of danger and as far as we could observe from male and female animals. Allomone function of the secret is more likely, it may put fish and small mammals on guard.

It seems as if the water beetles of the sub-family colymbetinae have developed their chemical defense mechanism according to a similar pattern as the water gliding *Staphylinides* which use terpenoids and an alkaloid as defense secretion. With the *Colymbetinae* however the two classes of substances are used by different species. So the defense substances which protect *Ilybius fenestratus* against insectivores on the land are steroids and an alkaloid, as already said.

In the defense secretion of *Platambus maculatus* we found besides the expected steroid not an alkaloid but a crystallizing sesquiterpene [20]. *Pl. maculatus* (Fig. 13) is a water beetle of about 8 mm length with a characteristic yellow, black, brown pattern on its wings. It can be found in the slow running side arms of the river Isar between waterplants or under pebbles. Against an attack of bacteria, fungi and vorticella *Pl. maculatus* is armed with a variety of phenolic compounds, like all the beetles of the family of Dytiscinae. The beetle stores this compound in high

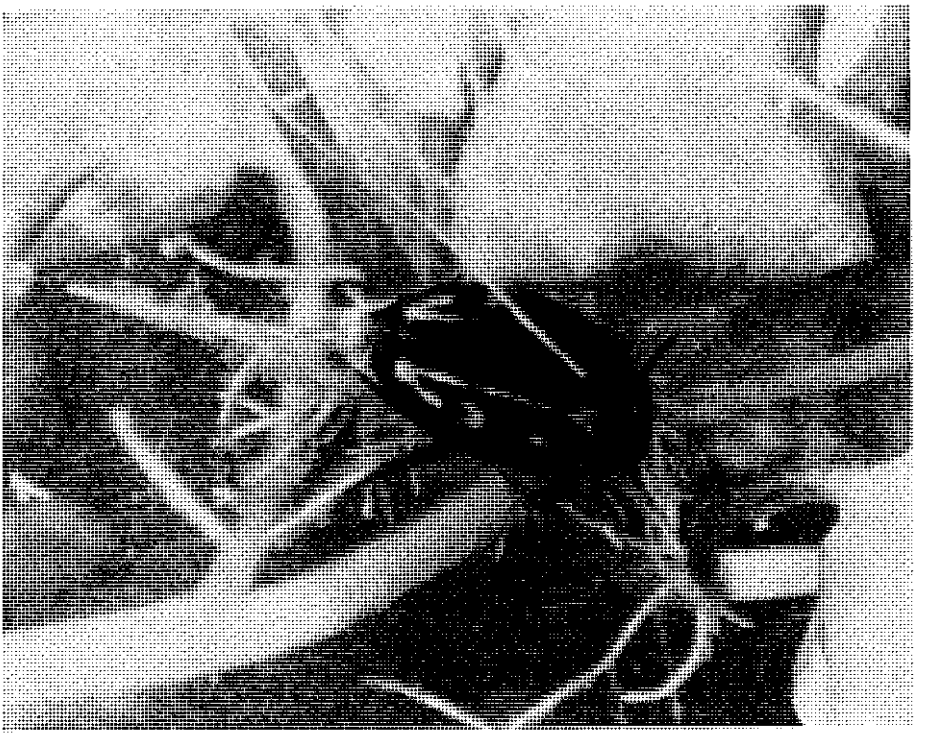


FIG. 13 — *Platambus maculatus*, between water plants.

concentration in its pygidial defensive glands. This gland system for the production of antiseptica exudes mainly p-hydroxybenzaldehyde, benzoic acid and p-hydroxy-benzoicacid-methyl-ester, a compound which was present in all the water beetles we have investigated so far, and finally hydroquinone.

Its prothorax is transparent, so one can realize through a window of chitin like glass its prothoracic defensive glands filled with milky secretion. One can see how the beetle depletes these glands when it is irritated by scratching it under its head. The secretion fluid can be sucked in thin glass capillaries and stored in methanol. We were able to isolate from 1000 beetles 1 mg of a steroid, corresponding to 4-pregnen-15α, 20β-diol-3-one (Fig. 14).

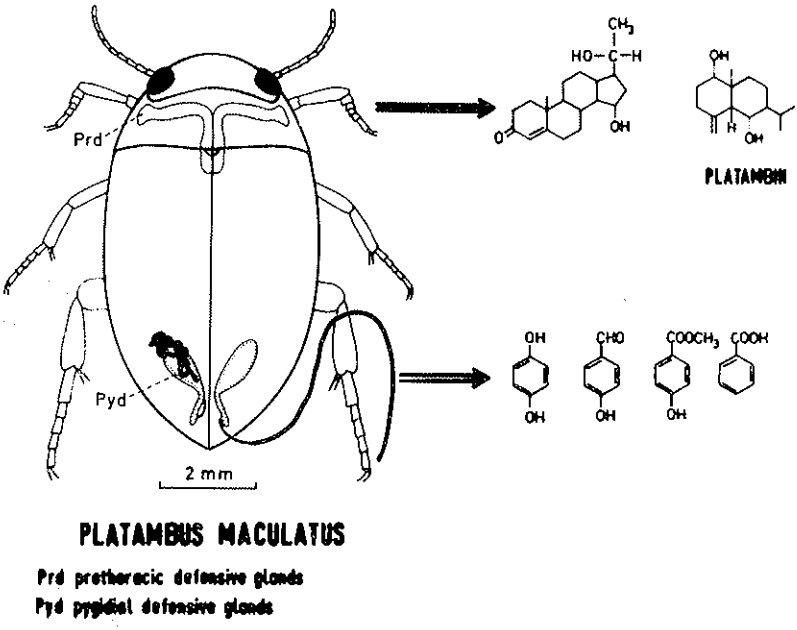


FIG. 14 — *Pl. maculatus* with the steroid and platambin.

Besides the steroid we isolated by thin layer chromatography 10 μg per beetle of a crystalline defense substance which we named platambin. The structure of platambin was elucidated with the help of physical methods and by chemical reactions in a micro scale. For additional proof dihydro-platambin was synthesized from dihydro-reynosin in several reaction steps and then oxidized to the diketo compound [21] (Fig. 15). The interpretations for the structure comparison were drawn from the ^{1}H-NMR spectra of dihydro-platambin and from the circular dichrograms of the diketones.

Nothing is known yet about the physiological properties of platambin, because we have to synthesize the compound to obtain an amount of substance sufficient for a screening test. It is to be expected that platambin is again a defense substance against small mammals, since protection against poikilothermic vertebrates is already provided for *Pl. maculatus* by the steroids in the secretion of its prothoracic glands.

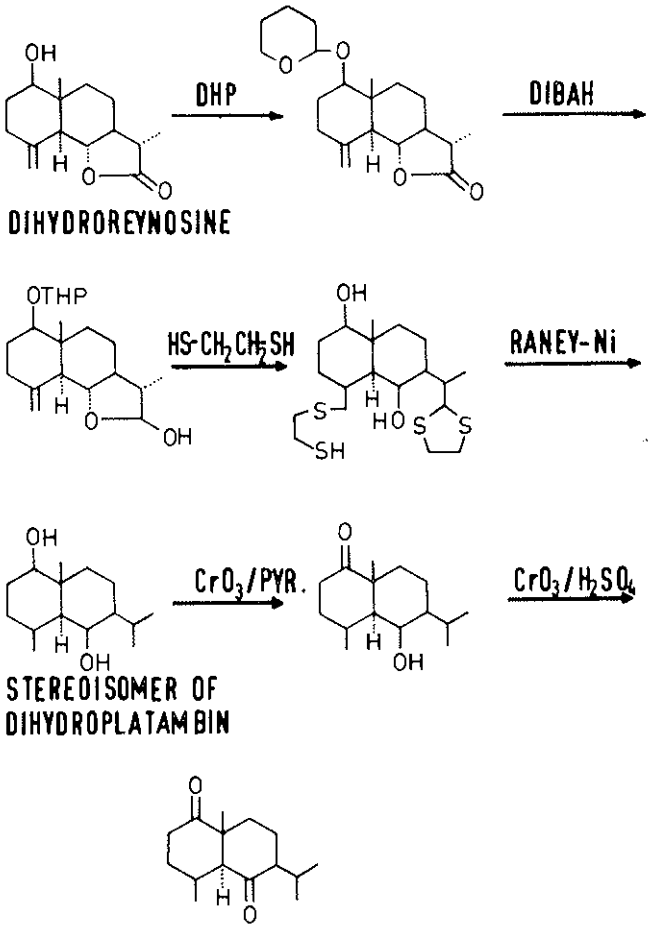
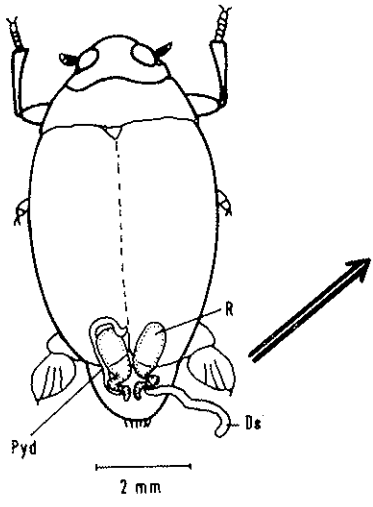
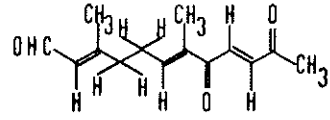


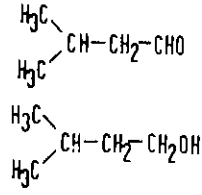
FIG. 15 — Synthesis of one of the stereoisomers of dihydroplatambin starting with dihydroreynosin [23].

The aquatic beetle *Gyrinus natator* possesses also a sesquiterpenoid as a defensive substance. We named it gyrinal and found for it the following structure [24] (Fig. 16). In this connection it is interesting to know, that the pygidial defense secretions of the aquatic beetles of the family *Gyrinidae* are composed largely of oxygenated norsesquiterpenes (Table 5), which have been shown





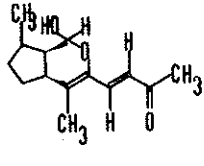
GYRINAL



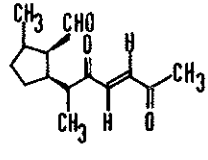
GYRINUS NATATOR

Pyd pygidial defensive glands

FIG. 16 — *G. natator* with the pygidial defense substance gyrinal.



GYRINIDON



GYRINIDION

TABLE 5 — Defensive compounds of gyrinid beetles [25, 26].

to be repellents to fishes and amphibians and to be toxic to mice as well [20]. Despite the fact that the gyrinid norsesquiterpenes are structurally dissimilar to steroids their toxicities are comparable. The anesthetic quality of gyrinal was less evident than that of the steroids [26].

The secretion of the whirligig beetles (*Gyrinidae*) is toxic to microorganisms living on the surface of water such as bacteria flagellates, algae, amoebae, and ciliates. Any of these organisms are killed within minutes by the action of the secretion. Thus gyrinal can be used as a defensive compound against both microorganisms and higher animals.

The Defensive Chemistry of Primulaceae, Urticeae and Euphorbiaceae

In 1959 we started with the investigation of the active principle of the glandular hairs of leaves and stems of plants causing dermatitis [27]).

The first plant which caught our interest was *Primula obconica*, the popular winter-blooming greenhouse primrose. Contact with the perennial herb causes severe dermatitis and conjunctivitis in some individuals. The poison is contained in the glandular hairs (Fig. 17) which occur especially on the leaves. Nestler isolated yellow needles from the secretion in the hairs. Bloch and Karrer suggested "primin" to be a lacton. We found it worthwhile to isolate primin and to investigate the structure of this real allergen. We suggested already in 1959 primin to be a quinone [27]. The infrared spectrum made a methoxyl quinone possible — micro methoxyl determination established one methoxy group — and indicated the substitution of the methoxyquinone with a straight pentyl side chain. The position of the side chain was found by the ^{1}H-NMR spectrum [28] and the "electronenbrenze" of primin (Fig. 18). On this basis the structure of primin was established as 2-methoxy-6-n-pentyl-p-benzoquinone. Final confirmation was given by the synthesis of primin [29] and its higher and lower homologs. They allowed interesting and valuable pharmacological tests [30]. Patch tests with 25 mg of a solution of 0,004 per cent gave positive reaction in all patients sensitive to the plant (Table 6). In most cases the

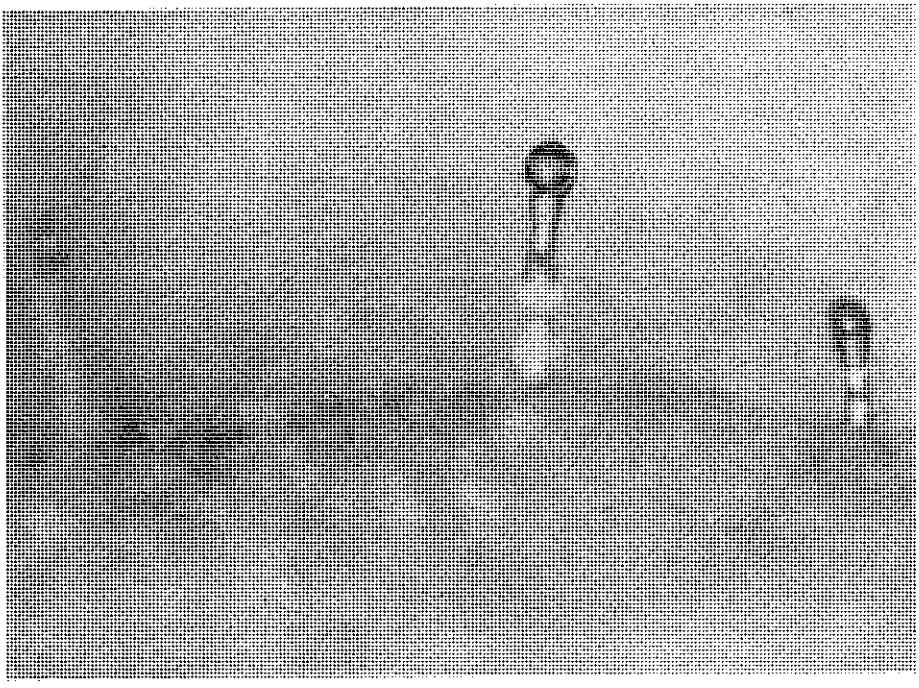


FIG. 17 — Glandular hairs of *P. obconica* with the yellow secretion.

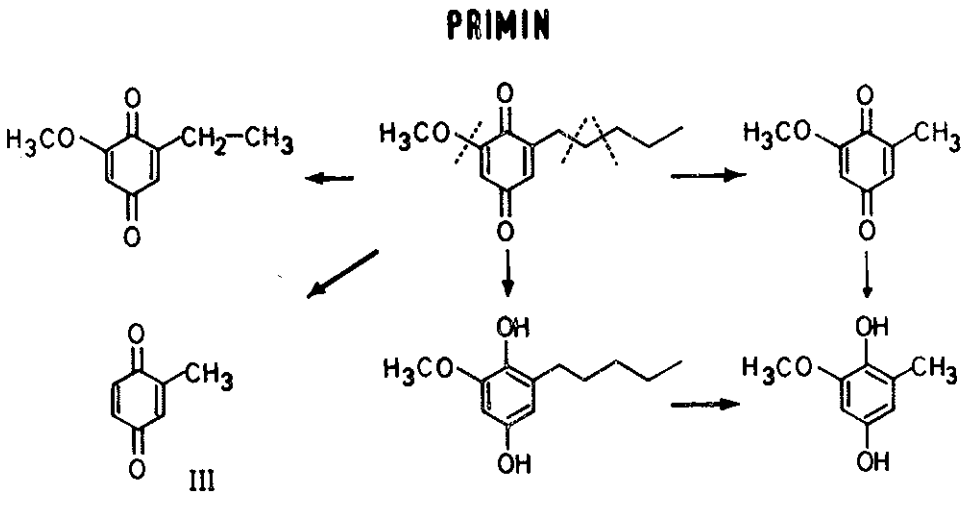
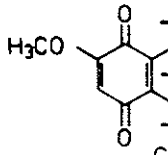


FIG. 18 — Scheme of the "Electronenbrenze" of primin.

[4] II, 3 - *Schildknecht* - p. 26



R_1	CH_3		C_2H_5		C_3H_7		C_4H_9	C_5H_{11}	C_6H_{13}	H		H	
R_2	H		H		H		H	H	H	C_5H_{11}		C_6H_{13}	
CONC.	10 xC	C	10 xC	C	10 xC	C	C	C	C	10 xC	C	10 xC	C
CASE NO. 1		+		+		++	++++	+++++					
2	+	–		+		++	+++	+++	+++		–		+
3	++	–		+		++	++++	+++	++		+++		+
4	+	–		+		+++	++	+++	+++		++		++
5	+	–		+		++	++	++	++		+		+
6	+	–	+	–		++	+++	+++	++		+		+
7	++	–	++	–		++	+	+++	+++		++		++
8	–		–			++	++	+++	+++		++		–
9	+	–	++	–		++	+	++++	+++		–		–
10	–	–	+	–		–	+	+	+	+	–	+	–
11		–		–	+	–	+	+	+		–		–
12	+	–	+	–	+	–	+	+	+		–		–
13	–	–		–	–	–	+	+++	++		–		–
14	++	–	+	–	++	–	++	++	++		–		–
15	–	–	+	–	+	–	+	+	+	+	–	+	–
16	–	–	+	–	+	–	+	+	+	–	–	–	–
17		–		–		–	++	++	++		–		–
18		–	–	–	–	–	+	++	+		–		–
19	+	–	–	–	+	–	++	++	–		–		–
20	–	–	–	–	–	–	–	+	–		–		–

C=0,00048 MOL./LIT. *1/4 C

TABLE 6 — Patch test reaction to primin and related quinones in 20 patients.

sensitivity extended to quinones with longer or shorter side chains in 6 position as compared with primin. Sensitivity also extended to quinones with a side chain in 5 position, but only in patients with a strong sensitivity to primin. No action was found with benzoquinone or toluquinone. The optimal action of primin for inducing sensitization may be related to immunological factors or to the cutaneous penetration of the chemicals.

We have heard that quinones are frequently used as defense substances by insects and the question arises if quinones have the same biological function in plants, especially when they occur in special plant organs.

The big stinging nettle *Urtica dioica* possesses besides glandular and shaggy hairs, stinging hairs which are 2 mm long and consist of sharp siliceous nettle cells which are embedded in epidermal cells. The nettle cells have a brittle tip (Fig. 19) which breaks off easily

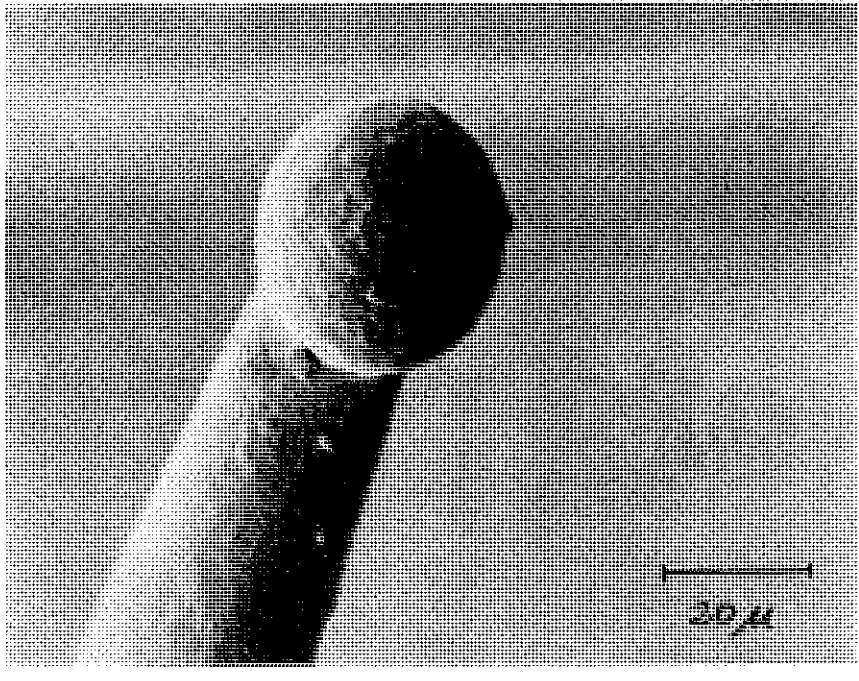


FIG. 19 — Trichome of the stinging nettle *U. dioica* with intact tip; Recorded with a scanning electron microscope (JSM-S1, Kontron GmbH).

when touched resulting in an oblique sharp opening which penetrates the skin and acts as injection needle through which the poison is injected (Fig. 20). The volume of the stinging liquid per hair is about 0,008 mm^3 and about a third of this volume is injected. The fluid causes the typical nettle rash.

In order to succeed in the isolation of the irritant principle of the stinging hairs we tried three methods. First we separated dried leaf powder by electro air separation (Fig. 21). Then we shaved fresh plants with a razor (Fig. 22). In a third method we avoided some disadvantages of these methods by cooling the plant in liquid nitrogen and shaved the hairs off the frozen plant.

Regarding the irritant principle of *Urtica dioica* again an interesting parallelism can be drawn to the defense substances of insects. *Urticaria* caused by the sting of the nettle is very similar to *urticaria* caused by insect bites. It is known that the venoms of

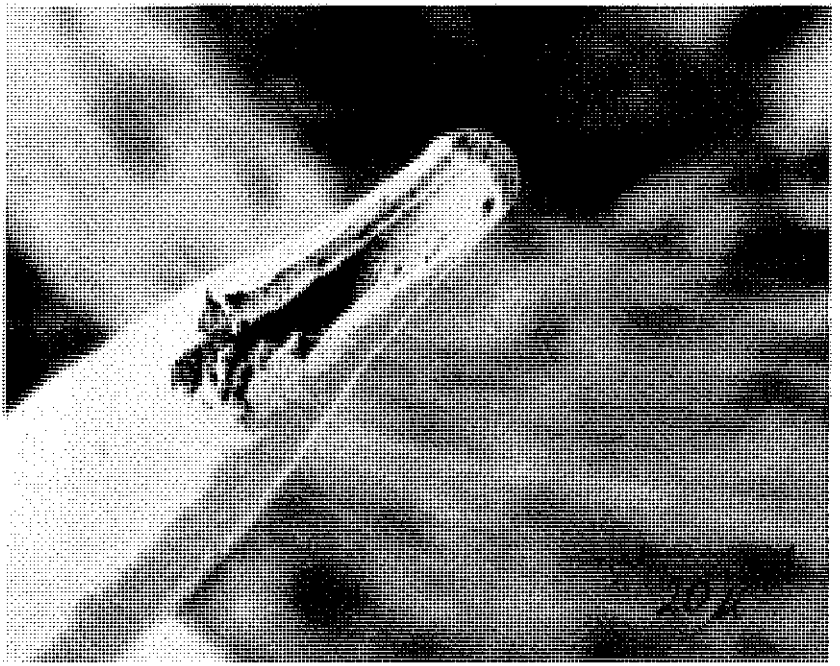


FIG. 20 — Trichome with broken tip of *U. Dioica*; Recorded with a scanning electron microscope (JSM-S1, Kontron GmbH).

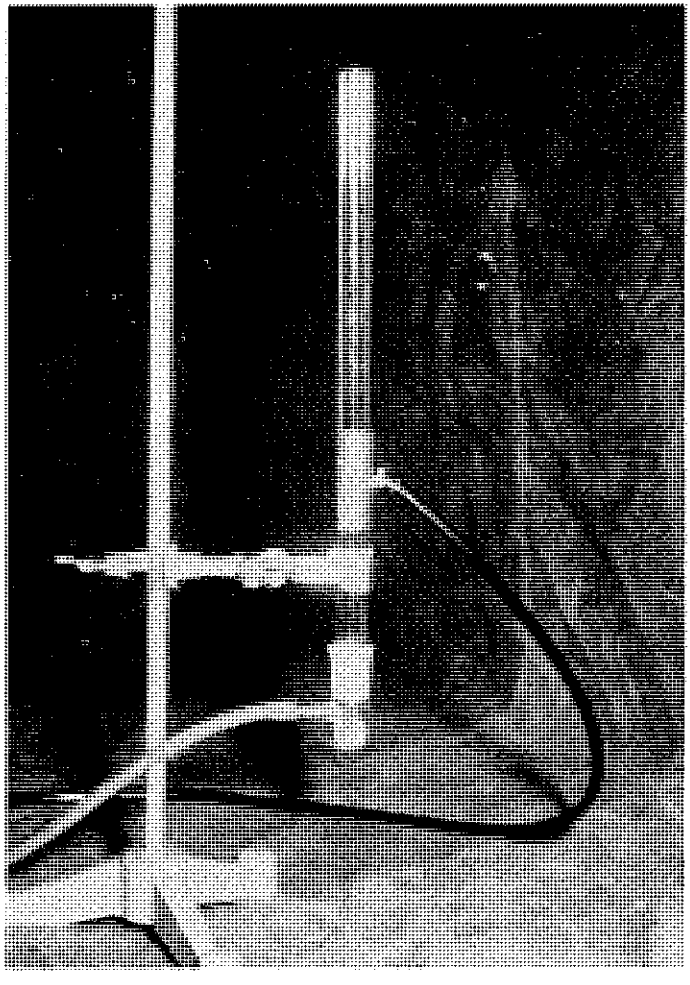


FIG. 21 — Electro air separator.

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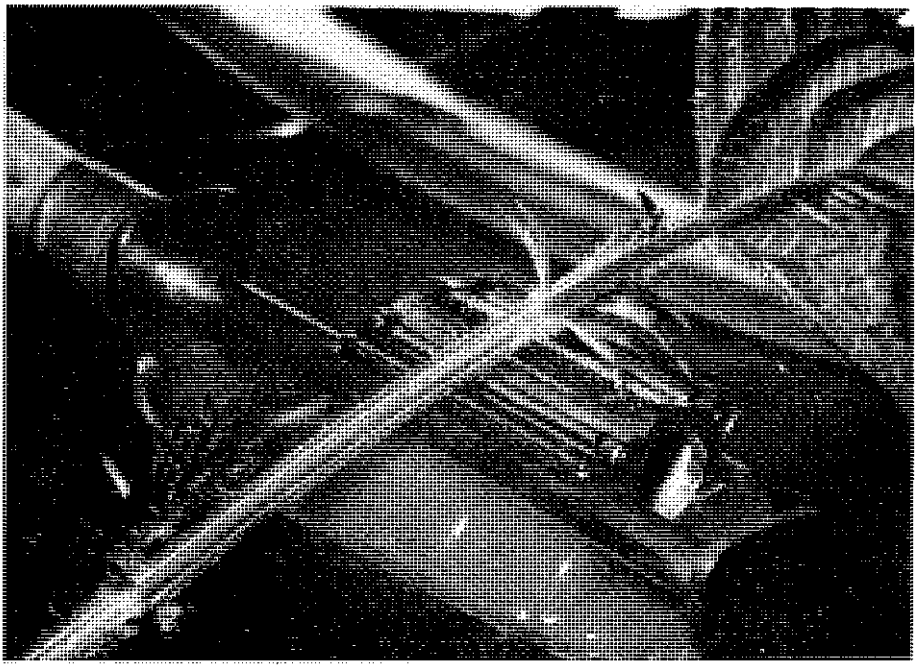

FIG. 22 — Shaving of fresh leaves.

some insects contain biogenic amines and actually already in 1947 EMMELIN and FELDBERG [31] suggested according to their pharmacological assay the presence of acetylcholine, histamine and a third not identified biogenic amine. In 1957 COLLIER and CHESHER [32] identified histamine, acetylcholine and serotonine by paper chromatography. Nearly at the same time we got the same results with the same method. In 1973 VIALLI *et al.* [33] used the condensation products of the amines with o-phthal-aldehyde to determine histamine and serotonine by fluorescence spectroscopy.

All determinations so far reported are not very specific. We converted therefore the biogenic amines to their 1-dimethyl-amino-naphthalene-5-sulphonyl derivatives, separated the DANS derivatives by thin layer chromatography guided by their fluorescence and analysed the separated fraction by mass spectrometry [34]. The principle of the fragmentation of DANS derivatives of biogenic amines under

electron impact is shown in Fig. 23. The mass spectrum of the DANS derivative of histamine obtained from preparative t.l.c. showed the expected molecular ion at m/e 577 with the elemental composition $C_{29}H_{31}N_5O_4S_2$ and the typical key fragment at m/e 82 with the composition $C_4H_6N_2$. The mass spectrum of a second separated fraction made the presence of β-hydroxy-β-phenyl-ethyl-amine or tyramine probable. Reaction with only one mole dansylchloride leading to a molecular ion at m/e 370 favours the former structure over tyramine, but a definite decision was not possible as yet (Fig. 24).

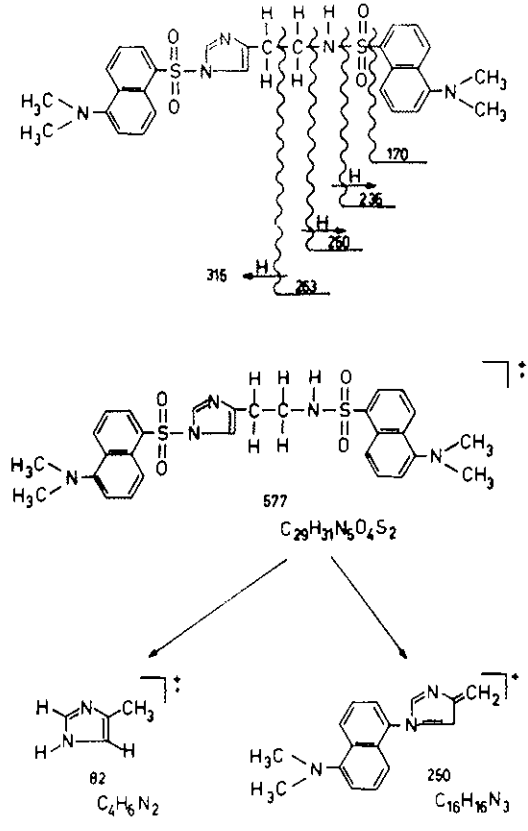

FIG. 23 — Fragmentation scheme of dansylated histamine.

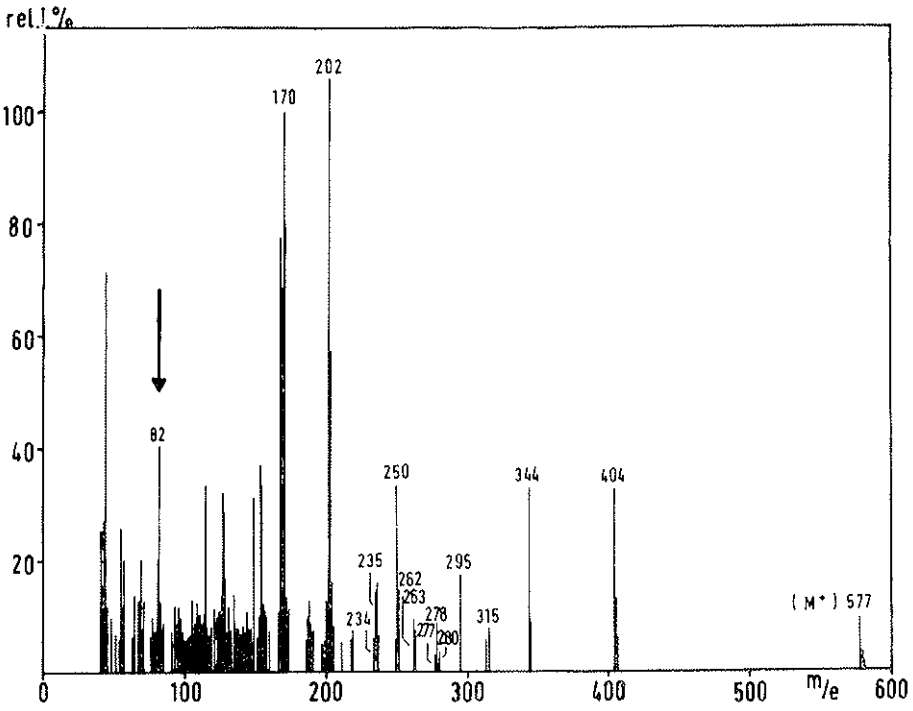

FIG. 24 — Mass spectrum of DANS histamine.

A second nettle with a much stronger irritant, *Laportea moroides* (Fig. 25), is found in Eastern Australia and New Zealand. The bush is considered to be a menace to stock and particularly to horses, which, if severely sting become violent and have to be shot. The stings of *Laportea moroides* cause painful inflammations which last for weeks. Every month we have harvested three or four leaves under great caution, using a gas mask, hecause the plant discharges irritant substances and causes sneezing which lasts for four to five hours. Mechanically *Laportea moroides* is much easier manipulated than *Urtica dioica.* In the secretion of *L. moroides* we identified again as DANS derivative histamine on t.l.c. and by mass spectrometric analysis. As shown by pharmacological tests reported in 1957 by ROBERTSON and MACFARLANE [35] there are more

FIG. 25 — *Laportea moroides*, grown in the greenhouse.

biogenic amines present in *L. moroides*. These can also be detected on t.l.c. and we are right now working on their exact identification.

Jatropha urens (Fig. 26) belongs to the family *Euphorbiaceae* and possesses bristly hairs which can be as long as 6 mm. Contact with the hairs causes painful inflammations. The affected skin is sensitive to change of temperature and pressure, even after a few days. The inflammation can become so severe that hospitalization for weeks may be necessary as in the case of a gardener at the botanic garden of the University of Heidelberg. But for the analyst

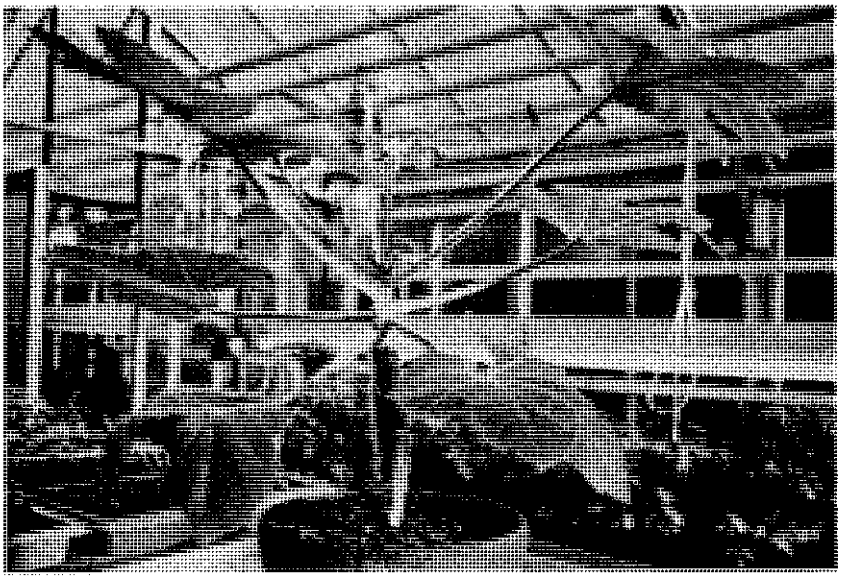

FIG. 26 — *Jatropha urens*, grown in the greenhouse.

Jatropha urens is a pleasant plant. The poisonous fluid is contained in stinging hairs of comparatively large volume. After cutting the tip of the stinging hair, the pure secretion is easily obtained by pressing the base of the hair (Fig. 27). The secretion is acid with pH 5,5. No formic acid was detectable and acidity may be caused by free amino acids (Fig. 28). The UV spectrum indicated the presence of aromatic compounds in the secretion. Comparative chromatographic analyses (Fig. 29) led to the detection of 5-hydroxy-triptamine (serotonine) and histamine. On the t.l.c. plates of the dansylated products one detects besides the spots for the dansylated histamine and serotonine, spots for the reagent, tbe hydroxylated reagent, DANS methanol, and most probably the DANS derivative of ethylamine. The DANS derivatives of histamine and 5-hydroxy-tryptamine were definitely identified by mass spectrometry (Fig. 30) from the fractions eluted from a preparative plate. Separation was done on the extract of 2000 stinging hairs.

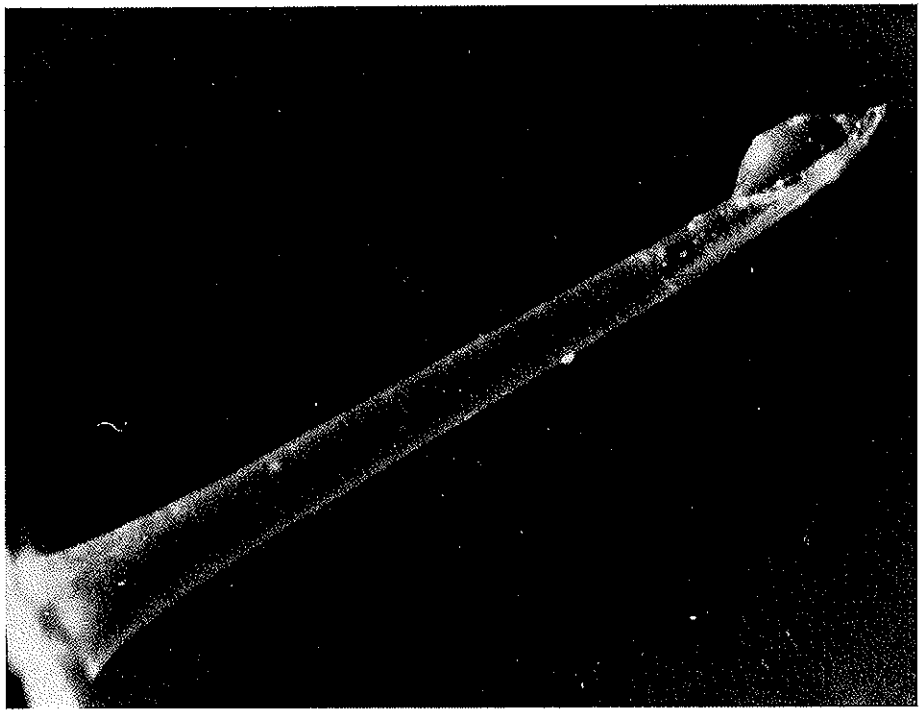

FIG. 27 — A stinging hair of *J. urens*, 4 mm long with a drop of irritant fluid.

It is remarkable that the three investigated poisonous plants and *Cridoscobus phyllacanthus* also [36, 37], which cause inflammation contain all histamine and other smooth muscle contracting substances. Yet histamine and 5-hydroxy-tryptamine (serotonine) are most probably not predominately responsible for the painful sting. If the substances are injected intradermally in human subjects in the same concentrations as they are found in the plants, they cause very little effect compared with the sting of the plant. It is very likely that other substances play a role in concerted action with the biogenic amines. Investigations to identify these substances are under way.

We have to discuss the biological function of these poisonous substances and the idea that they have defensive functions is

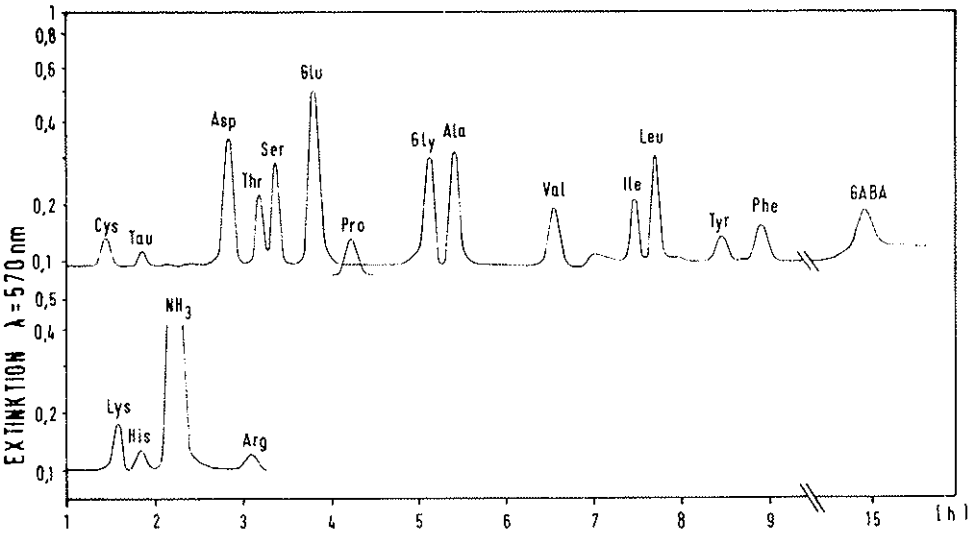

FIG. 28 — Elution plot of free amino acids of *J. urens*. Lower plot: elution of short column basic amino acids.

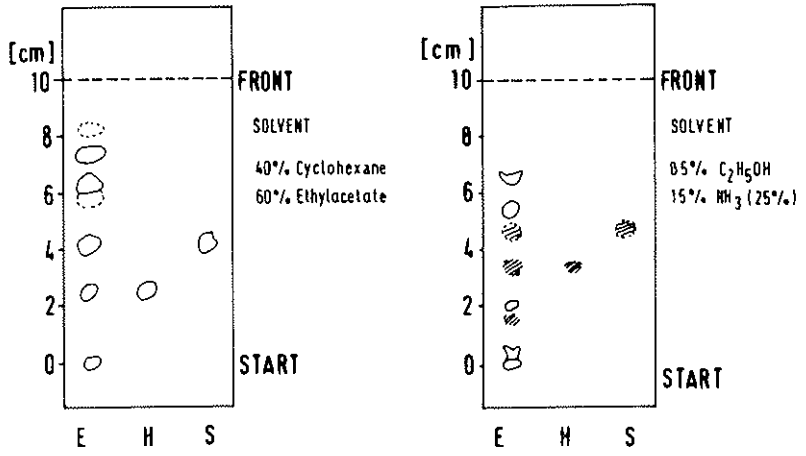

FIG. 29 — Comparative t.l.c. of the fluid of the stinging hairs of *J. urens* with the spots for histamine, and 5-hydroxytryptamine. Left side: t.l.c. of the dansylated products; right side: t.l.c. of the free amines, spotted with ninhydrine.

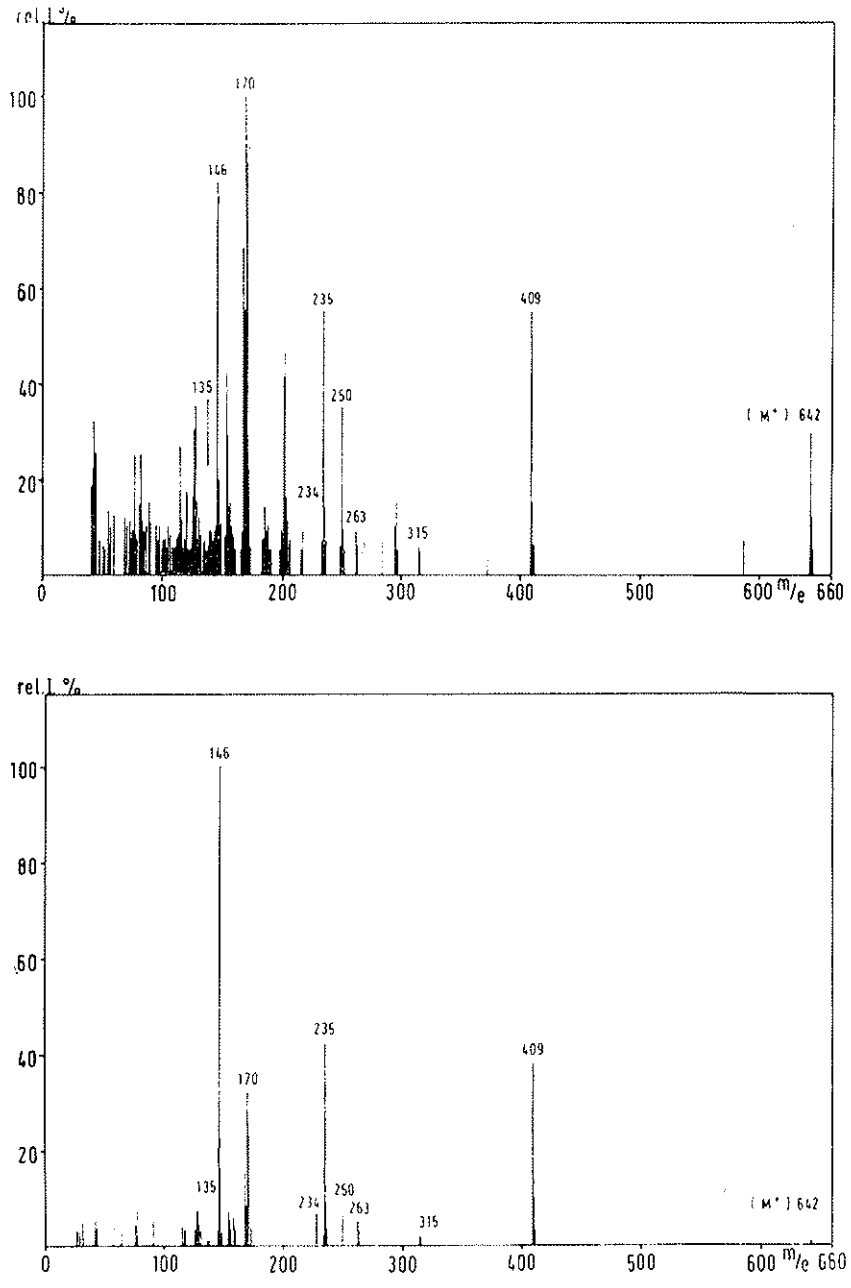

FIG. 30 — Mass spectrum of synthetic (below) dansylated 5-hydroxy-tryptamine and mass spectrum of dansylated 5-hydroxy-tryptamine from the irritant fluid of *J. urens* (above).

inevitable for people who have suffered from their action. We are perhaps permitted to attribute a defensive character to poisonous plant substances which occur in special organs and to those which are chemically very similar to defensive substances in insects.

The research described above was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie, which I gratefully acknowledge. I wish to express my special thanks to all my numerous co-workers.

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DISCUSSION

MARINI-BETTÒLO

Thank you, Professor Schildknecht, for this important presentation, which now makes clear the inter-relationships between natural products present in insects and those present in plants.

I think this point will be discussed further in subsequent meetings of this Study Week.

I should like only to underline here the importance of understanding exactly the meaning of the defensive substances of insects and their possible use in a wider strategy for the protection of plants.

Now I should like to have comments from all of you.

BELL

I should like to draw attention to the interesting transfer of methylazoxymethanol glycosides from leaves of *Cycas circinalis* to the larvae of the moth *Seirartia echo*. The foliage of *C. circinalis* contains various glycosides of the carcinogen methylazoxymethanol. Oral administration of these glycosides to mammals results in toxic effects as the aglycone is liberated by the β. glucosidase enzymes of the intestinal microorganisms. In the larvae of *S. echo* (which feed on the leaves of *C. circinalis*), the glycosides also appear to be hydrolysed in the gut but the methylazoxymethanol is detoxified by re-glucosylation to give the β. glucoside cycasin which accumulates in the insect as well as the plant, providing presumably protection for the larvae against their potential predators.

WAIN

I was interested, Professor Schildknecht, in your account, and especially on *Platambus maculatus* which exudes parahydroxy-benzaldehyde. Now it was reported that some insects feed on the leaves of

willow, which contains salicin. I should like to know if you have evidence that the p-hydroxy-benzaldehyde may be considered an endogen or exogen product of the metabolism of the insect.

Another question is this: is the salicilic aldehyde exuded by the sting glands as a repellent produced to protect the insect or is it merely an excretive mechanism, whereby the insect can get rid of the salicin which is oxidized by the insects to aldehyde?

SCHILDKNECHT

This phenomenon is well known: salicylic aldedyde is not only exogen, it is a real defensive substance. We have the same thing in other kinds of insects, which feed on plants and they store hexenol in their defensive bladders. But we have found that this hexenol comes not from the leaves — although it is very common in the green leaves of plants all over the world — but that insects produce their own hexenol.

In effect there are insects which do not feed on plants but only on blood and produce again hexenol. So I believe that salycilic aldehyde is a real defensive substance produced by the insect itself. On the other hand, we have an example with caterpillars. They live in the forest on trees, and they extract terpenes from leaves and needles and excrete them through special organs.

WAIN

It is quite remarkable that when an insect is eating the leaves of willows it is taking in a very large amount of salicin and it has to dispose of it. It seems to me a very simple mechanism; the insect may take energy first from the glucose and then from the oxidation of the salicylic alcohol to the aldehyde, the latter being excreted from the sting gland. You are saying that salicylic aldehyde is produced by another mechanism and not from salicin.

SCHILDKNECHT

Have you detected any HCN?

WAIN

There is no HCN in this particular group of glycosides. Salicin is simply the β-glycoside of salicylic alcohol. Now, the insect may hydrolize the glycoside and then oxidize the salicilic alcohol to salicilic aldehyde and push it out. I think that this is a philosophical question: is it pushing it out as an excretion product or as a defensive secretion?

MARINI-BETTÒLO

Thank you Professor Wain. I think we are now approaching a new problem: where do these compounds present in insects come from? are they synthetised directly from insects or are they present in plants from which the insects take them in with their feed?

I think that both mechanisms may be found in nature. Professor Schildknecht gave evidence of the formation of hexenol by insects. I remember that Prof. T. Reichstein reported that some steroids isolated from locusts were demonstrated to be taken directly from the plants. Are there any more examples of this?

KNÜSLI

Professor Schildknecht, you mentioned that you have worked with special urtical species. Now I wonder what is the active principle of the common urtical species?

SCHILDKNECHT

The active principle is histamine, especially histamine in high concentration and somewhat β-hydroxy-phenyl amine. But maybe there are also other substances, because, as I have said, the stinging action is not comparable to that of histamine. Therefore we are seeking other substances, but this is very hard with the common urtica, with *Urtica dioica,* and thus we have taken up our research work with other species as I have shown. They have bigger stings and more fluid in their base. So far the only poisonous substance found in *Urtica dioica,* the big stinging nettle, is histamine.

GRANITI

Certain plants like *Tagetes* and *Asparagus,* I think, produce root exudates which are active on nematodes living in soil.

Actually these plants are used, especially in the Tropics, to lower the nematode population in the soil. Is there any relation between these compounds and those which are insect repellents?

SCHILDKNECHT

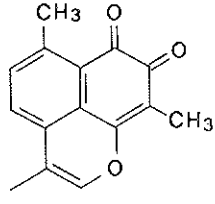
I do not know; I have heard of this phenomenon but I cannot say whether it can be related to the products used by plants as repellents.

MARINI-BETTÒLO

Something similar occurs in the woods of tropical trees. For many years Prof. Oswaldo Gonçalves de Lima in Recife, Brazil, has advanced the hypothesis that the woods of tropical trees were immune from the attack of insects and molds mainly because of the presence of special substances.

He isolated a certain number of quinones, and from literature we may find a great number of examples of quinones present in these woods; to begin with, Lapachol studied since the last century by Paternò.

Other examples are the wood of *Mansonia altissima,* which contains a group of quinones: the mansonones (*) generally together with the respective hydroquinone:



In the wood of *Miconia* Gonçalves de Lima and I were surprised to find the quinone primine, described by Professor Schildknecht in

(*) G.B. MARINI-BETTÒLO, C.G. CASINOVI and C. GALEFFI: Tetrahedron Letters, *52*, 4857 (1965); G.B. MARINI-BETTÒLO, C.G. CASINOVI, C. GALEFFI and F. DELLE MONACHE: Ann. Ist. Sup. Sanità, *2*, 327 (1966); ibid. *4*, 305 (1968).

Primula, which forms, with its hydroquinone miconidine, also present in the plant, a highly biologically active system (antibacterial and anti-tumoral) (*).

I am recalling these products, and this example in particular, in order to show how widely diffused are the same active principles in plants.

BOWERS

I am interested in stenusin. Is this a compound, as I understand, which repels insects across the water? Is this a spreading agent and has it also a repellent function as well? Do you know which came first: the repellent or the spreading properties?

SCHILDKNECHT

I think we have two types of substances in *Stenus*. It has two bladders: a small one and a big one. In the small bladder the terpenoids are present, which exert a toxic action especially against microorganisms. In the big bladder there is only stenusin, which is not toxic to microorganisms, and mainly is a spreading substance. The spreading reaction goes over the water at a speed of 40-70 cm per second.

GILBERT

I would like to ask more about these spreading compounds. First of all, is it a property of all substances of this type to have this high spreading velocity? Is this the reason why some insecticidal preparations for mosquito larvae contain amines?

SCHILDKNECHT

You are right: this is a special effect of the amines and in particular of piperidines or better substituted piperidines.

We have found that if in stenusin we cut the isopentyl chain to butyl, propyl and methyl groups the effect is progressively decreasing. The top spreading effect is present in stenusin.

(*) G. B. MARINI-BETTÒLO, F. DELLE MONACHE, O. GONÇALVES DE LIMA and S. DE BARROS COELHO: Gazz. Chem. Ital., *101*, 44 (1971).

SIDDAL

I would like to ask you about the origin of the steroid that you have described as a defensive substance.

SCHILDKNECHT

It is possible that a beetle prepares this substance from cholesterol or sistosterol.

NAKANISHI

Could you explain the technique of electron pyrolysis you mentioned concerning stenusin and primin?

SCHILDKNECHT

We put the substance in a very small vessel with 1 milliliter of tritiated water with high specific activity that is very important: 20 millicuries per milliliter.

In this solution the solvated electron can be detected in a very low concentration. When you freeze the solution, the ice is blue because of the presence of the solvated electron.

Now let me explain with a very simple example the electron pyrolysis. Let us consider α-alanine, that is α-amino-propionic acid. By the action of the solvated electrons a NH_2 group is eliminated and you get a propionic acid radical which in the presence of tritium is tritiated.

This part is radioactive and it can be detected by thin layer or gas chromatography. That was the idea of our method. The advantage in respect to mass spectrometry is that cleaving the molecule by the action of solvated electrons, you get always two parts which can be easily detected through their radioactive properties.

By this method it is quite easy from tbe breakdown products to write the structure of the substance under investigation as in the case of stenusin (*) and primin (**).

(*) pag. 69.
(**) pag. 84.

NAKANISHI

What is the most efficient way to generate this solvated electron? How do you do it?

SCHILDKNECHT

From tritiated water. THO is always emitting electrons. Tritium emits β-particles.

NAKANISHI

How much compound do you need?

SCHILDKNECHT

In the order of micrograms: 1 to 5. By this method we have studied quinones, hydroquinones, and nitrogen compounds.

SOME INSECT METABOLITES AND THEIR BIOLOGICAL SIGNIFICANCE

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Centro per lo studio delle sostanze organiche naturali del CNR
Milano

For centuries insects have been regarded as harmful to man, to domestic animals and to plants. The famous Milanese poet, Carlo Porta, in the 17th century, in a delightful sonnet describes the harm caused by insects to man.

Of course this bad reputation of insects is not entirely unjustified, for the losses to agriculture from the destruction of crops by insect attacks and the harm to man and animals constitute a sad reality. However, we must also recognize the fundamental functions of certain insects in the ecological equilibria, as for example, the pollination of flowers, the destruction of parasitic fungi and molds in plants, and the prevention of the spread of other harmful insect species.

This fact — clearly demonstrated by the harm which the indiscriminate use of potent insecticides has caused to the ecosystems — compels us to search for means of pest control having more specific activity and limited in the duration of their effectiveness. Such a program obviously requires an extensive knowledge of the physiology and behavior of insects. These studies, in which physiologists, chemists and entomologists have collaborated, were initiated in the late forties and were encouraged by the first interesting results, such as the isolation and the structure determination of those products which act as sexual attractants, and more recently the

hormones which regulate the development of insects, such as the molting and the juvenile hormones.

The products used hy insects for their defense and attack in their daily struggle for existence are also of importance in this new approach to pest control.

In the case of social insects these substances are generally combined with other substances which act as chemical messengers and are thus able to call together the memhers of the same family to warn them of the presence of an enemy and to guide them in the search for food.

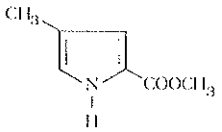
In this respect ants have received considerable attention for their remarkable social organization. In their foraging activity they usually follow the same trails which can be safely followed by a single individual ant. If the trail is interrupted by an object or the digging of a small furrow in the soil, the ants stop in bewilderment not knowing what to do.

CARTHY [1] observed that *Lasius fuliginosus* follows a trail marked by feces of ants of the same species, but he did not succeed in identifying the pheromone and the organ producing it. Recently VISCONTINI [2], using very refined techniques for collecting the pheromones, was able to demonstrate that it consists in a mixture of hexanoic, heptanoic, octanoic, nonaoic, decanoic and dodecanoic acids.

In *Iridomyrmex humilis* and in most of the *Dolicoderinae* ants PAVAN [3] identified the organ producing the trail pheromone in a gland which had never before been descrihed and which is now known as the "Pavan gland", which is situated between the fourth and fifth gastral urostern.

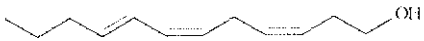
BLUM [4] has observed that tbe leaf cutting ant, *Atta texana,* and other ants of the same genus, when returning to the nest from the foraging areas, frequently press the abdomen to the ground at the same time pushing out their sting. This particular behavior, which in the opposite direction (i.e., in going out from the nest) is much less frequent, enables the ant to leave a trace which remains active for several months. From the secretion of the glands which are connected to the sting, methyl ester of the 4-methyl-pyrrol-car-

boxylic acid *1* has been isolated [5] and identified as the trail substance of these ants.



1

Other social insects also use pheromones in order to mark the trail. In the *Zootermopsis nevadensis* termites, the function is performed by hexanoic acid [6]; in the subterranean termite of the South *Reticulotermes virginicus* by cis-3,cis-6,trans-8-dodecatrien-1-ol *2* [7]. The other steroisomers are 10^3 to 10^4 times less active.



2

The specificity of pheromones is very interesting. More often they are active only on individuals of the same species; rarely do they show any activity on another species. Also synthetic substances which are structurally similar, at times evidence some activity but in a much lower degree.

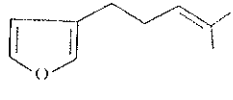
The complexity of the pheromone and the physical and chemical properties of its constituents are related to the functions that the pheromone itself must perform. For example, the trail substance of *Solenopsis saevissima* disappears in a few minutes and is deposited only by the workers when returning to the nest with food; those who return without food do not leave any trace [8]. The behavior of the workers and the nature of the pheromones are such that the intensity of the trail substance is directly proportional to the quantity of food collected and disappears when this is exhausted. The solubility, volatility and instability of these substances determine the duration of their effect.

The importance of the chemical and physical properties of pheromones is even more evident in the case of those substances which give the alarm or serve for recognition to the species. Generally they are associated with other substances having a true and positive defensive function for the insect. They are mixtures of saturated and unsaturated hydrocarbons, secreted by glands of the mandibula, of the anus, or of the abdomen, which usually are connected to the sting. Some secretions have been found that consist in very complex mixtures of saturated and unsaturated hydrocarbons, mainly from C-9 to C-19 [9] as well as terpenes like limonene [10] and farnesene [11]. According to some authors, these hydrocarbons have a lubricating function or act as vectors of the poison itself [9], while others consider them also as trail substances [12].

The function of alerting and alarm releasing or recruiting the population is performed by less stable products, in accordance with the requirement that the alarm should continue only for the time it is actually necessary. The secretion in the mandibular glands of *Acanthomyops claviger* contains citronellal and citral [13], and the secretion of *Lasius umbratus* contains citronellol [14] and tridecan-2-one [15] in addition to citronellal.

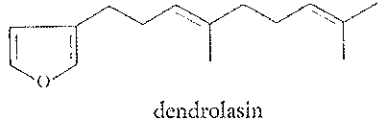
According to BLUM [14], it is only the citronellal that releases the alarm, whereas citronellol (which constitutes 85% of the volatile substances) and tridecanone have a defensive function.

The main component of the secretion of the mandibular glands of *Lasius fuliginosus* is dendrolasin (86%); the others are farnesal (7%), citral (1%) and perillen (traces) *3* [17]. Dendrolasin, whose



perillen

3



dendrolasin

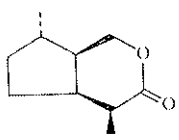
4

structure was shown to be the β-(4,8-dimetil-nona-2,7-dienil)-furan *4* [16], constitutes the first example of a furan derivative isolated from animal organisms and was subsequently found also in wood

of the trunk of the *Torreya nucifera* [18]. Dendrolasin has a selective toxic activity on the Formicidae [19]; this property might justify its production by a plant as a defense against ants.

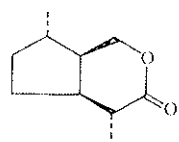
The complexity of the composition of the mandibular gland secretions is related to the complexity of their functions [14]. In fact, among the many components of the secretion of *Oecophylla longinoda*, the African ant, which is notoriously efficient in the defense of its territory, BRADSHAW and BAKER [20] have identified the specific activity which some of them have on ant behavior. The more volatile fractions serve to spread the alarm, whereas the more persistent indicate where the attack should be made and also mark the enemy [14, 20], acting thus as real defense substances even when they lack any specific toxic activity.

Pavan succeeded in demonstrating that other species of ants employ for defense substances, produced by anal glands, showing a strong insecticidal activity. He started from the observation that *Iridomyrmex humilis* in the struggle against other insects, brings the abdomen into contact with the victim and puts on it a liquid secretion. From said secretion he isolated a substance he named iridomyrmecin, which showed a strong toxic activity [21]. To iridomyrmecin FUSCO, TRAVE and VERCELLONE [22] assigned the structure of α-(2-hydroximethyl-3-methyl-cyclopentyl)-propanoic acid lactone *5*. Several other iridoids (substances chemically related to iridomyrmecin) were subsequently isolated in ants of similar species; among these were iso-iridomyrmecin *6* which was found in *I. nitidus* [23, 24], iridodial *7* in *I. detectus* [23] and *Tapinoma nigerrimum*, associated in this latter insect with 2-methyl-2-hepten-6-one and 2-methyl-eptan-4-one [25], and dolicodial *8* in *I. myrmecodiae* [26].



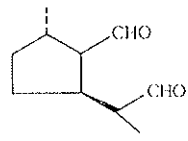
iridomyrmecin

5



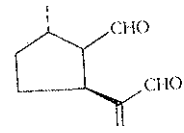
isoiridomyrmecin

6



iridodial

7

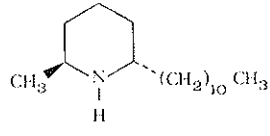


dolichodial

8

The contact toxicity of iridomyrmecin is highly specific. Generally high for the greater part of the arthropods, and for a number of them even higher than that of DDT and gammahexane, it is, however, surprisingly inexistent on the larvae of some lepidoptera and on some adult cockroaches [27] and practically nil for all the omeothermes animals [28]. These properties have suggested its use as an insecticide. After the first syntheses made independently by KORTE [29] and by Sir ROBERT ROBINSON [30], many others were achieved, but iridomyrmecin has never received the wide practical application that might have been expected.

Many substances of a different and uncommon structure have also been found in ants, which they use for defensive purposes. As an example I should like to mention the dimethyl-disulphide and dimethyl-trisulphide [31] isolated in the mandibular glands of *Paltotyreus tarsatus*, the African ant known for its unpleasant odor. These constitute the first known example of sulphurated defensive substances produced by Arthropods. I wish also to recall the solenopsin *9* isolated in the red form of the *Solenopsis saevissima* ant [32] which is an alkaloid not previously found in insects and shows haemolytic, insecticidal and antibiotic activity.

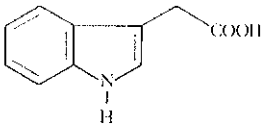


solenopsin

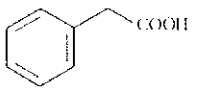
9

Antibiotic activity is also found in other substances produced by ants; at times it is associated with antimycotic activity and plant growth inhihiting properties, as in the case of iridomyrmecin [28]. Such findings induce us to study these substances also from another point of view. They would seem to have an indirect protective function for the ants by preventing the growth of fungi or other pathogenic agents in the nests. From this point of view the *Atta sexdens,* the leaf-cutting ant, is of particular interest; its metathoracic glands produce indolyl-acetic acid *10* [33], phenyl-acetic acid

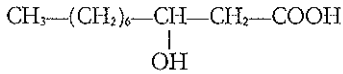
11 [34], which as well as myrmicacin (3-hydroxydecanoic acid) *12* [35] has high herbicidal properties.



10



11



myrmicacin

12

With its glandular secretion the ant cultivates its "fungus garden" which serves as a source of food. The phenyl-acetic acid inhibits the growth of bacteria, myrmicacin prevents the development of foreign spores, while the indolyl-acetic acid encourages the growth of the fungus symbiotic to the ant [35].

Despite the vast development in the chemical and biological study of insects in the last 20 years, which has clarified some peculiar aspects of their behavior, little is yet known of the function of some substances which have been known for some time. Such is the case of periplanetin produced by the laterocervical glands of *Periplaneta americana* and of other *Blattidae*, which was identified as 1-benzoyl-β-D-glucose [36], the first and thus far the only example of natural ester of the β-glucose. It might be logical to infer that for the cockroach this represents simply a means to eliminate the benzoic acid produced by its metabolism.

So far not even the function of cantharidine — the vescicant principle of the cantharide (*Lytta vesicatoria*), well known since the last century — has been clarified. Popular tradition attributes to it more or less imaginary properties, but the real function for the insect is still unknown. Recent research by H. SCHMID [37] at the University of Zurich may shed some light on the identification of its functions. By injecting the insects with labeled precursors, he has been able to prove that the male of *Lytta vesicatoria* is stimulated to produce cantharidine in mating and that during the mating he transfers the major part of the compound to the female. The female, however, is incapable of synthetising it. These observations lead us to infer that there is some connection between the production of cantharidine and the reproductive functions and they

immediately suggest to study the variations of the cantharidine content in the female body after mating.

Defensive functions are probably performed by the benzoate of mandelic nitrile contained in the secretion of the myriapode *Polydesmus collaris* [38].

The cossins (dienic and trienic C-14 alcohols and their acetates) *13* [39] contained in the mandibular gland secretion of the larva of *Cossus cossus*, which are toxic for ants invading the trunks where the cossus larvae live, can also be considered defensive substances for the larvae themselves.

$CH_2=CH-(CH_2)_5-CH_2-CH=CH-CH_2-CH_2-CH_2-CH_2-OR$

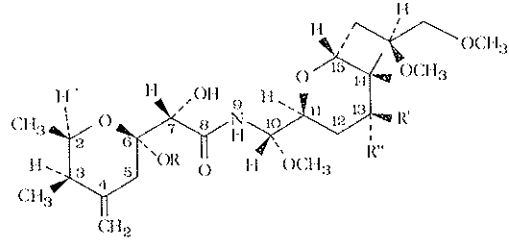
$CH_2=CH-(CH_2)_5-CH_2-CH=CH-CH=CH-CH_2-CH_2-OR$

$CH_2=CH-(CH_2)_5-CH=CH-CH=CH-CH_2-CH_2-CH_2-OR$

$R=H, CO-CH_3$

cossins

13



$R=CH_3$	$R'=H$	$R''=OH$	pederin	*14*
$R=OH$	$R'=H$	$R''=OH$	ϕ-pederin	*15*
$R=CH_3$	$R', R''=O$		pederone	*16*

Finally, I want to mention another substance, whose function in the insect has not yet been identified despite its unusually high biological activity. This is pederin. Ever since the beginning of the present century the lesions caused to man by insects of the *Paederus* genus have been described. At first these lesions were attributed to cantharidine, which it was believed the insects produced.

Later PAVAN turned to a study of this problem because of the frequency with which lesions attributed to this insect occurred among agricultural workers in the Pavia country side, where the *Paederus fuscipes* is rather widely diffused. This is a small coleopter staphylinide 7 mm long: the iridescent elythra partially cover the abdomen, which has a beautiful orange color due to carotenoid pigments (among these we have isolated and identified γ-carotene, β-carotene, β-zeacarotene, torulene, xanthophyl) [40]. The coleopter looks innocuous, but if accidentally a single individual is crushed on the epidermis, it causes a blister some centimeters long. Even more unpleasant lesions occur if the insects enter the conjunctiva sac. First PAVAN pointed out certain differences between the blisters produced by *P. fuscipes* and those caused by cantharidine; and subsequently he suceeded in isolating the active substance, which he called pederin [41]. The chemical study of this substance began a few years later and required the development of difficult new techniques to collect the insects and isolate the substance, which had aggressive properties of exceptional intensity. Just consider the fact that for this work 80 kilograms, or more than 20 million individual insects were collected, and that pederin even at room temperature has a considerable vapor pressure. We have learned to our regret that the vapors produced by ethereal or benzenic solutions used in the laboratory are sufficient to cause desquamation of the skin on the face and hands of persons present in the laboratory.

Along with pederin, which we have found in several other species of the genus *Paederus* [42] we have isolated a similar substance which we called pseudo-pederin. This substance is formed, at least in part, during the extraction process. We have also isolated a ketone, called pederone, derived from the oxidation of the secondary alcoholic group present in one of the two tetrahydropyran rings. To these three substances we have been able to attribute the structures *14*, *15* and *16*, but we could establish the absolute configuration of only two of the chiral centers [43]; the configuration of the other chiral centers has been estahlished by structural analysis by x-ray conducted independently in Japan [44] and in Italy [45].

Recently at the IUPAC symposium on natural products (New Zealand, August 1976) JERROLD MEINWALD, of Cornell University,

stated that he had arrived at the synthesis of two important fragments of pederin respecting the stereochemistry of all except one of the chiral centers. The authority of MEINWALD in this field permits us to foresee that we shall very soon have the total synthesis of this strange and fascinating product, which has an unusual structure, unique among all products of natural origin.

The biological properties of pederin are quite peculiar. It inhibits the growth *in vitro* of normal cells (chicken embryo cells and dog kidney cells) and tumoral cells (Hela and KB cells) in a concentration of one microgram per liter, reducing the cells in mitosis until they disappear completely and causing severe cytological alterations with precocious disappearance of the nuclear chromatin and of the cytoplasma [46]. In the cells cultivated *in vitro* the analysis of the macromolecular synthesis by radioactive labelled precursors indicates that pederin, in concentrations of 1.5 micrograms per liter almost immediately blocks the proteic synthesis and that of DNA without interfering with the synthesis of RNA. Also in cell-free extracts it inhibits synthesis of the proteins [47]. Subsequent studies have shown that pederin has a specific action on ribosome 80S of the eukariotic cells [48], and that it can block 99.9% of the cytoplasmatic proteic synthesis which is caused by the ribosomes 80S, but not the mitochondrial which occurs in 70S ribosomes [49]. It is interesting to note the insensibility to pederin of cells of *P. fuscipes* and probably of all insects. The development of knowledge regarding this substance arouses continually increasing interest. The production of a substance with an extremely complicated and uncommon structure, having a high biological activity, is certainly not accidental.

The obvious question is: what is the function of this pederin? It does not seem to be a defensive or an offensive product because it is present in the haemolymph of the insects and cannot be ejected at will by the insect. It does not appear to be a sexual attractant, since it is present in individuals of both sexes, although females contain about ten times more than males. It is not active as a trace substance, at least in the pure state. During preliminary studies on its biogenesis [50] we became aware that considerable amounts of pederin are present in the insect excreta. This could support the hypothesis that pederin might be one of the components of the

pheromone used by the insect to indicate its territorial limits or mark a trail. Sooner or later the entomologists will provide answers to all these questions while the biologists and chemists continue to investigate the exceptional properties of this substance at cellular level.

Even if sexual attractants, juvenile hormones and molting hormones have already attracted much interest — and during this Study Week authoritative scientists will illustrate the achievements of research and the prospectives that are opening up in the battle against harmful insects — it is not unlikely that future unexpected progress can result from the study of other substances of a different nature. The trail and alarm pheromones could offer useful means for controlling the spread of insects: either by using trail substances to direct the insects where their presence may be useful, or by using the confusion method to obstruct their migration toward areas where their presence is at present not desired. A contribution in this direction could result from substances which release the alarm or recruit the members of a family of insects.

The recognition that plants produce substances which in some way are active on insects and thus have a regulating function on the environment where they live, may also promote the utilization of these or analogous substances produced by synthesis, in order to protect for the time strictly necessary those cultures which do not have their own defense mechanisms.

The study of pheromones and other substances produced by insects, and the knowledge acquired regarding their mechanism of action lead us to evaluate positively the instability of potential defense agents, orienting us toward the use of easily degradable substances with a limited activity spectrum.

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DISCUSSION

MARINI-BETTÒLO

Thank you Professor Cardani for your very interesting report which represents only a small part of the outstanding work that the School of Milan headed by Professor Adolfo Quilico, your teacher and our common friend, has performed in the last years with the collaboration of the entomologist, Professor Mario Pavan of Pavia.

We are all here very sorry that both these distinguished scientists could not attend this meeting, because their contribution in the knowledge of insect chemistry and biochemistry has been fundamental, and their presence here would have been very important.

If we consider the substances presented by Professor Cardani, I think that the occurrence of so many new and uncommon structures may open some new paths of research. This means that we must go further on in the study of this aspect of insect biochemistry and physiology.

KNÜSLI

I feel that from all the compounds you mentioned iridomyrmecin has made the most progress. Now could you comment on the results you have got with this compound on the level of the solution of practical problems. That is my first question. Then the second: you have described so many interesting substances, but of course looking at the structures one sees already the limitations for an eventual practical use. Could you eventually qualify to which of them you would give the best chance to become of practical use in the field.

MARINI-BETTÒLO

Excuse me just a moment Professor Cardani. I want first to clear a general point which has been raised by Dr. Knüsli. The aim of

the present meeting is to study the practical possibilities of plant control through the new unconventional approaches which have been the object mainly of basic research in these years.

To this purpose we need, on this first day, to make a review of the present state of our knowledge on natural products which are involved in insect physiology, so as to discuss tomorrow the practical problems connected with their eventual use in the field for plant protection.

CARDANI

In fact iridomyrmecin was synthetized independently by the group of Korte and that of Sir Robert Robinson; the applications in the field were considered but the results were not sufficient for further experiments. Perhaps it could be possible, modifying the molecule of iridomyrmecin, to obtain better results simplifying the synthesis and lowering the costs. Under these conditions I think further field trials could be made.

CANONICA

Has the mechanism of the toxicity of poderin ever been investigated? Its kind of toxicity seems to recall that of yprite and related vescicators. The toxicity of these compounds is connected with their ability to block sulphydril groups and for this reason it is antagonized by some thiols, for example by dimercaptopropanol. It could be interesting to test the activity of these inhibitors on pederin.

CARDANI

I think the problem is quite interesting but practically impossible to solve because pederin, although a tremendous vescicant, is a very unstable substance. For instance, it is promptly decomposed not only in acidic solution at room temperature, but also in alkaline medium. In very mild conditions it suffers a demethylation losing one of its methoxy-groups. The product obtained in these conditions — named pseudo-pederin, which is also found in the insect body, is less vescicant than pederin itself.

If the alkaline treatment is stronger the substance is split in two

parts at the amidic group level. The two substances so obtained have no vescicant properties. So I think that it can not be possible to modify the structure of pederin in order to avoid the vescicant properties because in this case the pederin itself would be destroyed.

MARINI-BETTÒLO

Thank you Prof. Cardani. I should like to ask you now about the question which has been raised before by Prof. Schildknecht: is there any relation between the formation in ants of iridoids, like iridomyrmecin and that of the iridoids present in plants, not only the oxygenated compounds but also the nitrogenated like actinidine and skytanthine? I cannot forget that the first synthesis of skytanthine (*) was performed with a sample of natural iridomyrmecin provided to us by Professor Pavan.

CARDANI

The formation of iridomyrmecin in the insects and that of the nitrogenated and oxygenated iridoids in plants follow practically the same biogenetic scheme.

MARINI-BETTÒLO

This is not the exact point. I am asking if there is any evidence that the insect may take from the plant an iridoid in order to elaborate its own iridoid?

CARDANI

The insect takes from the plants all the substances necessary to its life and transforms them in its body. But this is not the case of iridomyrmecin: it is well known that insects can synthetise terpenes as citral and citronellal, therefore a conversion of these aldehydes into iridomyrmecin *via* iridoidal is most probable.

(*) CASINOVI G. C., DELLE MONACHE F., MARINI-BETTÒLO G. B., BIANCHI E. B., GARBARINO J. A. «Gazz. Chim. Ital.», *192*, 479 (1962).

WAIN

I am saying that when you get a trail followed by ants you do not get vegetation, there is in fact a pathway with no vegetative growth. It would appear that there is something coming from the insect which is preventing plant growth. And my question is: are these trail substances or any other chemical exuded by the insect, of any use whatever for killing plants, could they be used in the herbicide field?

CARDANI

Many of these trail substances are complex mixtures and surely there is not a single substance responsible for this activity. In many ant trail substances fatty acids are present. The selectivity of the trail substances is due not only to the nature of the acids but also to the quantitative composition of the mixture. With regard to the selectivity of the substance response I would recall the specificity of dodecatrienol for the subterranean termites, which is limited to only one of the geometrical isomers.

BALLIO

I wanted only to stress one point, which is the following: these dialdehydes you have shown have some resemblance with helmintosporal which is a phytotoxic substance quite highly specific. This fact is in agreement with the suggestion made by Professor Wain.

KNÜSLI

Again with respect to these trail substances you described one which is characteristic for termites. Now could you eventually with this material influence the termites in a colony, where so many other trails of natural origin are present, to follow in a preferential way the man-made trail?

CARDANI

Yes, I think that this is a very interesting matter. I believe that all suhstances from the insects are suitable for intervening in any way

for insect control in the way you have just mentioned and also in marking territory.

I think that pederin marks the territory of *Pederus* because we find always the *Pederus* in limited and confined areas, which are always the same. About the possibility of removing to other areas the *Pederus* with the use of pederin we would need the collaboration of the entomologists.

MARINI-BETTÒLO

Thank you Prof. Cardani, I think that just an entomologist or an insect physiologist could answer this question so I am asking Dr. Abo-Khatwa if he has any information about the use of trail substances in the control of termites.

ABO-KHATWA

At ICIPE we have an extensive program on trail pheromones of various termite species; both chemistry and physiology. One interesting problem facing us now is that some termite extracts as well as their synthetic analogues show trail activity under laboratory conditions, but in the field they failed completely to divert the foraging traffic of termites. The termites preferred their own natural trails. Now we are modifying our current trail bioassay to ensure its suitability to field conditions.

ANTI-JUVENILE HORMONES FROM PLANTS: CHEMISTRY AND BIOLOGICAL ACTIVITY

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Juvenile hormone analogs are effective in insect control primarily by interfering with adult development during the ultimate stages of metamorphosis, when the natural juvenile hormones (JH) must be absent. During the immature and adult stages, however, natural juvenile hormones are continuously secreted and therefore these stages cannot be controlled by treatment with juvenile hormone analogs. Since juvenile hormones are necessary during these stages the most effective endocrinologic method of insect control would be through the use of anti-juvenile hormones or juvenile hormone antagonists.

With this in mind I began searching for anti-juvenile hormones several years ago. Since we had isolated and identified a compound with juvenile hormone activity from the balsam fir and others had discovered numerous phytoecdysones in plants I wondered if plants might have developed chemical protections based upon anti-hormonal compounds. I developed assays which exposed immature insects to plant lipid extractives for a major portion of their immature life and hoped to find precocious metamorphosis. In addition, I exposed adult insects to plant extracts in anticipation of anti-gonadotropic activities.

These efforts were rewarded by the discovery of two compounds active in each of the above assays. The compounds were

extracted from the common bedding plant, *Ageratum houstonianum* and identified as 7-methoxy and 6,7-dimethoxy-2,2-dimethyl-chromene. In view of their induction of precocious metamorphosis I have named them precocene 1 and 2 (Fig. 1). By contact in a petri dish with the precocenes immature Hemiptera are induced to undergo precocious metamorphosis (Fig. 2). The resulting precocious adult females are sterile and the adult males are unable to inseminate normal females. Treatment of newly emerged female cotton stainers

FIG. 1 — Naturally-occurring anti-juvenile hormones; Precocene 1 (left) and Precocene 2 isolated from *Ageratum houstonianum*.

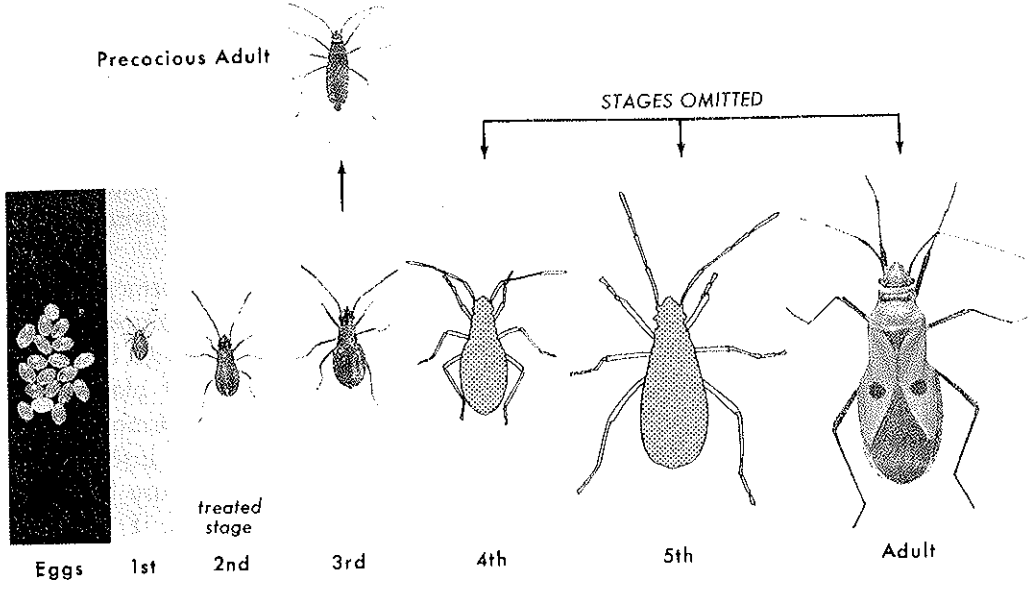


FIG. 2 — Precocene treatment of a 2nd stage cotton stainer nymph is followed by the development of a normal 3rd stage nymph which then molts into a precocious adult.

or milkweed bugs with precocene causes permanent sterility as shown in Fig. 3; however application of exogenous JH III to a sterilized female results in prompt ovarian development. These latter results show that precocene acts to turn off the secretion of JH rather than interfering with JH action at the tissue site.

We have been able to sterilize several holometabolous insects

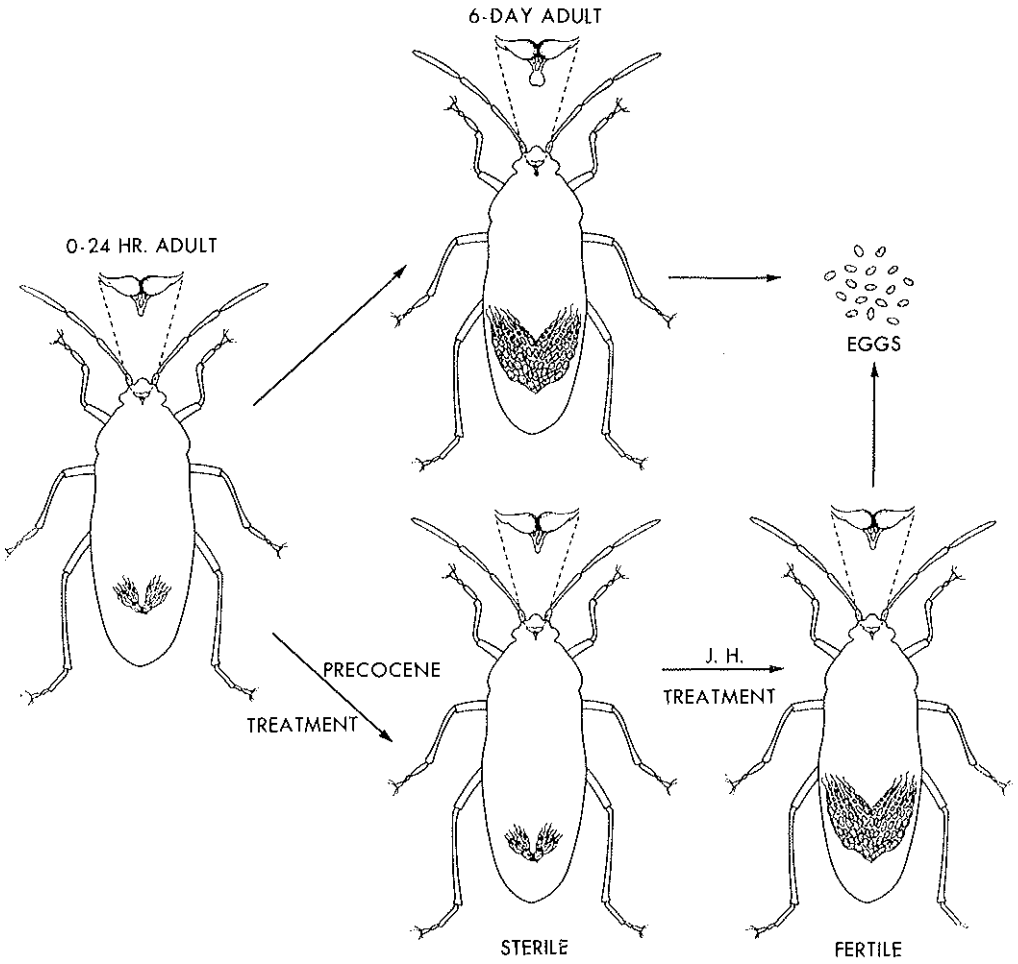


FIG. 3 — Action of precoces on ovarian development in Hemiptera.

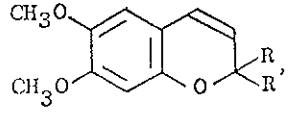
such as the Mexican bean beetle and the Colorado potato beetle, but have not been able to induce precocious metamorphosis in holometabolous larvae. Perhaps the control of allatal secretion in holometabolous larvae differs from that of paurometabolous insects and is not susceptible to the precocenes.

Chemical Structure Optimization Studies

We have investigated the possibility of optimizing anti-JH activity through the synthesis of several precocene analogs. In Table 1 we substituted several different alkyl groups for the dimethyl substituents in the two positions. Only 2-methyl, 2-ethyl substitutions induced precocious metamorphosis in the milkweed bug although at a higher concentration. Sterilization, however, occurred with all but the diethyl substitutions. Outside of the 6,7-position, all dimethoxy substitutions of the aromatic ring (Table 2) were inactive, as was 6,7-dimethyl substitution. Table 3 details mono-methoxyl sub-

Table 1 - *Structure Optimization - 2,2-alkyl substitution.*

Substituted 6,7-dimethoxychromenes	Precocious Metamorphosis 3.9	1.9 μg/cm²	Sterilization
	%	%	
R = R' = H	0	0	(+)
R = H, R' = CH_3	0	0	(+)
R = R' = CH_3	—	100	(+)
R = CH_3, R' = CH_2CH_3	50	0	(+)
R = R' = CH_2CH_3	0	0	(—)



[6] II, 5 - *Bowers* - p. 4

TABLE 2 - *Structure Optimization - Aromatic ring substitution.*

Substituted 2,2-dimethylchromenes	Precocious Metamorphosis 3.9 %	1.9 µg/cm² %
5,6-dimethoxy	0	0
5,7-dimethoxy	0	0
6,7-dimethoxy	100	100
7,8-dimethoxy	0	0
6,7-dimethyl	0	0

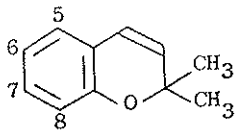
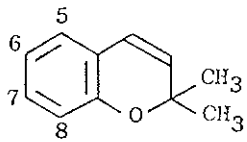


TABLE 3 - *Structure Optimization - Mono-methoxyl substitution.*

Substituted 2,2-dimethylchromenes	Precocious Metamorphosis 7.8 %	3.9 µg/cm² %
5-methoxy	0	0
6-methoxy	0	0
7-methoxy	100	60
7-methoxy	0	0

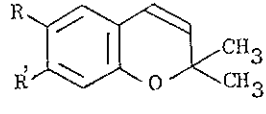


[6] II, 5 - *Bowers* - p. 5

stitution in all positions on the aromatic ring. Only the 7-methoxy derivative (equivalent to the naturally occurring precocene 1) induced precocious metamorphosis. The most important position for substitution in the aromatic ring is the 7 position while 6,7-substitution results in higher specific activity. The next step was to determine whether substitution at the 7 position with more complex ethers would be successful (Table 4). We also examined the effect of halogen substitution in the six position. Several ethers were active even a glycol ether, but propargyl ether even at high concentrations failed to induce precocious metamorphosis, although it was effective in sterilization. Bromine substitution in the 6 position decreased activity. In Table 5 we compare the activity of several simple ethers and find that the most active compound is the 6-methoxy-7-ethoxy-2,2-dimethyl chromene. Finally, we evaluated several dichromenes in the precocious metamorphosis test, and show

TABLE 4 - *Structure Optimization - Interaction of alkoxy substitution in position 7 with hydrogen or a bromine substitution at position* 6.

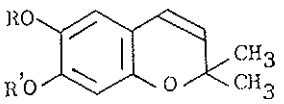
Precocious Metamorphosis 2.9 μg/cm²	Sterilization	Substituted 2,2-dimethylchromenes
	%	%
R = Br, R' = OCH_3	0	(—)
R = Br, R' = OCH_2CH_3	15	(+)
R = H, R' = OCH_3	60	(+)
R = H, R' = OCH_2CH_3	100	(+)
R = H, R' = $O(CH_2)_2OCH_2CH_3$	100	(+)
R = H, R' = $OCH_2C \equiv CH$	0	(+)



[6] II, 5 - *Bowers* - p. 6

TABLE 5 - *Structure Optimization - Interaction of alkoxy substitution in positions 6 and 7.*

Substituted 2,2-dimethylchromenes	Precocious Metamorphosis 3.9	1.9	0.8	0.4 μg/cm²
	%	%	%	%
R = R' = Me	—	100	90	15
R = R' = Et	—	100	50	50
R = Et, R' = Me	—	100	80	5
R = i—Pr, R' = Me	0	0	0	—
R = Me, R' = Et	—	—	—	100
R = Me, R' = i—Pr	—	—	100	80
R = Me, R' = Pr	100	50	0	0
R = Me, R' = Bu	50	0	0	0
R = Me, R' = Hex	15	0	0	0

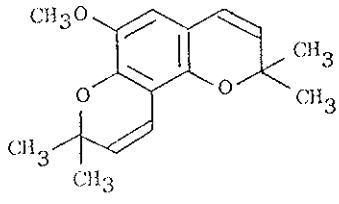
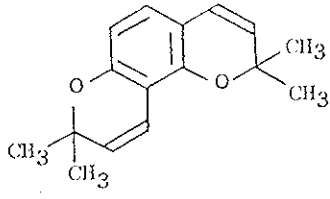
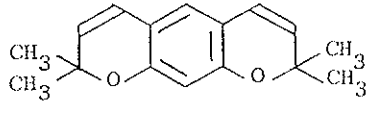


the activity in Table 6. It is seen in Table 5 that several simple analogs are more active than precocene 2, which is the most active of the two natural anti-JH's.

MODE OF ACTION OF THE PRECOCENES

Fundamental to our development of anti-juvenile hormones as probes for the study of insect physiology and as candidate insecticides is the need to understand the mode of action of the precocenes. In many ways the study of how precocenes work compliments our studies on the regulation of juvenile hormone secretion. Since al-

TABLE 6 - *Structure Optimization - Activity of Di-chromenes.*

Di-chromenes	Precocious Metamorphosis 3.9	1.9	0.8 µg/cm²
		%	%
	—	100	75
	—	45	0
	—	20	0
precocene II	—	100	90

latal volume has been associated with JH secretion we removed the corpora allata from female milkweed bugs for nine successive days following eclosion. We found (Fig. 4A) that the allatal volume began to increase on the 2nd day and continued until the 9th day. Treatment with precocene on day one (B) resulted in no allatal development. The application of exogenous JH III (C) did not affect allatal volume. Treatment of normal 5-day-old insects (D) with precocene 2 stopped allatal development and caused a slow regression in the allatal volume. Clearly precocene 2 could prevent or stop and reverse allatal development. Evidence of JH secretion

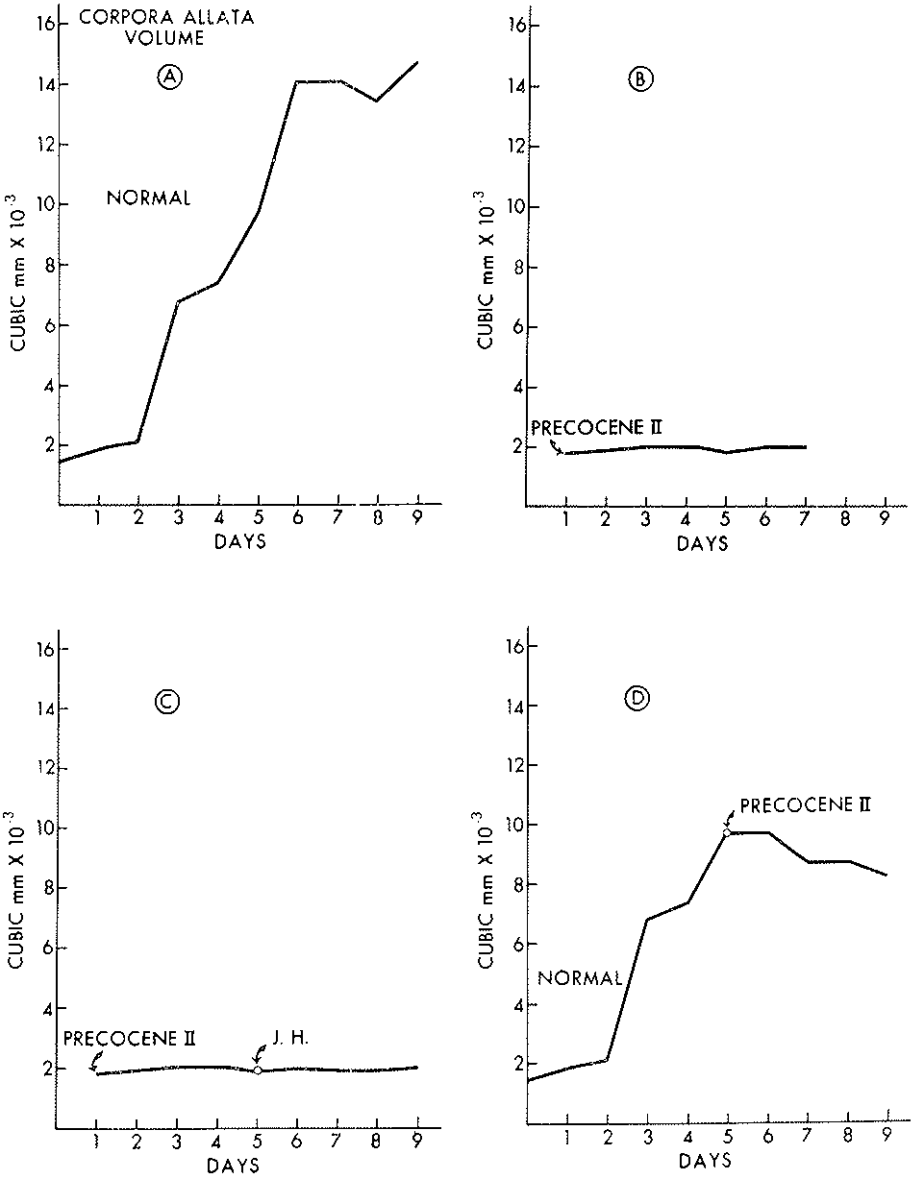


FIG. 4 — Effect of precocene 2 on allatal volume in female milkweed bugs following adult eclosion. (A) Change in allatal volume in untreated female; (B) allatal volume is unchanged following precocene treatment; (C) the administration of JH III to precocene treated females does not affect the allatal volume; (D) treatment of normal developing females with precocene on day 5 stops allatal development and causes regression.

in female milkweed bugs can be followed by measurement of the ovaries, especially the terminal oocyte length since ovarian growth is strictly dependent upon the secretion of JH. We measured the growth of the terminal oocyte for 9 successive days following eclosion. Fig. 5A illustrates the time course and relative growth of the terminal oocyte. In our laboratory culture the first eggs are laid on day 6. Treatment with precocene results in permanent sterility (B) whereas JH III treatment of precocene sterilized females on day 5 induced prompt oocyte development. This latter experiment gives ample testimony that the oocytes are poised target organs capable of response to JH and are unaffected by precocene directly. Precocene treatment of normal 5-day-old females about to lay eggs caused oocyte resorption and ovarian atrophy. In view of the inhibition of allatal volume caused by precocene shown previously in Fig. 4D, it is obvious that precocene causes allatal regression and stops JH secretion which results in ovarian atrophy.

Organ Culture Studies

In order to understand how precocenes interrupt allatal activity we have developed an *in vitro* culture technique. We find that undeveloped ovaries of one-day-old milkweed bug females cultured in Grace's medium plus five percent bovine serum albumen, ten percent foetal calf serum and containing JH III undergo oocyte development with partial yolking (Fig. 6). The gonadotropic action of JH III in organ culture is quite dramatic and reproducible. While we have no idea what the natural gonadotropic hormone of Hemiptera is, JH III is certainly adequate. We were quite surprised to find that when one-day-old milkweed bug brain corpora cardiaca-corpora allata complexes were cultured with undeveloped ovaries (Fig. 7A) no allatal or ovarian development occurred. When severed from the brain however the corpora allata increased in size and ovarian growth and yolking occurred (Fig. 7B). We have interpreted these results to indicate that the corpora allata are restrained from growth and secretion by neural connection to the brain. Similarly, undeveloped ovaries cultured with intact brain-corpora cardiaca-corpora allata complexes from precocene sterilized females do not develop

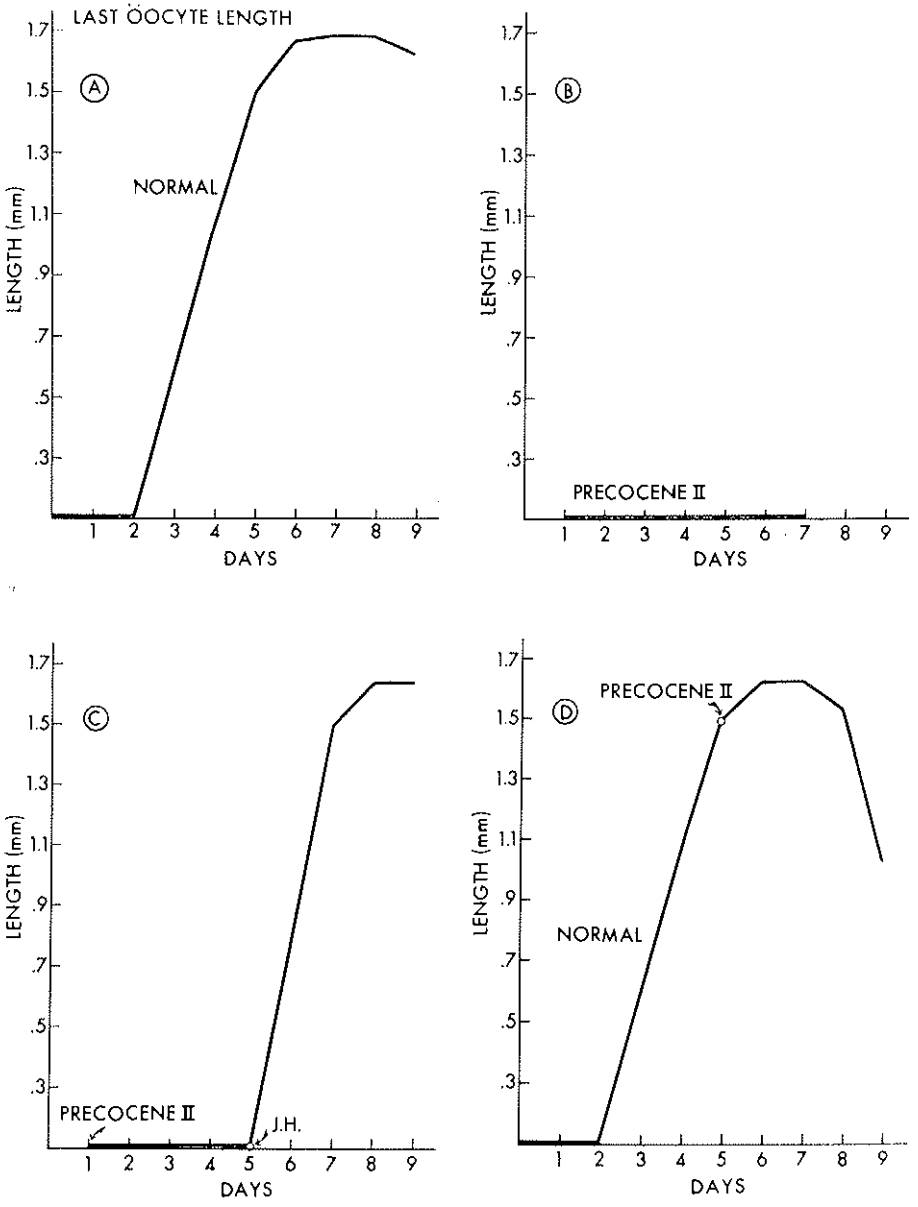


FIG. 5 — Effect of precocene 2 on oocyte growth in female milkweed bugs following adult eclosion. (A) Normal oocyte growth in untreated females; (B) oocyte growth is completely prevented by precocene treatment; (C) treatment of precocene sterilized females with JH III on day 5 causes rapid and complete ovarian development; (D) treatment of normal females on day 5 with precocene stops oocyte growth and promotes regression of the ovaries.

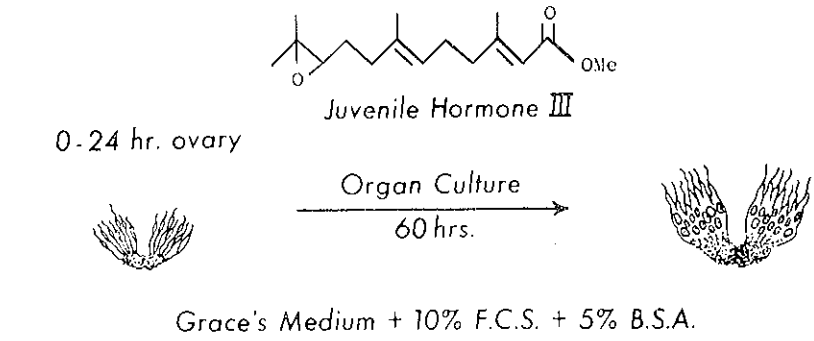


FIG. 6 — Undeveloped ovaries of newly emerged adult female milkweed bugs undergo oocyte growth and yolking when cultured in medium containing JH III.

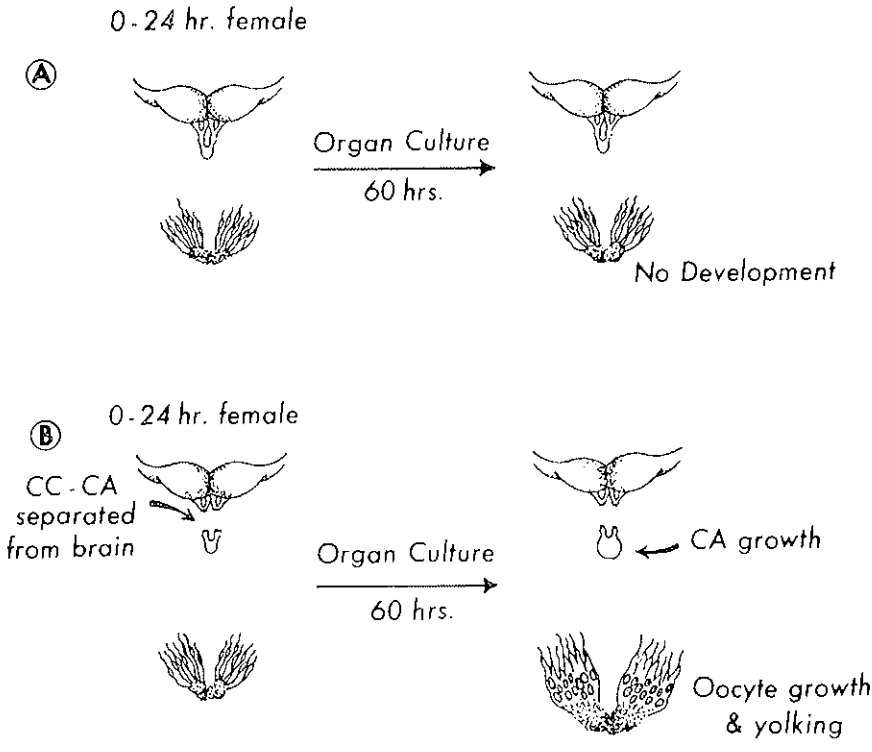


FIG. 7 — Development of the corpora allata and JH secretion are regulated *via* nerve connection between the brain and corpora allata. (A) Organ culture of intact brain-corpora cardiaca-corpora allata complex with undeveloped ovaries shows no allatal growth or ovarian development; (B) when the corpora cardiaca-corpora allata are separated from the brain the allata increase in volume and secrete JH since the oocytes grow and yolking occurs.

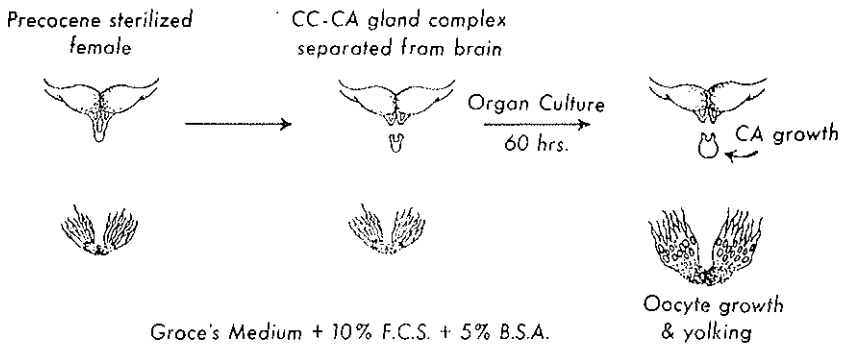


FIG. 8 — Precocene acts upon the brain to inhibt allatal growth and JH secretion. Brain-corpora cardiaca-corpora allata complexes from precocene-treated milkweed bug females fail to stimulate ovarian development in organ culture. Corpora cardiaca-corpora allata complex separated from the brain allows the corpora allata to increase in volume and the oocytes grow and fill with yolk. Precocene apparently acts *via* the brain to inhibit allatal development and JH secretion.

unless the nerve connections between the brain and corpora allata are severed. These results suggest that precocene does not act directly upon the corpora allata, but may influence brain control of allatal development by inhibition of the secretion of an allatotropin (Fig. 8). These experiments are clearly preliminary and much remains to be answered about the mode of action of the precocenes; however brain involvement in allatal development and secretion in the milkweed bug is strongly implicated.

SUMMARY

All of the physiological actions of the precocenes duplicate the effects of surgical ablation of the corpora allata which are the natural source of the juvenile hormones. Further, each of the anti-juvenile hormone actions of the precocenes can be prevented or reversed by the treatment with exogenous juvenile hormones.

In certain insects the precocenes interfere with all stages of insect life and therefore represent potentially important candidates for the development of a new class of safe and selective insecticides.

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BOWERS W. S., OHTA T., CLEERE J. S. and MARSELLA P. A., *Discovery of Insect Anti-Juvenile Hormones in Plants,* « Science », *193*, 542 (1976).

DISCUSSION

KARLSON

May I ask if you use one of the classical bio-assays for juvenile hormones and mix juvenile hormone with precocene, what happens?

BOWERS

Nothing, - you get juvenile hormone activity.

CHAPMAN

I must say I found your talk extremely stimulating. I am afraid I am going to ask some questions on things you probably have not done, that are of interest to me. Could you tell us what are the hemipterous insects you have looked at, and secondly, can you tell us whether *Ageratum* always has precocenes. The point I am getting at is that maybe there are insects that feed on *Ageratum* and that apparently are not affected by it. Now, what happens, again, if you feed in your precocene via the gut? Is this quite different from the sort of approach you take?

BOWERS

Yes, the precocenes fed to a number of insects are effective that way. Of course I explained that we fed them to holometabolous larvae, — cornearworms for example — and we did not get precocious metamorphosis; that is all there is to it. We also looked at precocene metabolism by holometabolous larvae and they tend to be very active in metabolizing the precocenes for one reason or another — much faster than Hemiptera. I have looked at *Ageratum* and indeed there are many insects which do feed upon it. On the other hand, in my experience I have not found any

insects which completed their development on it in nature. I found some wooly bear larvae and a number of other things feeding on them and they will take a few bites, sometimes even eat an entire leaf, but after a time they do not seem to like it and they leave the plant; given any choice at all, they go somewhere else. This has helped me to some extent in trying to develop a philosophy in plant selection. I am having difficulty developing a consistent approach to plant selection. One does not necessarily look in plants that nothing touches. In these plants there must be some repellency or even very toxic compounds which when the insect takes one bite it drops dead immediately. On the other hand there are those plants which may suffer some damage but are never completely destroyed. Insects will try them out and maybe even consume a little bit but then decide that the plant is not so good and then they leave it. I think that maybe these are the kind of plants that we might be more successful in looking at for growth regulators. Let me come back to one of your original questions; how many Hemiptera have we looked at? We have tested the precocenes on about 10 to 12 species including a coreid — one of the big leaf footed bugs. We get precocious metamorphosis and the adults are sterilized. Then of course there is the small brown leafhopper I referred to — but I did not do that work, that was done by Professor Yagi in Japan.

WILLIAMS

Would you repeat the species of that leafhopper?

BOWERS

The species of small brown leafhopper is *Laodelphax streatellus*. We have tried the precocenes on a couple of different Lygus bugs unsuccessfully. We have also looked at precocene metabolism in the Lygus bugs with precocene and it is very rapid. I suspect that is the reason precocene is not effective in Lygus.

CHAPMAN

This is very interesting because your insects that actually took it in via the gut, I think, were not affected. We have done some things

on acridids which also feed on *Ageratum,* and certainly over one generation they are not affected either, which of course does not mean to say there is no effect, but they do very well on it. But there are certainly other plants, which maybe we could talk about, that we have come across that do have adverse effect on acridids, which might be of interest.

STAAL

We have found some interesting effects in Lepidoptera, but we could not get any form of precocious metamorphosis by either feeding or topical applications of precocenes. Precocene II is a rather toxic compound; high doses will invariably kill insects rather unselectively. But there is a dose below this toxic level at which one observes a growth reduction in these lepidopteran larvae. Development is slowed down, but, if one leaves the larvae on the medium long enough, they eventually will develop into pupae after the usual number of molts, although they are smaller than normal. We tried several things: first, we transferred the larvae from the treated medium to untreated medium and found that the observed growth inhibition is reversible; normal development was resumed. We also tried adding, at the same time as precocene, a juvenile hormone analog to the medium. To our surprise we found some rescue effect on the growth inhibition. Therefore inhibition of growth could be indicative of a specific primary anti-juvenile hormone effect of precocene on Lepidoptera.

BOWERS

That mimics very much what we have done. We can slow down development tremendously. The slowdown is reversible by juvenile hormone to a large extent. I have tried to explain it a number of ways. Perhaps we do reduce juvenile hormone a little bit in holometabolous insects with precocene, but it is just not quite enough to cause precocious metamorphosis. If you allatectomize a cockroach it gets along very nicely in a rather chronic condition, but it gets very fat, it is quite sluggish and it is much slower moving. I think it is possible to say that its metabolism has slowed down. Perhaps that is not entirely accurate; at any rate it is a very sluggish insect. On the other hand, when you take certain insects and feed them or treat them with very large quantities

of either juvenile hormone or a very active mimic they become very nervous and they consume much more food. Tenebrio larvae fed on 500 to 1000 parts per million of some analogs can be seen constantly moving in the media. In other words, this large amount of juvenile hormone is acting in a way perhaps like the effect of thyroxine would on an animal. I will not say that JH is a high energy phosphate un-coupler like thyroxine but it does seem to stimulate metabolism. This almost gives us a tissue role — a metabolic role — for juvenile hormone. If we depress the juvenile hormone titer a little in holometabolous larvae, perhaps it results in a slower moving, slower developing, lower metabolic situation, which could be overcome by treating with juvenile hormone, by replacement therapy. I really don't have any better explanation for this phenomenon.

STAAL

In my presentation tomorrow I will indicate that in the substitution assay on *Manduca* larvae there is little or no effect of moderate amounts of juvenile hormone on the rate of development. On the other hand, exceedingly high doses of juvenile hormone analogs do have some sort of a toxic effect, leading to a slowdown in development. I have not noticed the effect on behavior you are describing in this particular lepidopteran species.

DORN

We were fascinated by the work of Professor Bowers and we also did some studies in our lab with precocene on *Oncopeltus*. We often saw that the precocious adults do not have a lot of adult features. They were very similar to larvae. All these precocious adults, however, were incapable to moult again, and we think that this is a most striking feature of these precocious adults. For this reason, my colleague Dr. Masner decided to take a closer look at the prothoracic glands which release the bormone responsible for the preparation and coordination of ecdysis. It was relatively easy to see the prothoracic glands in larvae by anatomical and histological methods, and he tried to find prothoracic glands again in precocious adults. However, in newly hatched precocious adults he could only detect traces of somehow disintegrated cells and 24 hours later even the traces had disappeared. This implies tbat the lack of ecdysone is

the reason for the failure of further development. The specificity of this effect was demonstrated by the injection of 10 micrograms of ecdysone into the precocious adults, and in six cases the synthesis of the new cuticle was induced. We tried to explain all these findings and we think that, as Professor Bowers pointed out, chromene inhibits in some way the formation of the endogenous juvenile hormone and eliminates thus the system limiting the activity of the prothoracic glands. This causes the overproduction of ecdysone, which is manifested by the precocious occurrence of the adult character after the next or the following ecdysis which, worked without juvenile hormone control, disintegrate precociously in a similar way as in adults.

BOWERS

I have not looked at the prothoracic glands, but I am quite sure that Doctor Masner has done a very good job on that. I am not surprised that they do not have prothoracic glands because they do disappear in adults — and these are adults. Only very occasionally have I found precocious Oncopeltus adults with immature characters remaining. This is something which was quite surprising to me when I first heard of it from Doctor Masner and apparently he finds them quite often. We have looked at thousands of precocious adults and have found very few that honestly had immature characters. But that is an honest difference in experience. The precocious adults are in my best judgment genuine adults and the fact that the prothoracic glands disappear I think is quite in line with what we would expect in an adult. As to the explanation of some of the alternate roles of ecdysone and the effect of precocene on the prothoracic gland, I can report an experience of some interest. If we take fourth instar nymphs and treat them with a near toxic dose of precocene in a petri dish (two or three milligrams) and hold them on this throughout the instar they will molt to fifth instar nymphs and then many of them will not molt any more. They stay as nymphs. These nymphs may live and grow quite large but eventually they just die as oversize nymphs. I think if these nymphs were dissected carefully, they would be found to have regressed prothoracic glands. Furthermore, if we treat these nymphs with ecdysone we can get them to molt. Sometimes they molt to adults, sometimes they molt to just intermediate things which die very quickly. They do not look like normal insects. The point is that they are

capable of responding to ecdysone if it is supplied to them. So, in these few circumstances where we produce these blocked insects, I would suspect there has been some effect on the prothoracic gland.

WIGGLESWORTH

In your slide of the premature, precocious adult of *Oncopeltus* I was intrigued by the extraordinary development of the proboscis. Is that a constant feature or just peculiar to that specimen?

BOWERS

No it is a rather usual feature, this very large proboscis in the precocious adults occurs frequently. It is almost as large as in a normal adult.

WIGGLESWORTH

It seemed out of proportion.

BOWERS

It certainly is — there is no question about it. It is just out of proportion in its size as the short wings of the precocious adults are to the body compared to those of a normal adult.

SIDDAL

I should like to ask, Dr. Bowers, have you specifically the evidence for your conclusion that precocenes do act via the brain? My impression was that the glands which were taken from precocene treated adults showed exactly the same phenomena in vitro as those taken from untreated adults. When you severed the brain from the Corpora allata complex, there was again a stimulation of ovarian development — so where is the evidence for action via the brain?

BOWERS

There is no difference really between the two. I did not mean to

imply that this explained everything — this is just the beginning. I am simply showing this technique and what I think we can eventually do with it. We hope we can work out the action of precocene. There are no final answers here as to how precocene works. I am simply showing that the brain-corpora allata complexes from adults treated with precocene must be severed just like the complexes from normal insects for them to produce JH, but that they are capable of producing juvenile hormone in vitro and that the glands themselves are not blocked by it.

MARINI-BETTÒLO

I should like to reassume some of the most important points raised by the reports and the research of Professor Bowers. First, these chromene derivatives are extremely specific. It would be very interesting to know if this specificity is linked to some solubility because I do not know if they have been tested — the hydroxy derivatives and not the methoxy derivatives — if they are less active or non-active at all. Secondly, we have a question of receptors — hormonal receptors — a mechanism which involves certain passages that we do not know completely yet. Moreover, so far as I understood, the precocenes were found in a single plant, although similar substances can be found also in other plants especially in plants which are not insect-attacked. I just raise these points because I think they may lead to other discussions and I should like to have the opinion first of all of Professor Bowers.

BOWERS

I am going to need the blackboard here for a moment. You asked about some of the substitutions on the chromene ring. Actually we learned pretty quickly what is detrimental to activity — what destroys activity. First of all, we must have substitutions in the aromatic ring at positions 6 or 7 and it has to be an ether. It has to be a relatively small ether. If we go much beyond propyl we have lost activity. Substitution is not necessary here in position 6, but if it is substituted by another small alkoxy ether, it increases activity. Now, we have made various substitutions in position 2 and by and large activity is decreased. These 2,2 di-methyls are necessary for optimum activity. There are some substitutions which can be made there but nothing of importance so far. Substitution either in

the 3 or 4 positions is detrimental — it destroys activity. The 3,4 double bond is absolutely necessary — if you take out the double bond you have the chromanes which are exceedingly easy to make, but which are completely inactive in terms of the things we have discussed. The chromanones are inactive — the chromanoles are not active.

MARINI-BETTÒLO

And the open ring?

BOWERS

We have made several open ring compounds of various kinds and...

MARINI-BETTÒLO

Not active?

BOWERS

Not so far. We are hopeful but not so far. We have made flavone type compounds, and they are not active. There are still things that possibly could be done but I think I will leave that to others.

WILLIAMS

You said that normal active corpora allata remain active when cultured in the presence of precocene.

BOWERS

Yes.

WILLIAMS

That suggests that precocenes do not act on the CA to block the synthesis or secretion of JH. How about precocene's action on cultured brain-CA complexes?

BOWERS

Well, if they are joined together they do not produce JH at all in our system. If we have the brain joined to the corpora allata, they are inactive.

WILLIAMS

After precocene treatment?

BOWERS

No, nor even before precocene. If we take them out of a newly-emerged female, untreated with anything, culture them with undeveloped ovaries we found that nothing happened. No JH apparently was produced because the ovaries did not develop. But if we cut the connections between the brain and corpus allatum taken from natural or precocene-treated insects, the corpus allatum produces JH and the ovaries grow. So in that respect the precocene treatment is no different from a normal insect.

WILLIAMS

How does a normal adult female mature its eggs if it cannot secrete?

BOWERS

I cannot tell you, but there is either an allatotropin produced which overcomes the brain's inhibition of the corpus allatum or in the intact insect other stimuli may release the corpora allata from this inhibition and allow JH secretion. In organ culture there would be none of the stimuli of feeding, mating etc.

WILLIAMS

Maybe the brain can turn off the CA by the hypothetical neurosecretion we have called allatoinhibin. And maybe this action prevails under the culture condition you have utilized.

BOWERS

Okay. Now allatoinhibin comes from...

WILLIAMS

The brain.

BOWERS

Now are you saying it comes down the tracks or is produced humorably?

WILLIAMS

Down the tracks.

BOWERS

Okay.

SIDDAL

I would like to make one comment. The timing activity of corpora allata may be critically important. You mentioned that you took your ovaries from a newly emerged female and it is quite possible that not until some days after adult emergence did the corpora allata normally become active.

BOWERS

We use ovaries from the newly-emerged females just because they are small. They begin developing by the middle of the second day. So we want to get them completely at the start but you can put singular active corpora allata from an older insect in with these ovaries, and they grow almost immediately or you can add synthetic hormone to the cultures of ovaries and they start growth immediately. They are perfectly capable of a response.

SIDDAL

Is it possible that the brain-corpora allata complex is not active in the newly emerged female. Is not this possible: that it requires perhaps one day of adult development?

BOWERS

Excellent question. If we take out the brain corpora allata complex from a six-day female which has already produced eggs, we still cannot get much growth at all in the ovaries. The brain corpora allata complex must stop producing JH, but if we cut the connections between the brain and corpus allatum they begin producing JH again.

ABO-KHATWA

Is there any evidence or is it possible that insects themselves do produce anti-juvenile hormone and they act — maybe by feedback inhibition mechanism? The second part is — if this is true that juvenile hormone is acting on a specific receptor site, one could wonder why through the hundreds of juvenile hormone analogs that have been synthesized no one came up with a proposal that some of these compounds are acting through competition as an anti-JH. Maybe this is the time now to look into these already existing materials for anti-JH activity.

BOWERS

I think they have been looked at. Whether all of the JH analogs have been looked at for anti-hormone activity or not, I cannot say, because there are several hundred analogs that have been made. We certainly looked at quite a number of them and found absolutely nothing. Of course we also looked at those obvious compounds that had no juvenile hormone activity in conventional assays. I think this was the hope of a lot of people that out of all of the optimization work done on juvenile hormone, one of them would compete at a tissue site or at some theoretical receptor site and prevent JH action and thus result in an anti-JH effect. I do not know of any success; perhaps Dr. Staal will illuminate this.

STAAL

As a matter of fact, we have been looking for this type of effect for many years and have investigated numerous JH analogs for possible antagonistic properties. This search so far has yielded two distinct groups of JH analogs with JH antagonistic properties. However, the effects are unmistakable but not very spectacular in terms of dose and stage affected. I will describe one of these compounds and its effects to you in my presentation tomorrow.

BERNAYS

Do the precocene doses you used compare with the normal dose that an insect might get if it fed on this plant?

BOWERS

I have no real idea honestly. The one time we measured the amount of precocene in *Ageratum* with some precision I guess — it was something like two tenths percent of the dry weight — that is a reasonable amount of material if the insect would eat very much of *Ageratum*. We have not found any insects that will eat *Ageratum* consistently. The insects we have tried to feed on *Ageratum* generally take a few bites, maybe eat as much as a leaf and go away in disgust. So I cannot really answer your question. However, I do not know how much they might take in if they were to continue to feed on it. If the compound would be absorbed from the gut they could get enough to do some damage. Certainly if we feed them on diets coated with precocene, their development is slowed down. I think this is an indication that it does go through the gut.

SIDDAL

You mentioned that your in vitro culture system was composed of a glass ring, fused to a glass surface with a silicone resin and you also mentioned that your brain gland Corpora allata complex is judged by various stimulation. Have you examined the residue for anti-juvenile hormone component?

BOWERS

We have not done it. This dish was arrived at after some monkeying around with various kinds of vessels. At first we used to do it in the open embryo dish but we found out that the dilution of hormone was considerable in this dish and that is why we went to the small ring. But we still find the same result with or without the silicone seal. We tried epoxy-glue, we tried various other glues, some of the other glues were toxic to the tissues. The silicone seal seemed to be very kind to the tissues. But I think we would get the same results. I cannot tell you about future experiments but most of these relationships were actually determined in other sorts of vessels. We have just standardized our technique using the ring cemented with silicone seal. We never re-use the dishes anyway, so there is no contamination of subsequent tests.

SIDDAL

One brief comment — some years ago we gave up the use of silicone seals because it turned out to be a very good reservoir for greater juvenile hormone than had been made in the culture system so instead we now use a very simple oval glass tube which is just a very small test tube and I do not see from your work so far that it poses any problem.

BOWERS

I appreciate that, I think we have considered it. It is something though that should be considered in our system. Very little of the silicone seal would be in contact with the medium but it is a consideration. To be on the safe side we do not re-use the dishes.

MARINI-BETTÒLO

Are there further comments? If not, I congratulate and thank once more Prof. Bowers and say that natural product chemists must investigate many plants to see if we can find other substances of this type which would explain the mechanism of anti-juvenile hormone activity.

Now we are going to discuss a new aspect of our program. We have so far shifted from the insect hormones to substances which are present in

plants, like the precocenes, but act on the juvenile hormone cycle. Now with the presentation of Dr. Elliott's work we are approaching the study of a classical natural insecticide the pyrethrins.

These substances and all the work dedicated for years to the determination of their structure has formed the basis of the synthetic work developed by Dr. Elliott in the aim of obtaining substances on the model of natural pyrethrins with improved properties of activity and resistance to atmospheric agents.

SYNTHETIC INSECTICIDES DESIGNED FROM THE NATURAL PYRETHRINS

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INTRODUCTION

Chemical insecticides with combinations of favourable properties will certainly be needed for as long as can be predicted, and will be increasingly used in rationally conceived pest management programmes.

Most current insecticides are organophosphates, carbamates or organochlorine compounds. The first two categories, mainly relatively lipophobic materials, act by phosphorylating or carbomoylating acetylcholinesterase, in processes which involve cleavage of the active molecules [1, 2]. Although these classes include very valuable insecticides, the activity attainable, expressed as the median lethal dose in mg per kg of insect body weight, appears limited, despite the wide range of structures explored. Other compounds, such as the organochlorine insecticides and pyrethroids are more lipophilic, and their activity depends on the intact molecule, modification of which, by processes such as hydroxylation or cleavage, usually produces inactive compounds. Of these two categories, the organochlorines have been intensively investigated, in the search for useful compounds for modern regimes of pest control, but most suffer the disadvantages of undue persistence, of diminished effectiveness because resistant strains have developed, and, to some extent, of intrinsically low levels of activity against important insect pest species. In contrast, the natural pyrethrins and related com-

pounds have many desirable features but are too unstable and expensive to contribute much to solving the insect control problems of modern agriculture and silviculture [3]. It is therefore appropriate at this conference on natural products and the protection of plants, to consider how these natural esters may be modified to give compounds of more general practical value whilst their favourable characteristics of low mammalian toxicity, restricted persistence and very high levels of intrinsic activity are retained.

Relation of Insecticidal Activities with Structure

a) *General Principles*

The six insecticidal components of natural pyrethrum (Fig. 1) are all esters of dimethyl cyclopropanecarboxylic acids with either

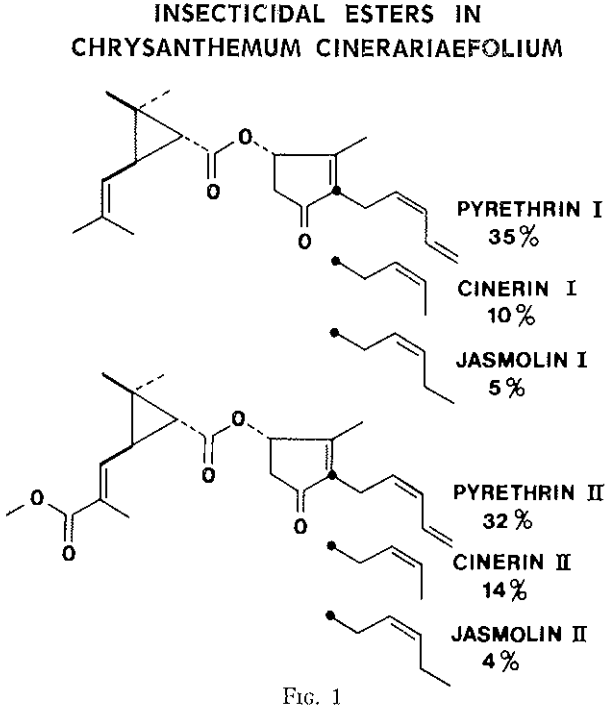


Fig. 1

of two unsaturated side chains in the *trans* position at C-3. The alcoholic constituents are all nearly planar cyclopentenolones with methyl groups and unsaturated side chains at the sides of the ring remote from the hydroxyl substituents [4, 5]. The well known killing power of pyrethrum is due largely to the presence of pyrethrin I and the rapid knock-down action, for which the pyrethrins are widely used, depends chiefly on the presence of pyrethrin II. Pyrethrin I is therefore a suitable prototype from which to assess how structural features determine the activity of these insecticides. Figure 2 shows the structure of pyrethrin I divided into six segments, within which the influence of structural changes can be examined as if independent of changes in the rest of the molecule.

STRUCTURAL VARIATIONS

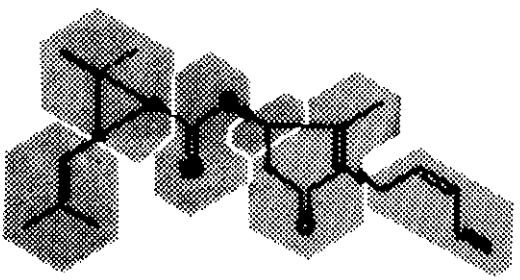


FIG. 2

An important structural component of these compounds where activity is greatly influenced by changes in the substituents is the unsaturated side chain of the cyclopropane acid (Fig. 3). Pyrethrin II, which has a methoxycarbonyl group instead of a methyl group in this segment is more polar and knocks down flying insects rapidly although its toxicity to most insect species is diminished [6]. Other changes which have been investigated are replacing the unsaturated side chain with a dimethyl group, a dichloro group or with a spiro structure. However, the greatest insecticidal activity is obtained, with appropriate alcoholic components, with a *cis*-

STRUCTURAL VARIATIONS — 1

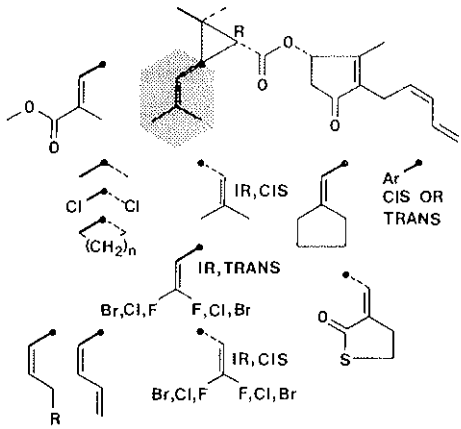


FIG. 3

butadienyl or methylbutadienyl group in the *trans*, but less effectively in the *cis*, configuration. Alkenyl compounds where R is methyl or ethyl are also active, but less so than the butadienyl compounds. An important change which improves the properties of the esters is to introduce dihalovinyl side chains at this site in the molecule, either *cis*-, or *trans*- to the carboxyl function [7, 8]. These compounds will be discussed further below.

If an ethano-bridge is placed on the isobutenyl side chain of natural chrysanthemic acid both insecticidal activity and mammalian toxicity are increased [9]. An important improvement in knockdown activity is obtained, with some alcoholic components, by a thiolactone group *cis*-, but not *trans*-, to the ester function [10].

In the next segment of the molecule (Fig. 4), the *cis*, methyl group is more important than the *trans*. A significant innovation in pyrethroid structures was made by workers in the Sumitomo Chemical Company, Japan [11] who showed that the methyl groups essential for activity need not be supported on a cyclopropane ring but may be present as an isopropyl group on the α-carbon of a phenyl acetic acid with aromatic methyl, halogen or

STRUCTURAL VARIATIONS — 2

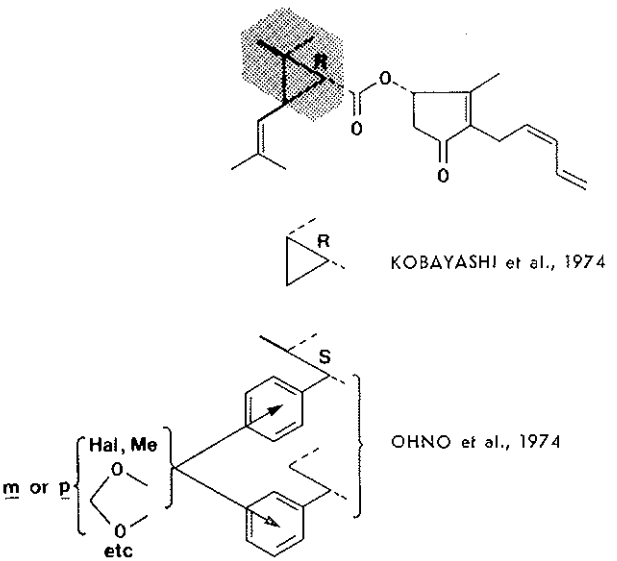


FIG. 4

methylenedioxy substituents, this ring being in a position sterically equivalent to the unsaturated side chain of the cyclopropane acids. This modification gives greatly increased scope for structural diversification in this group of insecticides; the relation of these acyclic compounds to the cyclopropane series is confirmed by the correspondence in absolute stereochemistry of the active components of the diastereoisomeric mixture of esters in both series [12]. Inverting the optical form of the acidic component of either series of esters (Fig. 5) at this central site changes the shape of the molecule and diminishes greatly insecticidal activity, as does substitution of keto, amido, or hydroxyl groups for the ester linkage.

The potency of esters derived from cyclopentenolones (Fig. 6) depends on the absolute configuration at the secondary alcoholic site, but considerable insecticidal activity and low mammalian toxicity is attained in derivatives of various primary alcohols in which a methylene group is present at a position equivalent to that

STRUCTURAL VARIATIONS — 3

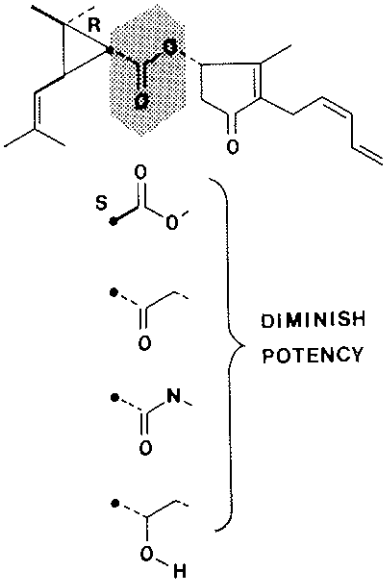


FIG. 5

STRUCTURAL VARIATIONS — 4

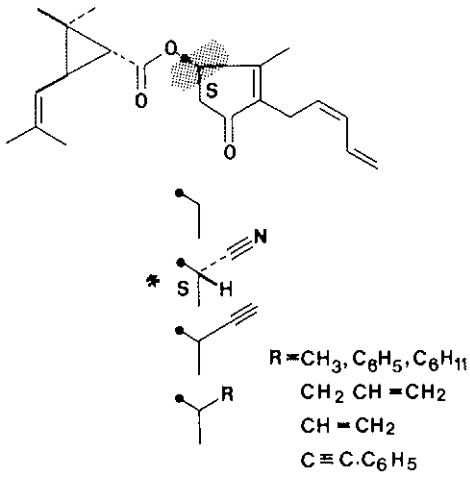


FIG. 6

of the methine site of the cyclopentenolone. With substituted benzyl alcohols, activity is increased by inserting a cyano, (see below) or somewhat less effectively, an acetylene group at this site, in the appropriate steric configuration. Other structures with various substituents (R) have been reported but the level of their insecticidal activity is not clearly defined.

An important requirement for activity is that the alcoholic component of the ester should maintain an unsaturated centre in the appropriate configuration with respect to the rest of the molecule, just as the relative conformation of the acidic side chain depends on the structure of the acid component. Many variations have been investigated, (Fig. 7) including heterocyclic rings, 2,5 and 3,5-disubstituted furans, *m*- and *p*-substituted benzene nuclei and acyclic structures. The greatest insecticidal activity is associat-

STRUCTURAL VARIATIONS — 5

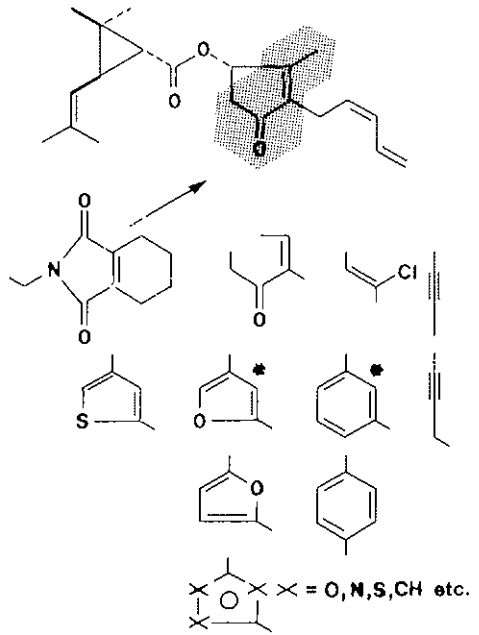


FIG. 7

ed with only a few of these compounds, in particular with 3,5-disubstituted furans, and *m*-substituted phenyls. The most effective non-cyclic compounds are derived from acetylenes and chloro-substituted olefins, but except against an atypical species such as the housefly, these are relatively inactive; the tetrahydrophthalimido-methyl compound is most useful as a knockdown agent against flying insects.

Tbe development of practical compounds related to the natural pyrethrins, but with modified properties, has been considerably furthered by the demonstration that the pentadienyl side chain on the cyclic alcoholic function is not essential for activity (Fig. 8) and that shorter olefinic substituents (e. g. allyl) and especially aromatic rings are effective in this position. Alcoholic components with

STRUCTURAL VARIATIONS — 6

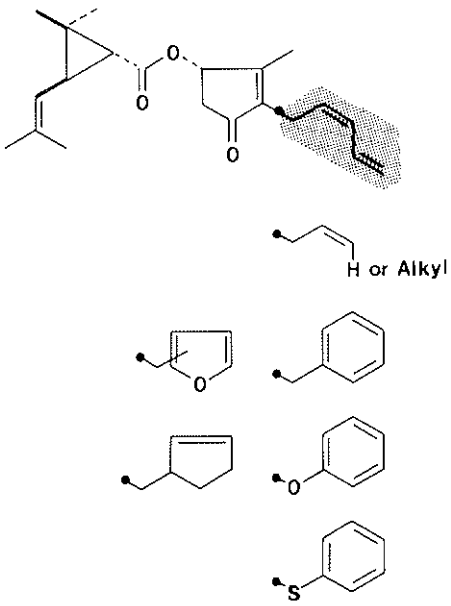


FIG. 8

aromatic side chains are much easier to synthesize than their olefinic equivalents, an important factor in evolving photostable pyrethroids [13].

b) *Specific Examples*

These various structural considerations indicate that a great number of synthetic compounds will have some insecticidal activity. Some of these have already been synthesized, but this brief survey will concentrate on some modifications leading to the greatest insecticidal activity, variations conferring stability, and finally structural changes by which mammalian toxicity may be manipulated.

The biological activity of one form of an asymmetric molecule frequently differs greatly from that of the other. For example, one

optical isomer of allethrin, which has an asymmetric centre at C-4 of the cyclopentenolone ring, is much more active than the other (Fig. 9) [14]. When some esters of (±)-α-cyano-3-phenoxybenzyl alcohol, which has a chiral centre at a position in the molecule equivalent to that in allethrin, were shown to have exceptional insecticidal activity, it was obviously important to discover if either of its two isomers gave the more active esters. Resolution of cyanohydrins by conventional chemical techniques is difficult, because even when the forms have been separated by combination with a suitable chiral agent, racemization may occur in the process of cleaving the resolved components. In this case the desired separation was achieved by esterifying the racemic cyanohydrin with the dibromovinyl analogue of (+)-*cis*-chrysanthemic acid, known from other work to give exceptionally active, and frequently, crystalline esters. One of the two cyanohydrin esters separated in crystalline

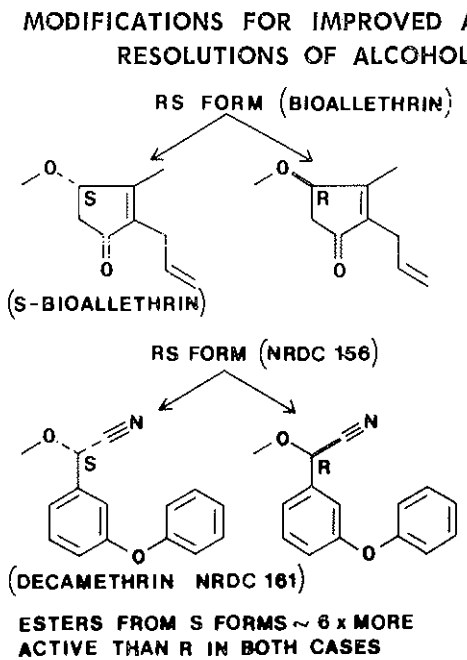


FIG. 9

form (m.p 100°) from the mixture and was at least ten times more active than any previous natural or synthetic pyrethroid. The absolute configuration of the active centre in the cyanohydrin ester was determined in two ways. First 3-phenoxybenzaldehyde cyanohydrin was generated in the presence of D-oxynitrilase, a reaction known from investigations with benzaldehyde to give cyanohydrins (by relation to mandelic acid and thence glyceraldehyde) of the R series. This enzymically produced cyanohydrin gave an ester with the [1R, *cis*] dibromo acid which was much less active than the crystalline compound which was therefore deduced to be derived from the [S] series because their nmr spectra were characteristic and different in the region of the –O.CH.CN proton [15]. Assignment of the crystalline active cyanohydrin to the [S] series was fully confirmed by X-ray analysis by JOHN OWEN of the Molecule Structures Department at Rotbamsted [16].

Figure 10 compares the structure of pyrethrin I, the most active natural ester, and decamethrin, the compound eventually derived from it by progressive systematic modification. Precise bioassays show that the changes have increased insecticidal activity on a moler basis by nine times to *Periplaneta americana*, twelve times to *Phaedon cochleariae*, one hundred and forty times to *Glossina austeni*, one hundred and ninety times to *Anopheles stephensi* and by over eight hundred times to *Musca domestica*.

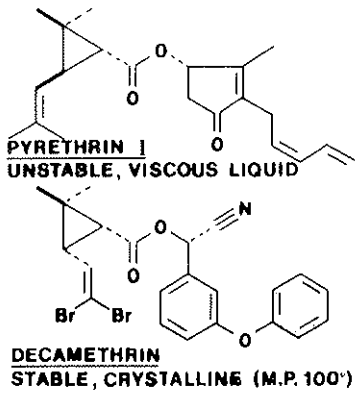


FIG. 10

Relation of Photostability with Structure

Such increases in insecticidal activity alone might not widen the range of application of pyrethroids, but they are accompanied by a gain in photochemical stability. To consider in turn the alcoholic and then the acidic components of the insecticidal esters in this respect, pyrethrin I (alcoholic component shown top left in Figure 11) has at least three centres unstable in the presence of oxygen and light. Insecticidal activity is increased, in the sequence of compounds shown, by replacing the cyclopentenolone ring with furan and the unsaturated aliphatic side chain with a benzyl unit.

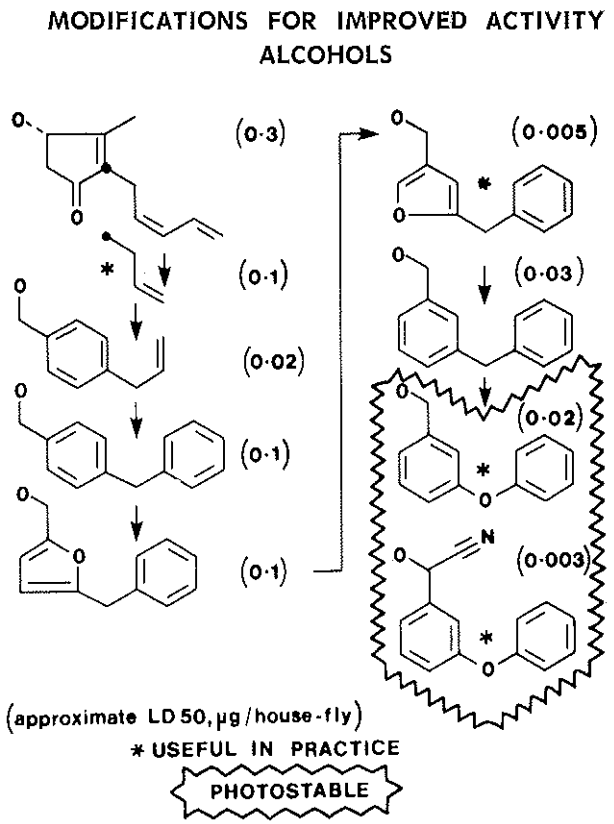


Fig. 11

Yet, despite the greater activity of the 5-benzyl-3-furylmethyl ester (top right), the furan unit is photochemically labile and this compound, bioresmethrin, is not stable enough for any application requiring more than one or two hours persistence in sunlight. The related 3-benzylbenzyl and 3-phenoxybenzyl units however, although somewhat less effective, are stable, as are their α-cyano analogues which are again highly active (see data in parentheses in figure). Photostable replacements for the acidic components have been discovered in a comparable development (Fig. 12). Although some of the modifications shown increased activity, only *cis*- and *trans*-dihalovinyl compounds were both more active and photostable. The parallel development of isopropylphenyl acetates has provided

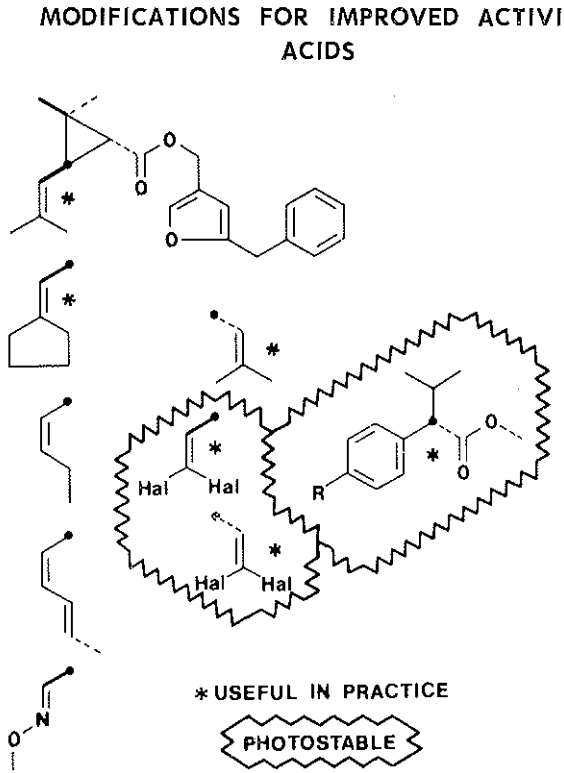


FIG. 12

an alternative series of photostable acids. Pyrethroids constituted from these components have stability in sunlight comparable or superior to that of many of the commonly used organophosphates and carbamates; Fig. 13 shows four compounds at present being assessed for field development.

Relation of Mammalian Toxicity with Structure

The discussion above has illustrated how the insecticidal activity of pyrethroids may be optimised and photostability developed. Low mammalian toxicity is also an important general characteristic of this group of insecticides but amongst the many different structures and stereoisomers now available, wide variations in toxicity

CANDIDATE PHOTOSTABLE PYRETHROIDS FOR FIELD USE

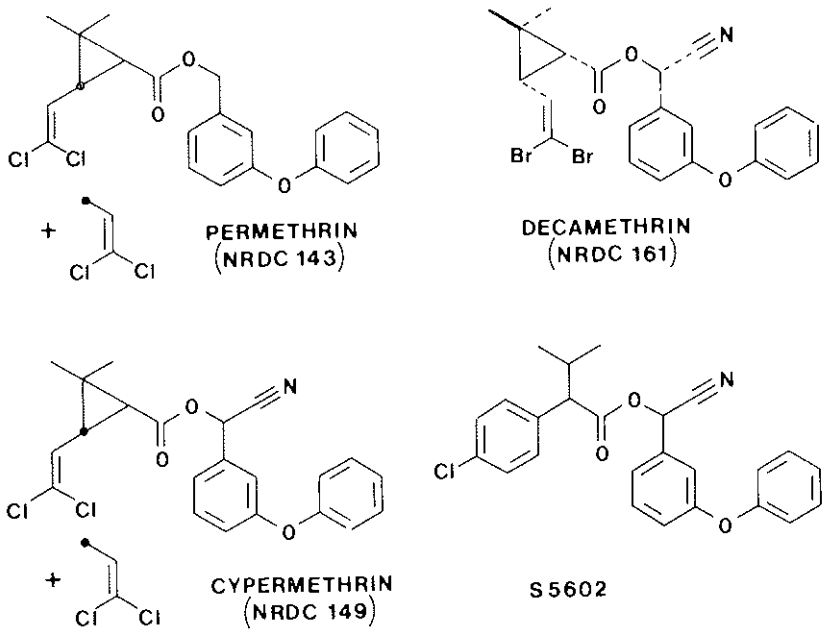


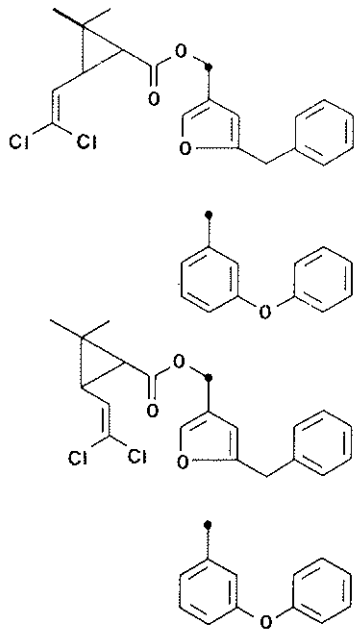
Fig. 13

to mammals are encountered. It is therefore important to consider how this property, too, is influenced by structural changes [17].

Insects are much more susceptible than mammals to the action of pyrethroids, a difference attributed to ability to metabolise and eliminate these compounds before they can penetrate and accumulate at the site of action [18]. The presence of readily oxidised centres and easily cleaved ester bonds may thus favour low mammalian toxicity [19].

Because many pyrethroids have very low oral toxicities it is easiest to demonstrate such effects by comparing intravenous toxicities which are usually higher, as shown by the data in Figure 14 [20].

MODIFICATIONS TO DIMINISH MAMMALIAN TOXICITY
I. OXIDISABLE CENTRES



	INTRAVENOUS* TOXICITY mg. kg⁻¹	ANOPHELES† STEPHENSI ng/female
	26 – 33	0·8
	>270	2·0
	1·4 – 2·8	0·3
	>270	0·8

* Compounds injected neat or in glycerol formal into female rats (BARNES AND VERSCHOYLE)

† LD50 values by topical application (BARLOW AND HADAWAY)

FIG. 14

The 3-phenoxy benzyl esters of both the *cis*- and *trans*-dichlorovinyl acids are considerably less toxic by this route than the 5-benzyl-3-furylmethyl esters, although all four types differ relatively little in their toxicity to insects. This effect may be associated with the greater susceptibility of the 3-phenoxybenzyl esters to mammalian mixed function oxidase detoxification [21, 22]; figure 15 shows comparable differences in mammalian toxicity due to varying sensitivities to ester cleavage [24]. Pyrethrin I, the ester of a secondary alcohol, has relatively high intravenous toxicity, yet bioresmethrin, a more active insecticide and derived from the same acid but a primary alcohol is much less toxic. This alcohol esterified with (+)-*cis*-chrysanthemic acid where the isobutenyl side chain can interfere with ester cleavage is more toxic to mammals. Similarly the ester of the *cis*-dichlorovinyl acid with α-cyano-3-

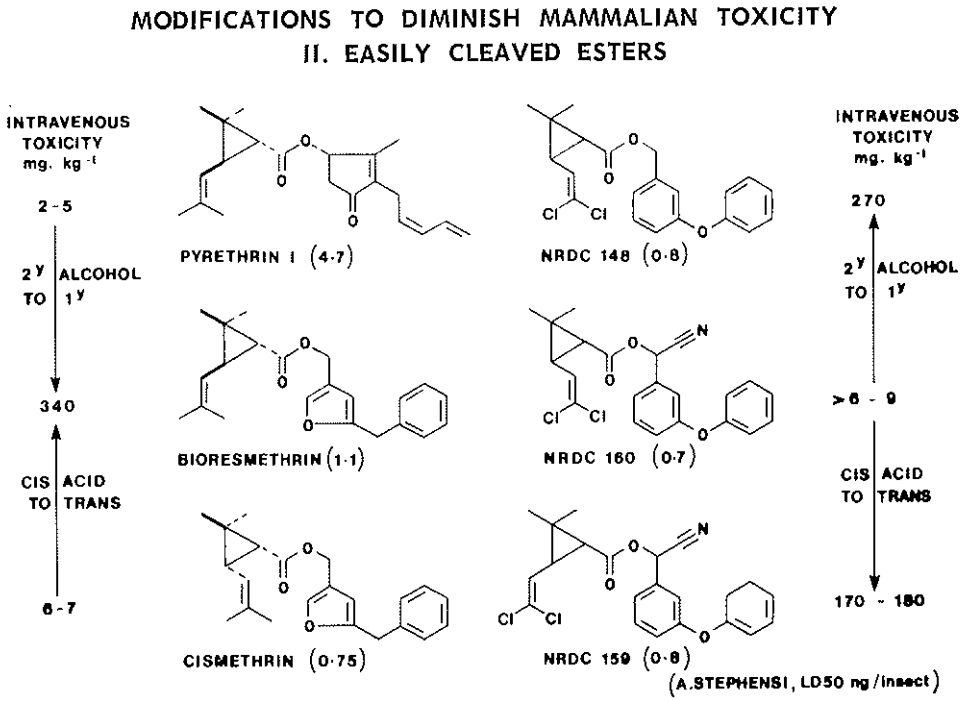


FIG. 15

phenoxybenzyl alcohol is more toxic to rats than two related esters, one with the primary alcohol without an α-cyano group (top right), the other with the *trans* isomer of the acid, in both of which steric hindrance to ester cleavage is diminished. These examples illustrate how mammalian toxicity is affected by structure, for the differences between the insecticidal potencies of the six compounds are relatively small.

Pyrethroids compared with other Insecticides

The discussion so far has indicated how pyrethroids with appropriate activities, stabilities and toxicities to mammals may be designed. To show how such products compare with other insecticides, Table 1 gives toxicities to the locust [25] and to the rat

Tab. 1 — *Relative Toxicities of Insecticides to* Schistocerca Gregaria *(Mature Males) and Rats.*

	Approximate LD50 Values		
	Insects, ug.g^{-1}	*Rats, mg.kg^{-1}*	*Rat/Insect.*
Toxaphene	100+	69	1
DDT	100+	250	2
Chlordane	50	500	10
Carbaryl	31	540	17
Malathion	31	1400	45
DNOC	13	45	4
BHC	9	190	21
Aldrin	8	44	6
Sumithion	6	375	62
Diazinon	5	185	37
Dichlorvos	2	65	33
Parathion	1	11	11
Permethrin	0.7	2000	2900
Decamethrin	0.05*	135	2700

* 2000 × DDT, 20 × Parathion.

of some common insecticides and of two synthetic pyrethroids, stable enough for field use. The ratios in the third column indicate that these two compounds are much safer than the others. Table 2 shows results, adapted from data of BARLOW and HADAWAY [26] and their co-workers which simulate conditions of practical use for endosulphan, dieldrin and these same two pyrethroids. Whereas the deposits of endosulphan and dieldrin at 500g per hectare fail to control insects after only three weeks exposure, permethrin (25g per hectare) still behaves well at six weeks as does decamethrin at 5 and even 1 g per hectare. The pyrethroids are probably not lost significantly by volatilization in wind currents, as are the other two compounds. However, although persistent under these conditions on the waxy leaf surface, preliminary results show that these more stable pyrethroids are rapidly degraded (half lives 15-30 days) in many types of soil, and so active residues should not persist to contaminate the environment.

TAB. 2 — *Residual Toxicity to Tsetse Flies (Glossina Austeni) of Insecticidal Deposits on Ivy Leaves.**

Compound	*Rate* $g.ha^{-1}$	*% Mortality of flies exposed for 1 minute to deposits (age in weeks)*					
		0	1	2	3	4	6
Endosulfan	500	100	92	50	17	—	—
Dieldrin	500	100	88	54	4	—	—
Permethrin	25	96	96	—	—	100	69
Decamethrin	5	100	100	100	—	100	100
	1	95	—	88	—	79	—

* Data adapted from HADAWAY, BARLOW, TURNER and FLOWER (1977).

An important feature of synthetic pyrethroids is therefore the diversity and versatility of their structures, which offers the possibility of synthesising compounds with a range of useful combinations of high insecticidal activity, low mammalian toxicity, and controlled environmental stability.

ACKNOWLEDGMENT

I thank all my colleagues at Rothamsted Experimental Station for sustained collaboration and help in all aspects of this work, which has made this report possible.

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DISCUSSION

MARINI-BETTÒLO

Thank you Dr. Elliott for your excellent presentation of the features of the new pyrethroids and I wish now to open the discussion and ask for comments.

ABO-KHATWA

According to Hansch and his biological correlations of which I am sure you are aware, it is possible to express in quantitative terms the biological activity of any given compound in terms of its lypophilicity, electronic make-up, steric effects and so forth. Have you ever tried to quantitate and thus predict the activity of pyrethrins according to this model?

ELLIOTT

Yes, we have done a considerable amount of work to try to relate insecticidal activity to all the properties — chemical, stereo-chemical and physical — of the compounds we have investigated, and in particular we have measured the octanol-water partition co-efficients, as indications of polarity, for about 300 of the synthetic pyrethroids we have made. Our conclusion is to some extent a disappointing one from the point of view of prediction and correlation following Hansch principles. We consider that there is in general an optimum polarity for insecticidal activity and one, less lipophilic, for knockdown activity. But within quite a broad band of both of these there is a wide variation and for any one particular structural modification for example, the α-cyano-3-phenoxybenzyl compounds, there seems to be an optimum polarity. So that we have found polarity most useful as a guide to indicate the type of compound we should try to synthesize rather than to indicate precisely

what structural features will give the greatest insecticidal activity. The Hansch approach in the simplified form in which we use it indicates that compounds of polarity comparable to the systemic organophosphates and carbamates would be unlikely to have pyrethroid-like insecticidal activity. For example, hydroxyl groups introduced into the molecules seem to produce compounds of such different polarity that they lack insecticidal activity and are rapidly eliminated from mammalian systems, either as the parent compounds or as conjugates. We find this broad type of classification most useful rather than precise correlation with calculated parameters such as Hansch uses. I think the biological target is too complex and too many factors such as detoxification and species specificity are involved for it to be possible to obtain adequate data at this stage in our knowledge for reasonably precise correlations. I look forward to the future when we understand far better the biochemical lesion and can define much more precisely the biochemical system with which we are dealing.

KNUSLI

I think Dr. Elliott and his group really can be congratulated for this beautiful work and I think a real break-through can be claimed in the field of insecticides. As Dr. Elliott mentioned, the field is now wide open for further modification and I think everybody wants to go along such lines. In my group, for example, these is now a big debate between my entomologists: on the one hand they confirm the qualities you mentioned; but on the other hand they claim that these pyrethroids are not selective, that in this respect they are as "antiquated" as Parathion and other P-esters which kill also the predators. And so, we are again involved in the debate which started this morning: what is requested from a new modern insecticide? I think again we will end with a compromise. I also see the possibility that further structural variation may lead to compounds with a more selective behavior.

ELLIOTT

I think the range of structural modifications of pyrethroids is now so great that amongst new compounds we shall find some that are species specific. However, I couldn't agree more strongly that if we could

avoid using potent insecticides in the future this would be the best solution: to control insects without killing them. But I do not think this is a feasible proposition to contemplate within the next 10-15 years or within the period for which we have to design our research programs. My first point was to indicate that I hope the compounds will be used rationally in pest management schemes which would not necessarily involve killing pests and predators indiscriminately. I think this a very important point. Also I think it important to plan for development of resistance to any new synthetic pyrethroids which are introduced because if used indiscriminately I think it must be recognized that many insects will develop resistance to the newly introduced compounds. However, there is an opportunity, before they are used too widely, to study ecological factors and the pest-predator relationship and to use the compounds to the greatest extent possible in a rational approach based on a view of the whole system.

WAIN

I think Dr. Elliott has just answered one of the questions I was going to ask him. The natural pyrethrins I and II possess three well known advantages: firstly they are non toxic to man and animal — or virtually non-toxic —; secondly they can be synergized by various dioxymethylene compounds and thirdly insects do not readily build up resistance towards them.

I would like to ask Dr. Elliott whether his new compounds can be synergized. Secondly in making these pyrethrin type compounds, containing bromine atoms, and so on, we are no longer in the realm of the naturally occurring insecticides and I would like to know how far the insect is capable of developing resistance towards them. I am sure quite a lot of work is being done on this aspect.

I remember in the early days of DDT it took only about one year before the workers at Beltsville in the USA had produced houseflies which had developed a complete resistance to the insecticide.

I remember seeing them walking on DDT crystals in their cages — and showing no effects whatsoever. How far have the resistance studies gone with your new synthetic pyrethroids?

ELLIOTT

The problem of resistance is one which has occupied us at Rothamsted very considerably. In Denmark where every new insecticide was tried in succession against populations of flies invading pig sties, farm buildings, etc., over the years very serious levels of insect resistance have built up, so that flies are resistant to all the known insecticides, even decamethrin. Development of resistance under these circumstances in Denmark has been analyzed by my colleague Roman Sawicki, and he considers that one of the main factors involved in the build-up of resistance to the natural synthetic pyrethroids has been the extensive use of dimethoate and it is from cross resistance induced by various agents that very serious levels of resistance develop. I understand that the house fly is a highly evolved insect, capable, by various mechanisms, of developing resistance to practically any compound with which it comes in contact. Other agricultural pests, I think, are less highly evolved (I stand to be corrected) and in practice resistance has not yet reached the very disastrous levels in Denmark. So I think there is hope with the rational approach to the use of these compounds that for some time resistance can be staved off. This is the only comment I have to make about resistance.

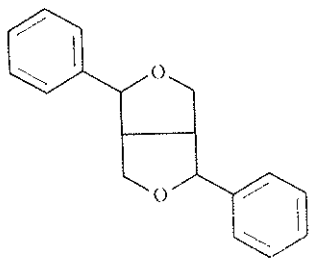
Professor Wain also asked me to discuss synergism of the compounds — a large subject which I deliberately avoided because there was limited time. It is considered that the methylenedioxyphenyl synergists act by suppressing the ability of insects to detoxify compounds. With a special technique of predosing house-flies 2-3 hours before the insecticide with a large enough dose of sesamex — a methylenedioxyphenoxy compound — to suppress all the detoxifying mechanisms, a synergistic factor of perbaps 400 times can be obtained with pyrethrin 1. (A. W. Farnham, Rothamsted). In an exactly similar situation, bioresmethrin can only be synergized 10 to 15 times, but the level of insecticidal activity attained then both by pyrethrin 1 and by bioresmethrin is about the same. So that in bioresmethrin we seem to have a compound less easy to detoxify. Synthetic pyrethroids in general are less well synergized than is the naturally occurring compound pyrethrin 1. Decamethrin is remarkable because with a high level of activity of about 0.03 mg. kg^{-1} LD_{50} can yet be synergized about 15 rimes by this special technique with sesamex so that LD_{50} falls to the level of 0.002 $mg.kg^{-1}$ which makes it a remarkably active compound by any standards. Many syn-

ergists are rather photolabile compounds, difficult to formulate for field use. Further, I think there might be difficulties with the regulatory authorities who would require evidence of safety both with and without synergists. Therefore I think that a number of factors indicate that synergists may be less useful with the more stable agricultural pyrethroids than with the earlier naturally occurring compounds where synergists were particularly effective.

NAKANISHI

Excuse me, I am not too familiar with this field so my things may be quite out of your interest.

One brief comment. I just thought it was rather intriguing that sesamin,



according to preliminary results, acts on the human sickle cell anemia blood and it changes the morphology of the red blood cells.

ELLIOTT

I am very interested indeed to hear that observation.

NAKANISHI

We have not done it in detail, and I am not an haematologist but preliminary results do indicate that.

ELLIOTT

Which is exactly the compound?

NAKANISHI

It is sesamin, a common synergist used in application of pyrethroids.

ELLIOTT

It would obviously be well worthwhile to try the other pyrethroid synergists.

NAKANISHI

On the photostabilization of pyrethroids by using cyclodextrins? Have you experience in this and how does it work?

ELLIOTT

No, but I've talked to Dr. Yamomoto who has developed this application for cyclodextrin. It seems a very valuable advance in formulating the naturally occurring and the unstable synthetic compounds but difficulties appear to be the relative expense of the formulation and the limited level of its insecticidal activity. I think it is most useful where the insecticides are ingested as stomach poisons, for instance when the insect is eating leaves on which the insecticide has been sprayed. Greater stability of insecticidal activity is perhaps better obtained by more stable compounds which will inevitably give a more economical formulation.

NAKANISHI

But the stable ones, on the other hand, may be more toxic too. I mean they are not that biodegradable.

ELLIOTT

Well, the mammalian toxicity of some of the more stable compounds is remarkably low. The oral toxicity of permethrin is 2000 to 3000 mg. kg^{-1} in rats which is a perfectly acceptable level.

NAKANISHI

One other question: I should like to know as you have a substituted cyclopentadienone ring and thus R and S carbons, how have you determined the absolute configuration?

ELLIOTT

In S-bio-allathrin the absolute configuration of allethrolone was determined by Crombie and his collaborators at Nottingham University using the 6-bromo-2,4-dimitrophenylhydrazone in X-ray crystallographic techniques.

BRADER

I was tempted to come back to the question of selectivity, but I think we will do this in our talk later this week. However, I would like to make one point. Dr. Elliott said that part of the speedy resistance development in house flies in Denmark could be explained by the closed surroundings in which the insecticides were used, and he doubted that in agriculture such examples existed on a large scale. I can inform you that there are many examples in agriculture where the pest control situation has completely deteriorated due to the fact that the pests became resistant to almost all chemicals currently used for their control. There is one example here in Northern Italy where in the pear-growing industry *Psylla pyri* became completely resistant. The classical example is the one in the cotton industry in Northern Mexico where a whole area of 150,000 hectares went out of production due to resistance. Another example is hop-growing in Central Europe and in the U. K. where we will probably soon be in serious trouble due to the fact that the spider mite and the Damson hop aphid are becoming completely resistant to most of the chemicals used in their protection. So I do not think that the house fly of Denmark should be too easily seen as an exception or that these cases cannot occur in agriculture.

ELLIOTT

Yes, I'm sure, that's a very valuable comment and my remarks were just a general indication that possibly fewer examples were known

in agricultural practice than those with domestic situations but I'm quite sure your observations are very relevant. Thank you.

MARINI-BETTÒLO

Thank you Dr. Elliott for this discussion and the presentation. Myself I am considering that it is a wonderful example of model of natural substances and tailor-made synthetic derivatives. The only thing I fear is that there has been retained from the original molecule of the natural products only the conformation and the cyclopropane ring and I hope that these substances will not induce resistance, or toxicity for man.

INSECT GROWTH REGULATORS FROM PLANTS

KOJI NAKANISHI
Department of Chemistry
Columbia University - New York, N. Y.

I. *Stereospecific Conversion of Diosgenin to Alpha-ecdysone* [1, 2].

The insect moulting hormones, the ecdysones [3] were found to be widely distributed in the plant kingdom in 1966 [4, 5] and since then more than forty phytoecdysones have been isolated from plant sources [6].

OKAUCHI *et al.* [7] reported that the cocoon spinning of a silk-worm colony can be synchronized when ecdysones are added to the diet (15 mg per 20,000 larvae) at a particular stage during the fifth instar. Extensive field tests carried out since have verified this, and further have shown that simultaneous application of lauryl alcohol repels larvae, automatically moving them towards the nestling area where spinning of high-quality cocoon is assured. If the entire silk industry in Japan were to adopt this new hormonal method, a rougb estimate shows that 40 Kg of ecdysone per annum would be necessary [8].

Diosgenin 2 which is one of the major natural sources for the industrial preparation of steroidal hormones, can be cleaved between C-22 and the six-membered oxygen bond in 95 % yield to give dihydrodiosgenin [9]. Examination of molecular models indicated that the C-22 configuration in dihydrodiosgenin would be R [10], i. e., the same as that in all zoo- and phyto-ecdysones [11]. Hence a reductive cleavage of the C-16/O bond would lead directly in a stereospecific manner to the alpha-ecdysone side-chain.

This has been achieved [1] in 16 steps in an overall yield of ca. 1 % (not optimized) by going through the key intermediate *3* shown in Fig. 1.

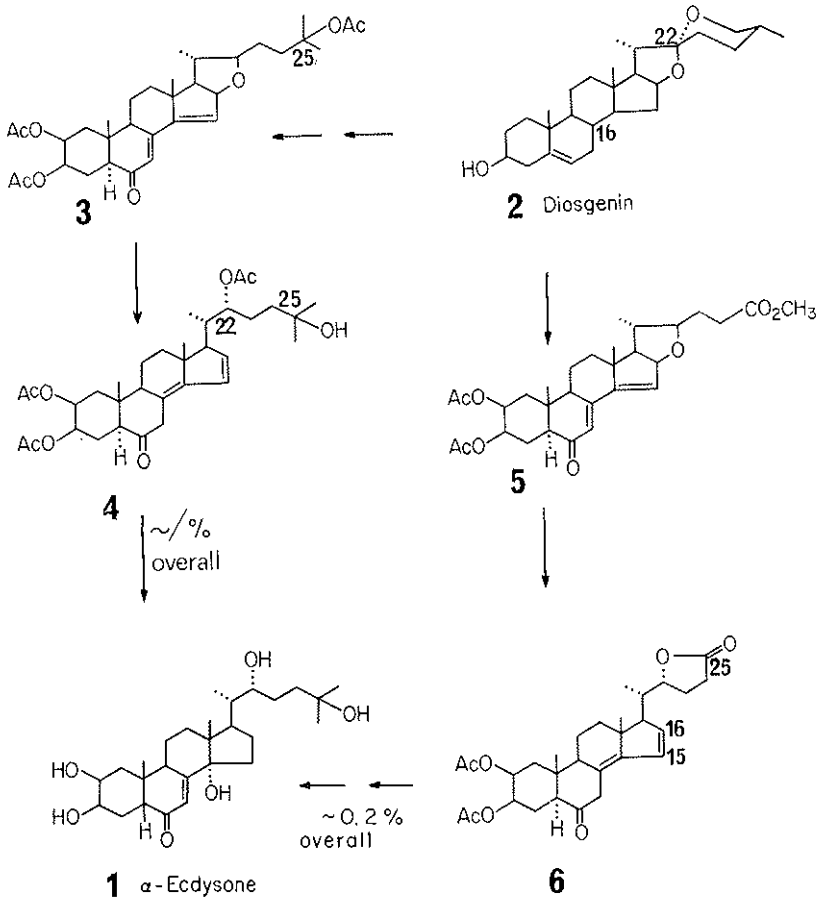


FIG. 1

Treatment of 3 with zinc and acetic acid gave *4*, 53 % yield, in which the 25-OAc had undergone an unexpected but fortunate concomitant migration to C-22 in *4*. A modification of this scheme going through the ester *5* has also been completed [2]. The intermediate *6* allows one to introduce tritiums at C-15 and 16, and also two ^{14}C labelled methyl groups thus leading to double-

labelled ecdysone which would be of significance for metabolic and related studies of ecdysones; the syntheses of these labelled ecdysones are in progress.

II. *Insect Antifeedants*

Antifeedants are defined as substances which when tasted can result in cessation of feeding either temporarily or permanently depending upon potency. Most antifeedants described are those against the African army-worm, *Spodoptera exempta* (monophagous) and *S. littoralis* (polyphagous), widely occurring crop tests. The antifeedants appear to be specific to certain insects; for example those described in the following are only weakly active or non-active against *Manduca sexta* (tested on tomato leaves) and *Schistocerca vaga* (tested on corn seedlings) [12]. On the other hand, preliminary tests have shown that some compounds which are inactive against army worms elicit activity against the Mexican bean beetle, *Epilachna varivestis.*

The plants were collected in Kenya, The International Centre of Insect Physiology and Ecology (ICIPE). Except for azadirachtin, the rest of this topic describes the preliminary results stemming from Dr. Isao Kubo's [13] systematic survey of East African insect antifeedants.

The plant extracts were fractionated by the leaf disk bioassay (Fig. 2) with *Zea mays* (corn) which was found to contain sucrose and adenosine as feeding stimulants [14]. The test consists of dipping 2cm diameter leaves into acetone solutions of fractionated extracts for 2 seconds and giving them to insects with the blank control. If the leaves were soaked for longer periods, the palatability for insects decreases due to extraction of constituents.

III. *Azadirachtin* [15].

Azadirachtin was isolated during attempts to isolate the factor(s) present in leaves of *Azadirachta indica* (Indian neem tree) which induced pronounced morphological changes in *Antestiopsis*

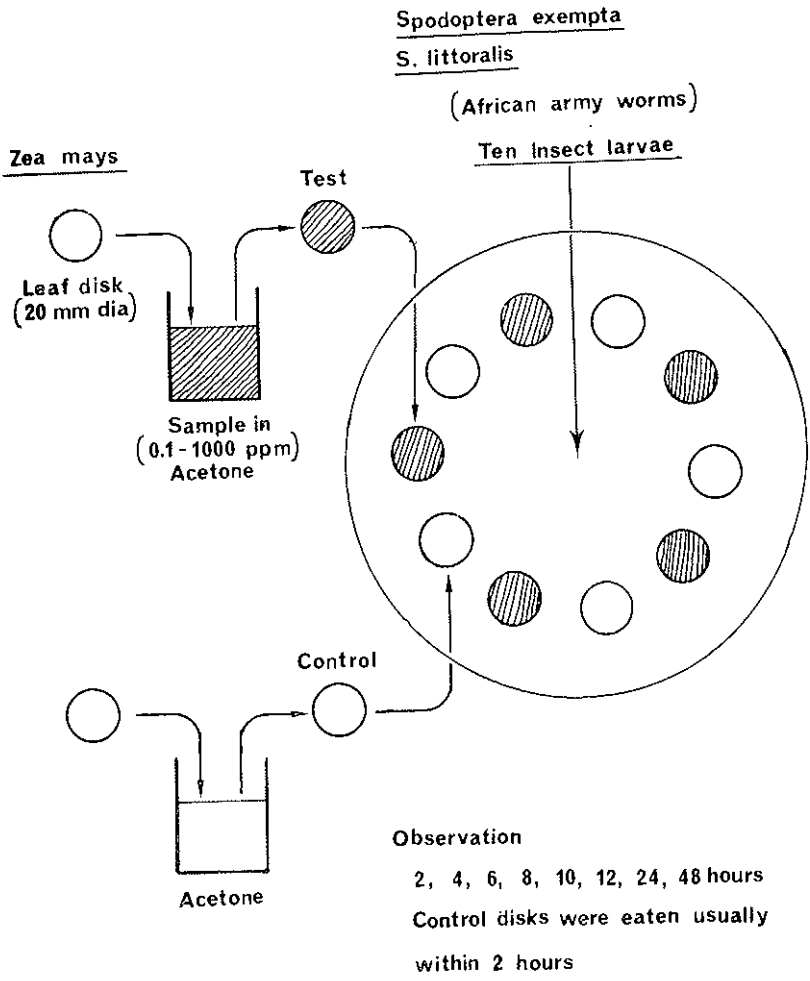
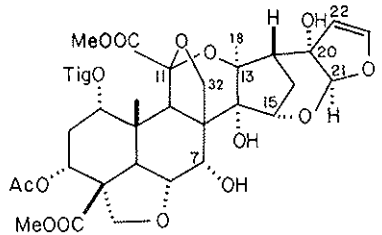

Spodoptera exempta
S. littoralis
(African army worms)
Zea mays
Ten insect larvae
Test
Leaf disk
(20 mm dia)
Sample in
(0.1 - 1000 ppm)
Acetone
Control
Acetone
Observation
2, 4, 6, 8, 10, 12, 24, 48 hours
Control disks were eaten usually
within 2 hours

FIG. 2

(coffee bug). Azadirachtin had already been isolated [16] from *A. indica* and the closely related species, *Melia azedarach*, together with the locust antifeedant meliantriol [17], and was known for its potency against certain insects. Namely, when tested against the desert locust *Schistocerca gregaria*, the limiting concentration to cause 100 % inhibition of feeding is 40 micrograms/liter, or when impregnated onto filter paper 1 nanograms/cm^2 [18]: it is also a systemic growth disruptor [19].

In spite of extensive and clever chemical studies [16c] it was not possible to deduce the entire structure due to its noncrystallinity and great complexity. However, development of the new nmr technique of combining partially relaxed Fourier transform with simultaneous off-resonance decoupling led to the proposed structure 7.



7 azadirachtin

It is one of the most potent antifeedants, although slightly less active than warburganal and its analogs (see below). When locusts are left with leaves topically treated with azadirachtin at the level of few nanograms/cm^2, they starve to death. It is not toxic to birds since the berries are favorite food for local Kenyan and Nigerian birds. A yield of 800 mg is obtained from 300 g of the large fruit seeds. The complexity of its structure precludes synthesis, and therefore if it were to be used at all for insect control purposes, there seems to be no alternative but to grow the tree.

IV. *Ajugarins* [20]

These are three moderately strong antifeedants isolated from the leaves of *Ajuga remota* (Labiatae) which are not attacked by

African army worms. The *ent*-clerodane structures of ajugarins (Ar)-I *8*, -II *9*, and -III *10* are based on chemical and spectral data and represent the absolute configurations as well. It is interesting to note that caryoptin and 3-epicaryoptin which have the antipodal clerodane skeleton have also been reported as being antifeedants [21]. The antifeedant activity level for the ajugarins is 100 ppm and 300 ppm, respectively, against *S. exempta* and *S. littoralis*.

V. *Harrisonin* [22]

The East African shrub *Harrisonia abyssinica* Oliv (Simarubaceae) ("Msabubini" or "Mpapura-doko" in Swahili) is widely used in various folk remedies. The chopped root bark (650 g) of the shrub collected near Mombasa gave after work-up ca. 70 mg of harrisonin *11* and 25 mg of obacunone [23], both in crystalline form. The nature of all 27 carbon atoms in harrisonin was clarified by proton-noise decoupled, undecoupled, and partially relaxed Fourier trans-from cmr spectra, the chemical shifts of which are shown in structure *11*. Comparisons of cmr data with the known obacunone *12* and other spectral measurements including clarification of the CD curve led to the structure shown. The unusual hydroxy-hemiketal ketone moiety may be an artifact derived from a hydroxy diketone grouping during extraction or it may exist as such in nature.

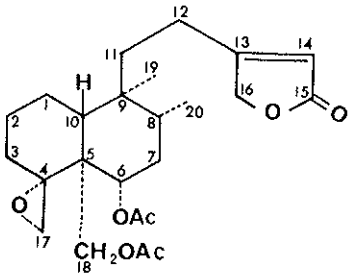
The extraction of the root bark was monitored both by anti-feedant and antibiotic activity (gram-positive microorganisms) tests. Both bioassays led to the same compound, harrisonin *11*, the final activity being 20 ppm for antifeeding (quite potent) and 5 μg/ml against *Bacillus subtilis*. In addition it was found that harrisonin exhibits cytotoxicity at the level of 2.2 μg/ml (KB test).

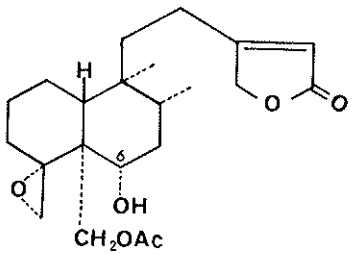
VI. *Xylomolin* [24]

During the course of studies on *Xylocarpus molluscensis* (Meliaceae), we had occasions to study its unripe and bitter fruits. The fruits each weighing ca. 200 g are used as aphrodisiacs when unripe,

Ajuga remota (Labiatae)

AJUGARIN Insect antifeedants (locust, army worms)





8 Ar-I $C_{24}H_{34}O_7$

m.p 155 157°

CI-MASS: CH_4 435(M+1)
i-Bu, 435(M+1)

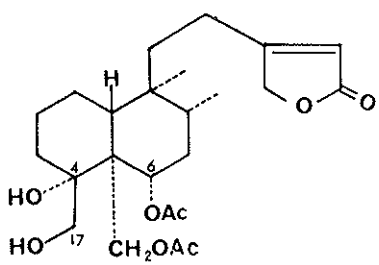
EI-MASS: M-30

IR(KBr): 1780, 1750, 1730 cm^{-1}

UV(MeOH): 212 nm(ε~10,000)

9 Ar-II

IR: 3400 cm^{-1}

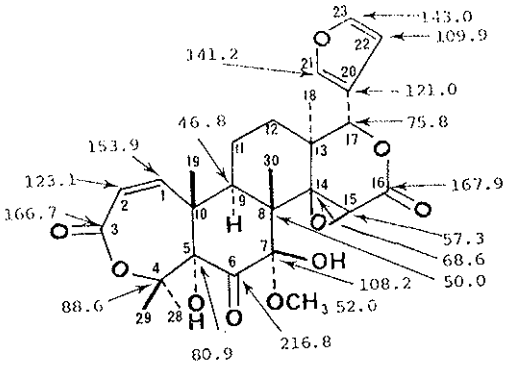


10 Ar-III

m.p 243-245°C

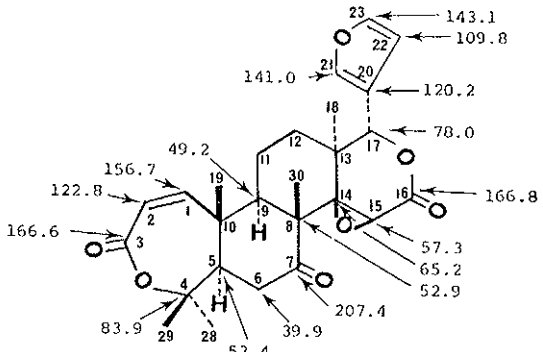
IR: 3400, 1780, 1735 cm^{-1}

FIG. 3



Harrisonin **11**

:cmr data in $CDCl_3$
$C_{27}H_{32}O_{10}$, m.p. 155-156°
CI(isobutane)/MS: 517(MH^+)
uv(CH_3OH): end
ir($CHCl_3$): 3490(intra. H-bond),1760(sh)
1741,1709,1627(C=C),875(furan)



Obacunone **12**

:cmr data in $CDCl_3$
$C_{26}H_{30}O_7$, m.p. 226-228°
CI(isobutane)/MS: 455(MH^+)
uv(CH_3OH): 288(ε 1,363)
ir($CHCl_3$): 1747(sh),1735,1717(sh),170
1620(C=C),880(furan)

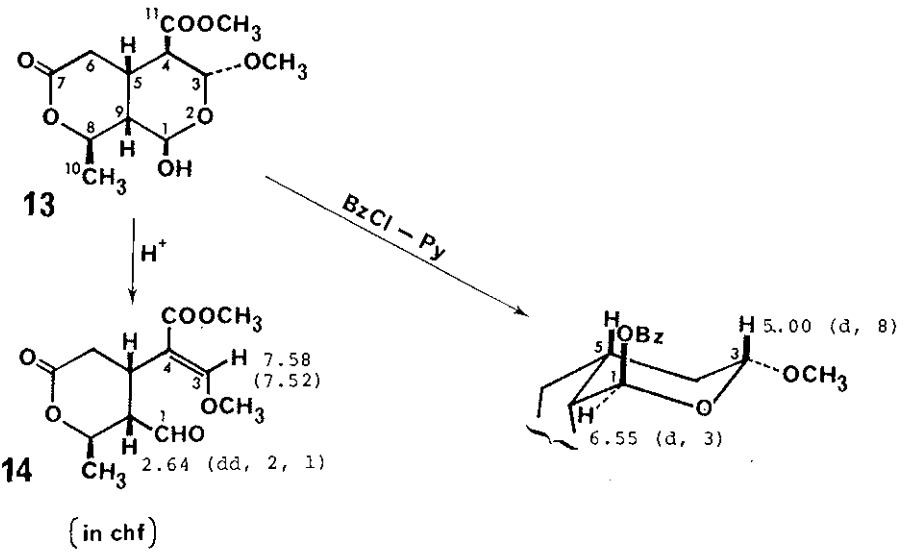
FIG. 4

Xylocarpus molluscensis (Meliaceae)

Xylomollin, $C_{12}H_{18}O_7$, mp 138-139°

High-res. EI : 275 ($M^+ + 1$), 243 (275 - MeOH)

IR (chf) : 3600, 3400, 1734, 1720 cm^{-1}



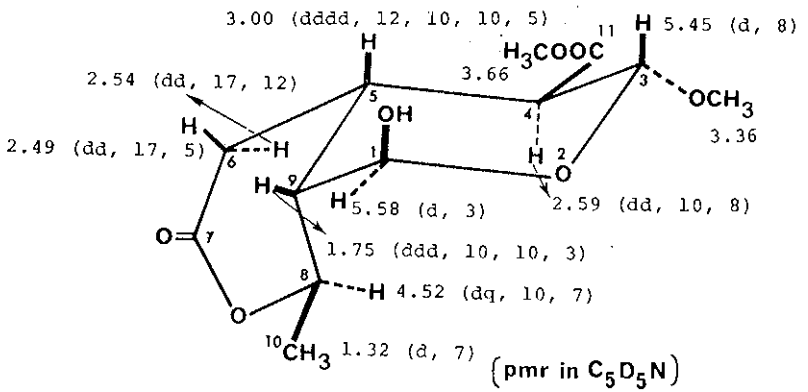


FIG. 5

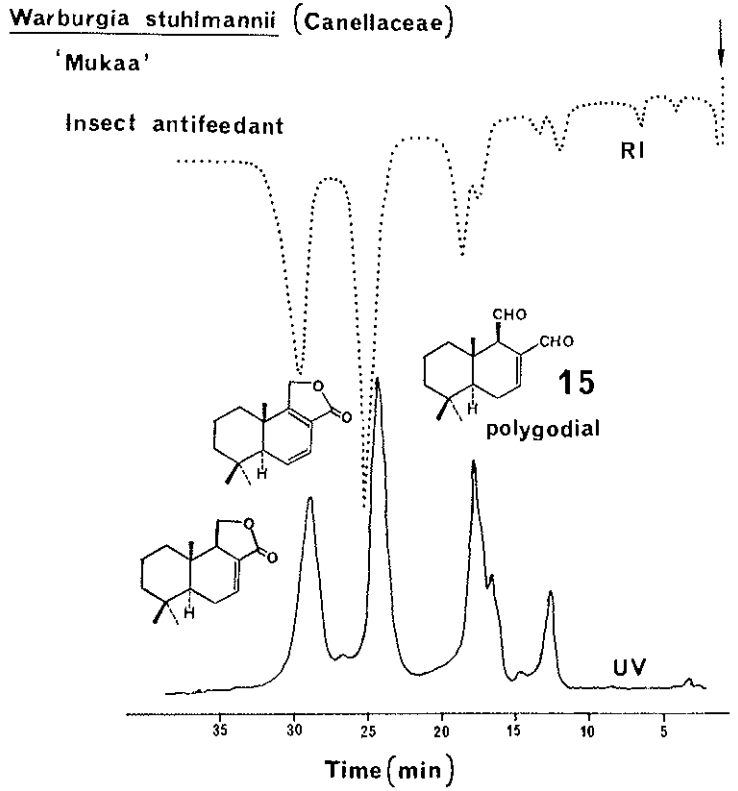
but with ripening the bitterness is rapidly lost and the fruits become edible. From the unripe fruits has been isolated xylomolin, which besides being an antifeedant, strongly inhibits the respiratory reactions of mitochondria from rat liver (N. ABO-KHATWA and I. KUBO, to be published). Spectral data led to the unusual monoterpenoid secoiridoid structure *13* having a non glycosidic hemiacetal function at C-1 and an acetal function at C-3. Xylomolin was readily obtained by extraction of the fruit flesh with aqueous metbanol, removal of methanol, extraction of residual aqueous concentrate with ether and concentration; yield 0.1 % of wet weight. Repeated chromatography of the ethereal mother liquid of the extract afforded numerous compounds, the major product being the enol etber aldehydes *14*, mixture of cis and trans 3-ene isomers. The mixture was also obtained by a room temperature acid treatment of xylomolin in 100% yield.

It is quite conceivable that xylomolin is a key intermediate subsequent to secologanin in the biosynthesis of indole alkaloids.

VII. *Polygodial, warburganal and ugendensidial* [25]

The East African genus *Warburgia* (Canellaceae) consists of two species, *W. stuhlmannii* and *W. ugandensis*, the barks of which are employed widely in folk medicine and as spices in food. The extraction of ground barks of *W. stuhlmannii* led to a syrup possessing strong antifeeding activity. The mixture was submitted to several high-pressure chromatography separations (Fig. 6) to finally yield the two lactones [26] (inactive) and polygodial *15* [27]. Similarly, *W. ugandensis* barks afforded in addition to the two lactones, warburganal *16* (new compound) and ugandensidial *17* [28]. (See Fig. 7).

These series of sesquiterpenes exhibited very potent activity, the level being 0.1 ppm against army worms; however, the antifeedant level was nonsignificant against *M. sexta* and *S. vaga* [12]. Electrophysiology studies using the army worm sensilla [29] showed that the antifeedant activity was suppressed upon addition of equimoral quantities of L-cysteine to the test solution. In addition the derivatives shown in the middle and right columns of Fig. 7 were all devoid of activity. These results together with the inhibitory action of L-cysteine suggest that the enal moiety acts as a nucleophile (SH) acceptor,



μ-Bondapak-C_{18} 4mm I.D. x 30 cm. 2 ml/min
3500 psi 55:45 MeOH:H_2O λ = 255 nm
Waters 6000 psi pump
Schoeffel variable uv detector with RI detector

FIG. 6

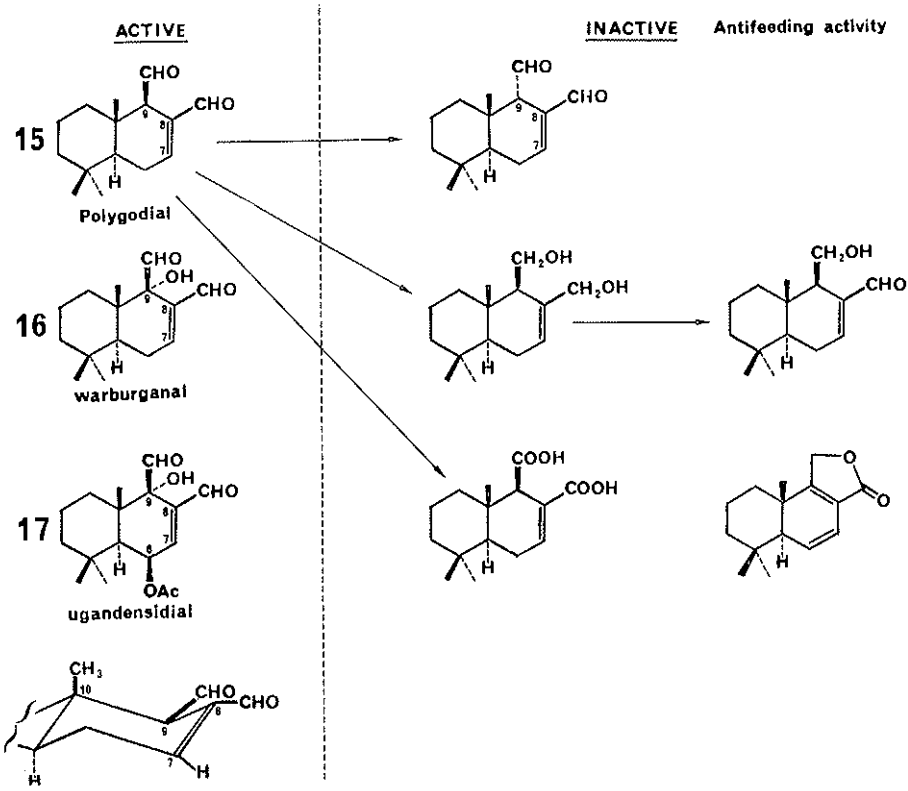


FIG. 7

and that the 9 β-aldehyde acts as a hydrogen bond or nucleophile acceptor which is located at a critical distance from the enal. Interestingly, the army worm antifeedants taste hot to humans, while the nonactive compounds listed in Fig. 7 are not hot.

ACKNOWLEDGEMENT

I am most grateful to Dr. I. KUBO who initiated the systematic studies on antifeedants at ICIPE. In addition to the people who carried out the chemical studies, the names of whom are listed in the references, I am also greatly indebted to all of my colleagues who are working at or related to ICIPE for numerous discussions. The studies have been supported by NIH Grant AI 10187 and ICIPE. A grant to I. KUBO from the Society of Promotion of Science, Japan, is also gratefully acknowledged.

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Note added in proof: Professor D.A.H. TAYLOR of the University of Natal, Durban, has informed us in a letter dated February 9, 1977, that the plant from which we isolated xylomolin [25] was *Xylocarpus granatum* and not *X. moluccensis*. The fruits from the former plant are large whereas those from the latter are not more than 5 cm in diameter. We thank Prof. Taylor for this information. However, the name « xylomolin » is now not appropriate.

DISCUSSION

KNÜSLI

My question is again dictated by « professional deformation ». I am always amazed by seeing such a brilliant work, and by hearing of such results. But then, all this ends more or less on the level of a laboratory curiosity. Now I ask is that inevitable? Must it remain on this level? Of course I very often see the limitations — the structures are so complicated they hardly could be made synthetically, but in view of these tremendous activities — were all other possibilities exhausted? For example: we grow orange trees, we grow apple trees, pear trees — is it out of reality to grow neem trees — to collect the fruits and to apply these fruits or extracts — would that not eventually be a chance just for the developing countries? Maybe you have already a comment that it is impossible but it is a question which comes to one's mind, especially in view of these 40 micrograms per liter you mentioned.

NAKANISHI

Well, a way out is to grow the neem trees, although we won't do it. On the other hand, take warburganal, the much simpler compound. It would be a rather simple compound to synthetise and make many modifications; you may also wonder what about the chemical degradability because of the dialdehyde functions. I think for example — we have not done this — that they could be protected as hemiacetals or derivatives. If it is active against some other pest insect it could at least be used during storage, for example, in which one would not have to worry about photochemical consequences. The antifeedant compounds do not necessarily belong to any group. They include all sorts of structural variations. Now the neem oil is produced in ton quantities in India. It is one of the most abundant of plant oils, and for example, I sup-

pose Dr. Jacobson has started looking into this in a more systematic way. The neem tree is a very common tree, and I don't think it is impossible to cultivate it. But as John Siddal showed yesterday, it is going to take years if this is to be developed into pratice. Probably all of us will be retired by that time. But I still feel optimistic and that is the reason we are going to continue this. I was getting disappointed and disillusioned with plant chemistry until Isao Kubo came out with all his new series of medicinal plants, quite a few of which overlap with the plants the witch doctors use. They have thousands of years of history and the nontoxicity at least on an oral level is proven and so that is the reason I am starting to feel optimistic again about natural plant products.

KNÜSLI

Well I'm still not yet completely satisfied by the answer. You said you cannot grow for example...

NAKANISHI

No, but we are in a Chemistry department... in New York.

KNÜSLI

I asked here at this round table — who would eventually be in a position, what would you think for example, gentlemen, of FAO, — in the case it would be useful on the practical level?

NAKANISHI

I think the first thing is to do a detailed activity testing azadirachtin, which is almost impossible to synthesize and if you really think it is worthwhile, then one can start growing the trees and the seeds. The pyrethrin, I think, at the early stage was like that too. And it is just a matter of how important one thinks these compounds are. It is still too early to say this.

CHAPMAN

If I could just say a word on that last point, I think Professor Nakanishi is absolutely right because we have done some work with azadirachtin and clearly its potency is very variable. It is much more effective against *Schistocerca* for instance than even other acridic species, but the point I really wanted to make was: I was really most interested in what you were saying about Ma's work because I think this emphasizes the importance of using electrophysiological techniques for your assays. Because I think the key point this brings out, or would bring out in parallel with other work, is that anti-feedance of the kind you are talking about falls into two classes. There are those which actually stimulate the nervous system in the same way that a phago-stimulant does which the insect then interprets within the central nervous system as an anti-feedant and I think these vary very greatly in their specificity from insect to insect. But with a compound like warburganal I get the impression from the way you have described it that what you are doing there is actually interfering with the sensory system because the sensory system does not recover after you take the stimulus away; and I think if you are dealing with this kind of anti-feedant it is much more likely to have general applicability than the sort which is specific and is simply perceived as a stimulus and I think it is an important distinction to make and I think it does emphasize the importance of the electro-physiological work.

NAKANISHI

If I was the head of an institute I think the first thing I would do for bio-assay is to set up an electro-physiology study.

Coming back to the irreversibility, if based on this very simple scheme I mentioned, you can explain that in terms of organic chemistry, in other words, you have this enal moiety and the SH which is sticking out of the sensilla. The SH will add to the enal in a so-called Michael addition type, which for some reason becomes irreversible. This is very simplicistic but it is based on organic chemical reactions and I think it makes sense. And the other aspect one has to consider, and I think this is quite critical, is the critic geometric requirement. I mentioned that the beta aldehyde has to be pointing upwards, for if you make it pointing down, it is not active and it is not bitter either. The second aldehyde group could

react with another second SH group or it could react, for example, with the terminal amino group in an amino acid residue. Although it would be difficult to set up these things in a chemistry department, the advantage of electro-physiology is that it gives us some semiquantitative idea because you can measure this in terms of times and impulses per second.

GILBERT

I think this group of compounds in these plants is one of the most interesting potentially practical advances we have seen mentioned for the tropics. In answer to the question about whether one can cultivate these trees and use their extracts, there is no question that one can do this and quite easily. I have myself planted *Melia azedarach* and it grows very rapidly indeed. I think in a few years one could have adult trees of the size we saw illustrated of the neem tree. It is just a question of organization and money. One could find in Brazil people able to do this with a given plant and have them tested on crops. This is perfectly practical and we might discuss later how this could be developed.

NAKANISHI

I would like to add one more comment and that is this tree — the twigs of the trees — are used as toothbrushes, as you know, by the Nigerians and Indians — none of their teeth are decayed — they are beautifully white. We haven't looked into the antibacteria component; again the leaves are used against malaria.

JACOBSON

I can add to what Professor Gilbert has said in so far as the neem tree is concerned. My group is at present doing a considerable amount of work on the anti-feeding compounds in both the neem seed and neem leaves. We know that there are at least three or four different components in the fresh neem seed — one of which is azadirachtin, another is melantriol which has already been worked on by several other groups but there are also two or three others in the seed

that are highly effective as feeding deterrents. We find azadirachtin and also several of the others are extremely effective in preventing feeding on host plants by about seven or eight different species of chewing insects that occur in the United States, one of which especially is the Japanese beetle; it is also effective against several types of scale insects. We are so exuberant about the neem tree and its possibilities in preventing — or in plant protection — that we have looked into the possibility of growing these neem trees in the United States and indeed it has proved to be possible. We have several neem trees growing on a federal reservation right outside of Miami, Florida. They grow very well in that warm climate. And we also have a number of chinaberry trees — *Melia azedarach* — growing at the same place. Although we can not get enough seed from these trees as yet in the United States to carry out our investigations, we have ordered through the International Programs Division of United States Department of Agriculture 100 pounds of fresh neem seed. I must stress that in order to get these compounds we must use the fresh seed. Once the seed is allowed to dry, these materials no longer occur in the seed. Now we are also looking, as I said, into the use of the neem leaves because it had been reported by some investigators in India, a couple of years ago, that the leaves contain attractants for some sugar beet pests and these are something that we need very badly in the United States in the areas where sugar cane is grown. And we have used the neem leaves from the trees which we have growing in Florida and they do indeed seem to be attractive. So I think I can give Dr. Knüsli a little more hope for the practical use of these materials.

BOWERS

I do not think we have really enough possibility of using the structures such as Professor Nakanishi comes up with from these plants for molecular models for optimization and study of the fundamental aspects of chemistry which are involved in antifeedants and other things. I think this is one of the very great possibilities for these studies. It is one thing to find a compound in a plant such as azadirachtin and find that it has such unusual activity, and I suppose at the same time it is very natural to be depressed about the possibility of making it synthetically, but these are models that Nature has given us. I think that some-

times Nature does tend to embellish chemistry a little bit for its own particular purposes perhaps, but these compounds can certainly be modified and be made more useful. I would simply cite the juvenile hormones and what has been done with them. It is a natural product that if one were to try and synthesize — let's say the JH one and all the proper stereo-chemistry — the most active form on a commercial scale would drive men mad; but look what happened to the optimization attempts which came up with compounds far far far more active than the natural compounds. I believe that we should continue this work with more intensity than ever and look at these compounds as molecular models that Nature provides us with and then try to understand the threat of activity which is in these molecules and then with our own creative chemical instincts try to develop the simple forms that can be manufactured and used.

NAKANISHI

We would like to do that, but it has only been, well, less than a year since we got the first structure so we haven't had enough time but on the other hand I would just like to make one comment on the relative activity of warburganal and azadirachtin. As far as electrophysiology studies are concerned warburganal is about twice as active as azadirachtin. And I would rather play around with this molecule than with azadirachtin of course.

DORN

An anti-feedant applied in the field should also protect newly growing leaves of the crop plant from feeding damage. For this reason it would be highly desirable to have antifeedants with systemic activity. As far as I know, azadirachtin possesses this interesting quality. Do you have similar indications about the other anti-feedants?

NAKANISHI

No we don't. It is only azadirachtin. On the other hand I will be quite delighted to send these compounds to whoever could kindly test this for us because we are simply not set up to do this type of thing. And

the point you raised is true — if it is simply an anti-feedant. I have been criticized — several people have mentioned these comments which are very valid. Namely, the plants grow, then the insects will soon find a place in which this is not topically applied. So it has to be systemic I believe.

WAIN

I have some sympathy with Dr. Knüsli; he stresses the importance of finding chemicals which have practical value and which can be developed for commercial use. Here I would like specifically to refer to hydroxy-methoxybenzaldehyde which we have heard is an attractant for cockroaches. Now cockroaches are tremendous pests. So we had to find out whether this is a good cockroach attractant or not in practice. It is useful, for example, for attracting cockroaches into an area treated with insecticides. I would just like to make one observation ahout the neem tree which is very interesting to me because thirty years ago now in the Sudan I saw them using the fresh neem leaves either as a repellent for insects or as an insecticide. I am not sure which. The leaves were put into drawers where clothing was stored and were very effective. So effective was the treatment that I brought back some of the neem leaves with me, extracted them and tested fractions of the extract for insecticidal activity unfortunatly without success.

BALLIO

I have a few questions and comments. First of all, I should like to know if there is anything known about specificity of this compound in respect to different insects?

NAKANISHI

They have not been studied widely enough. However, warburganal has negligible activity against locusts or tobacco horn worm. On the other hand, there are cases where an extract has only weak activity against army worms but is very potent against the Mexican bean bettle. So there is definitely a specificity and my feeling in that so far as the army worms are concerned, the antifeedants against them taste hot to humans too.

BALLIO

Are your antifeedants also active on economically useful insects?

NAKANISHI

What do you mean, I didn't quite...

BALLIO

Is anything known about the specificity of these antifeeding compounds towards different insects?

NAKANISHI

I can not answer that because simply the tests have not been enough.

BALLIO

You told us that many of these compounds are very bitter. I wonder if, from the practical point of view, the bitterness influences the palatability of edible plants treated with them, and in that case how stable these compounds are.

NAKANISHI

Well, are you just simply asking of stability — chemical stability aside from the bitterness?

BALLIO

Yes, linked with the fact that many of these compounds are bitter I am thinking of the application, say, to plants.

NAKANISHI

Or palatibility? Are you speaking of?

BALLIO

Yes.

NAKANISHI

If it is at the level we are speaking of, for example, if you take azadirachtin — for which more quantitative studies have been done — two nanograms per square centimeter stops locusts from feeding: now this is negligible. Just to get an idea, this corresponds to 20 gr per kilometer square. Warburganal is twice as active, although this is an unrealistic multiplication. I do not think that palatability would be affected. I also forgot to mention that warburganal is contained in a common rather expensive medicinal plant and that the local people use it to spice their food.

BALLIO

I should like to make a comment about the mode of action of these compounds. If your hypothesis is correct and, for instance, the dialdehyde reacts with SH-groups of cysteine residues as well as with ε-amino groups of lysine residues, the compound might be very useful as a specific label of active sites in some enzymes. I wonder if your compounds can he made available to people interested in this type of studies and if they can remain soluble on addition of aqueous buffers.

NAKANISHI

Regarding the availahility of azadirachtin it is pretty easy; and I could send you some warburganal, too, in the future. And the reason I showed the LC trace there was that it comes as a very close mixture of — a few other active compounds.. It took the two LC people, a postdoctorate and a graduate student, two solid weeks on the LC to get 20 mg of pure compound. We are quite experienced in hplc but nevertheless the resolution is not enough to separate the two active compounds which would be required for detailed studies. As far as warburganal is concerned, I think by far the easiest would be to synthesize these compounds. Azadirachtin is easier to obtain from plants,

but as Dr. Jacobson mentioned, once you crush the fruit, disappointingly, the yield is drastically low. Also it appears that the fruit has to be fresh. We think there must be some enzyme contained in the flesh of the food which could degrade azadirachtin which is contained in the seeds.

MARINI-BETTÒLO

Professor Nakanishi has stressed a very important point that is: plants and active principles of plants should be also tested for their toxic, antifeedants, repellents etc. properties versus insects.

There has been demonstrated in many plants the occurrence of *iridoids*, which are rather common. We should now investigate if the plants containing these substances are attacked by insects or not.

About the mechanism of action of warburganal I consider that it could be rather similar to that proposed by Cavallito in 1945 and later by M. Kupchan for the anti-tumoral substances. That is: the highly reactive aldehyde or epoxy groups should react with the SH groups of the enzymes necessary to the life of the insect.

Many other products present in plants, mainly quinones and terpenes with epoxy groups are also very reactive and can explain the protection of the plant.

I just wanted to quote these examples to show that more research is necessary at biological level on natural products in order to find new ways for plant protection.

NAKANISHI

One common factor with all these plants we've been studying is none of them are attacked by insects; Another comment I would like to add to ajugarins is that according to Abo-Khatwa the respiration of mytocondria in rat liver is suppressed.

Because of the presence of electrophilic moieties, it is possible that these antifeedants possess other activities.

ABO-KHATWA

Since some of these antifeedants do bind SH groups, I investigated the inhibitory action of some of these compounds on some SH-enzymes

such as succinic and lactic dehydrogenases. Two of these compounds exhibited marked inhibition at concentrations ranging from 10^{-5} – 10^{-6}M.

CANONICA

You have mentioned a crude ecdysone preparation, used by Japanese industries in order to improve say, silk production by the silk worm. If you can answer, I would like to know what is really this crude preparation and whether the active component is ecdysone or whether there are other active components present.

NAKANISHI

As far as the ecdysone preparation is concerned it is an extract of a plant rich in phytoecdysones and it is now being sold by Takeda Pharmaceutical Industries under the particular trade name which is mentioned in this paper; the other preparation is that of a mixture containing dodecanol as the main constituent. But the reason that the entire "automated" silk production has not gone into practice yet is because of its far-reaching effect on individual silk-worm farmers. The Altoside, I understand, has been now approved by the Japanese government, I am sure that John Siddal knows much more about this but if you apply Altoside to the last instar silk-worm larvae, they spend a longer time in this instar and end up making a 10-15% larger cocoon of the same quality of silk. Now this is already in practice. The third aspect is artificial diet. The Japanese sericultural stations have been looking into this for the past twenty years or so and they have almost come out with a totally artificial diet without using mulberry leaves. But a small amount of mulberry leaf extract still is required, otherwise the same quality cocoon is not obtained. They are still trying to find out what this extra factor contained in mulberry is. If they succeed in doing this, then we don't have to use the mulberries any more. It will be just an artificial mixture of vitamins, antibiotics, minerals, cholesterols, and so on. But if this is put into effect, it will have a big impact on the people who are raising mulberry leaves. The Japanese government is still contemplating whether to switch or not at this stage. But at least one step which will be going into practice is the juvenile hormone.

[8] II, 7 - *Nakanishi* - p. 25

SOMERVILLE

This remark rises out of what Dr. Abo-Khatwa said but I think that it should be stated that we might be a little sanguine in assuming that because these compounds form part of the normal diet there are no toxicological problems and surely their success in the field will in the long run depend on their passing the normal toxicological tests.

PHYTOECDYSONES: ENVIRONMENTAL DEGRADATION

LUIGI CANONICA

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Centro di Studio delle Sostanze Organiche Naturali del CNR

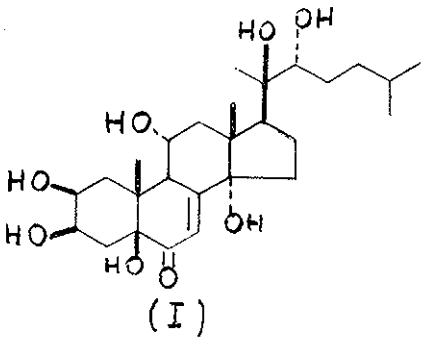
In these past years it has been found that ecdysone and many derivatives of it are widespread in different plants. Many of these compounds are more or less active as insect moulting hormones, but there is no evidence that after the death and the degradation of the plant tissues which produce them, these compounds are accumulated in the environment acting in the living cycle of the insect: so, probably, they are quickly degradated in the environment.

The problem of the stability of the ecdysones in the environment is also interesting from another point of view: these compounds are actually considered as possible biological insecticides, and moreover their hormone activity can be utilized in special applications as in improving and increasing the production of silk. The investigation of these applications has been firstly hindered by the lack of the required amounts of ecdysones; nevertheless, in these last years a rich source of ecdysones was found in the seeds of an Indian *Ipomoea* and besides a new method of partial synthesis has been published which is more convenient than the first known methods.

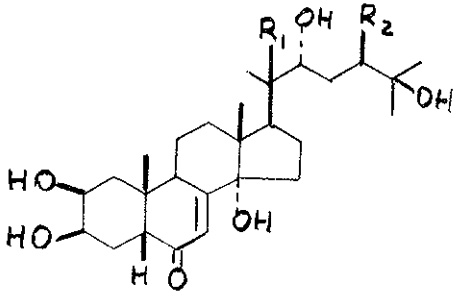
As usual, the problem has to be investigated not only because it is fundamental in tbe possible practical applications but even considering the danger of an eventual toxicity of their degradation products.

One degradation agent can be the degradation performed by the microorganisms. Our work on the microbiological transformations of ecdysones started in the hope of being able to introduce in this way an hydroxyl group at position 11 of these compounds.

The interest of this research was justified by the high activity as moulting hormone of muristerone A (I) [1].



(I)



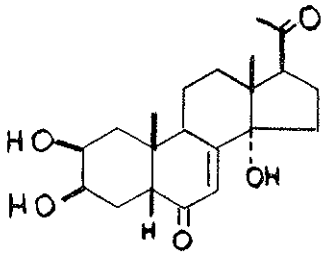
(II) $R_1 = OH$; $R_2 = H$
(III) $R_1 = OH$; $R_2 = CH_3$
(IV) $R_1 = R_2 = H$

This compound is one of the new phytoecdysones isolated from the before mentioned *Ipomoea*; its moulting hormone activity on the silkworm is some times larger than that of ecdysterone, and in this test it seems to be the more powerful agent known up till now.

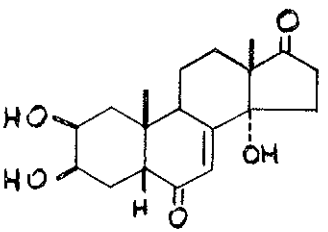
Comparing the structure of muristerone A with those of other ecdysones, its large activity can be connected with the presence of the oxygenated group at position 11. Besides, this group can be eliminated leading to an unsaturated compound which probably can be utilized as starting product for the synthesis of interesting substituted derivatives, for instance of 9-fluoroderivatives.

Owing to these considerations, we have fermented ecdysone, ecdysterone and makisterone A in *Rhizopus nigricans, R. arrhizus* and *Curvularia lunata* cultures [2]: these microorganisms are known to be able to introduce a hydroxyl group at position 11α or 11β

of many progesterone derivatives. Their whole cells are not able to transform the checked compounds, but ecdysterone (II) and makisterone A (III) are quickly oxidized by the autolyzed cells; ecdysone (IV) is not transformed even under these conditions. The microbiological oxidation of both previous compounds does not give the corresponding 11-hydroxyderivatives but a mixture of poststerone (V) and rubrosterone (VI).

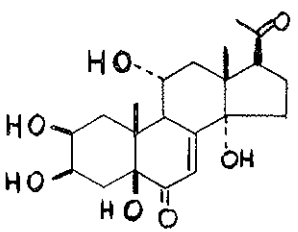


(V)



(VI)

Rubrosterone could hypothetically derive from the starting compounds through two different degradation pathways: nevertheless, at least mainly, it is the product of a further degradation of poststerone and not the product of a direct C-17 : C-20 fission of the starting compound. In fact, if poststerone isolated from a fermentation is introduced again in autolyzed cells, it is transformed into rubrosterone. Muristerone A itself is degradated through a pathway parallel to that now shown, but in this case the degradation yeilds a poststerone derivative (VII).



(VII)

A short time later, other Authors [3] reported the microbiological degradation to rubrosterone of a different phytoecdysone, i.e. ponasterone A. The microorganism utilized was *Fusarium lini*, which hydroxilates androstane and pregnane derivatives at position 15α, some cardenolides in position 12β [4]. In this research poststerone has not been isolated and its possible role as intermediate has not been proved.

All these results suggest some general remarks. The microbiological C-20 : C-22 side-chain fission of phytoecdysones seems not to be affected by an alkyl group at position 24, as in makisterone A, or of hydroxyl groups at positions 5β and 11α, as in muristerone A; the presence of the hydroxyl group at C-25, as in ecdysterone and in makisterone A but not in muristerone A and in ponasterone, is not necessary. The pair of hydroxyl groups at C-20 and C-22 seems instead to be a required feature because ecdysone is not transformed by any tested microorganism. The enzymes responsible for the degradation of other ecdysones probably are not induced enzymes, as indicated by the inactivity of the whole cells in comparison with the activity of their lyzates.

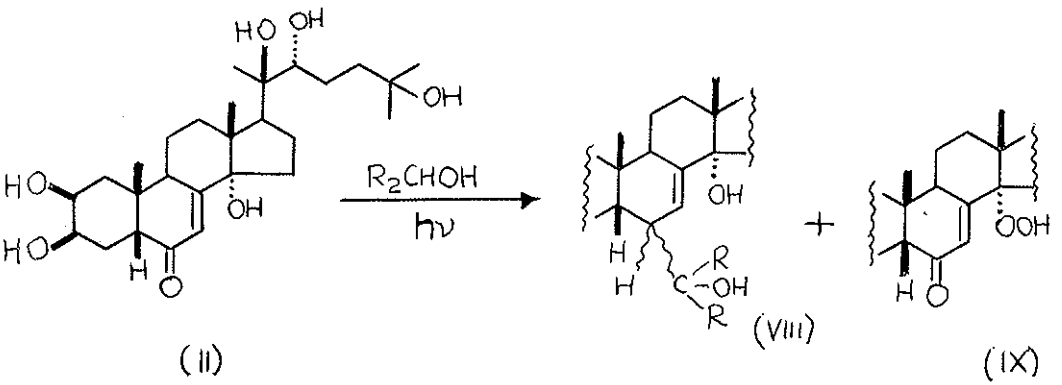
The degradation of ecdysterone to poststerone has been observed in *Bombyx mori* too [5]; moreover, the small amounts of poststerone and rubrosterone isolated from different plants [6] probably derive from the same degradation pathway we have found in microorganisms. The analogy with the degradation of cholesterol to sex and adrenocortical hormones via its (22R)-20α,22-dihydroxy-derivative which takes place in mammals is also evident.

Poststerone and rubrosterone, at least if fed to insects under the usual test conditions, appear to be inactive as moulting hormones. Besides, they do not show any significant activity on the mammals: so, they seem to be only degradation products deprived of ecological or toxicological interest.

As we have observed, another agent of environmental degradation of ecdysones can be the sunlight. Their photosensitivity probably could not be an insurmountable limitation for the pratical applications even in agriculture, because it can be easily controlled by the addition of small amounts of suitable photostabilizers. Nevertheless, it was interesting also from the technical point of view to

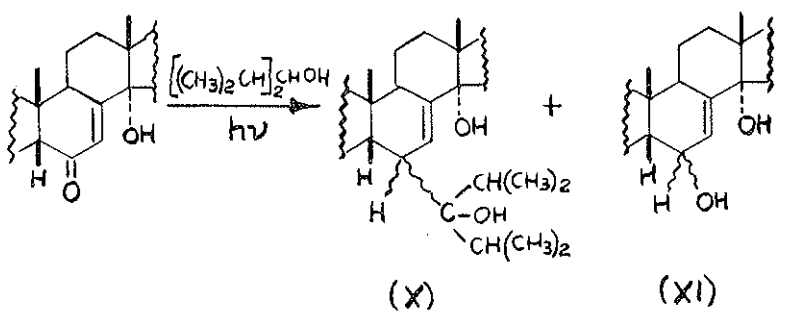
know the fate of ecdysones under irradiation. We carried out our experiments in argon atmosphere utilising a normal high pressure U.V. source equipped with Pyrex filter.

The photochemical transformation of ecdysterone is strongly influenced by the nature of the solvent. In simple alcohols as methanol or isopropanol it is very fast and gives mainly a mixture of two stereoisomeric adducts (VIII); together with these, minor amounts of 14-hydroperoxide (IX) are formed.



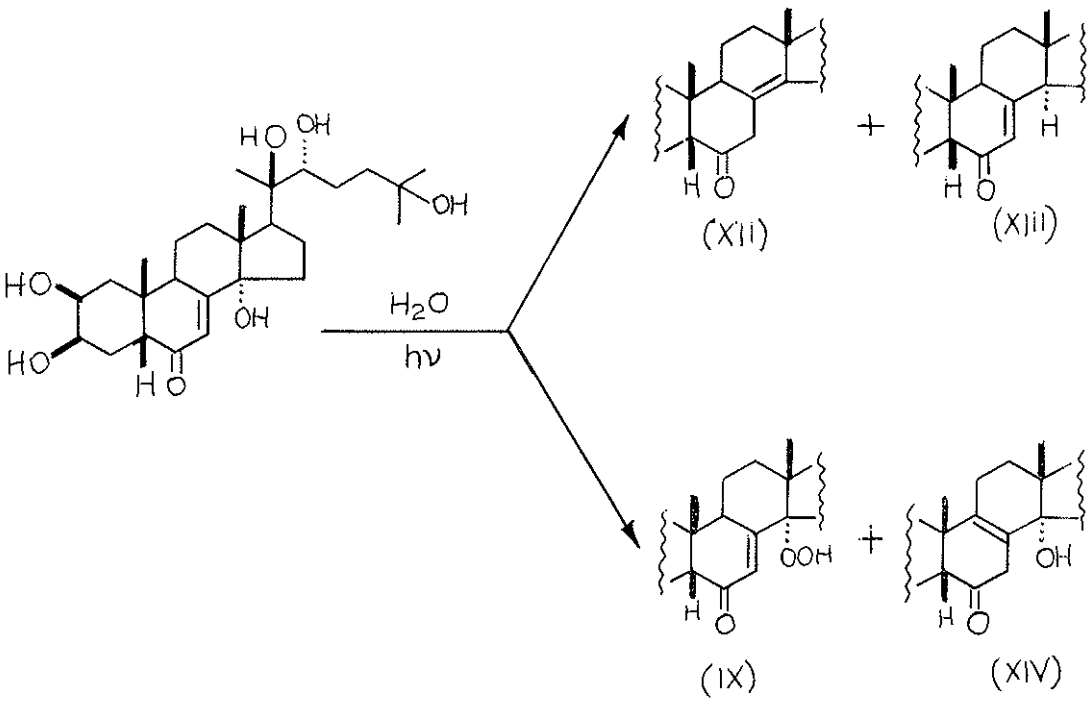
(II) (VIII) (IX)

In a strongly hindered alcohol as diisopropylcarbinol, the photochemical process is partly modified and, together with the adducts (X) and other transformation products obtained in simple alcohols, the epimeric reduction products (XI) are formed.

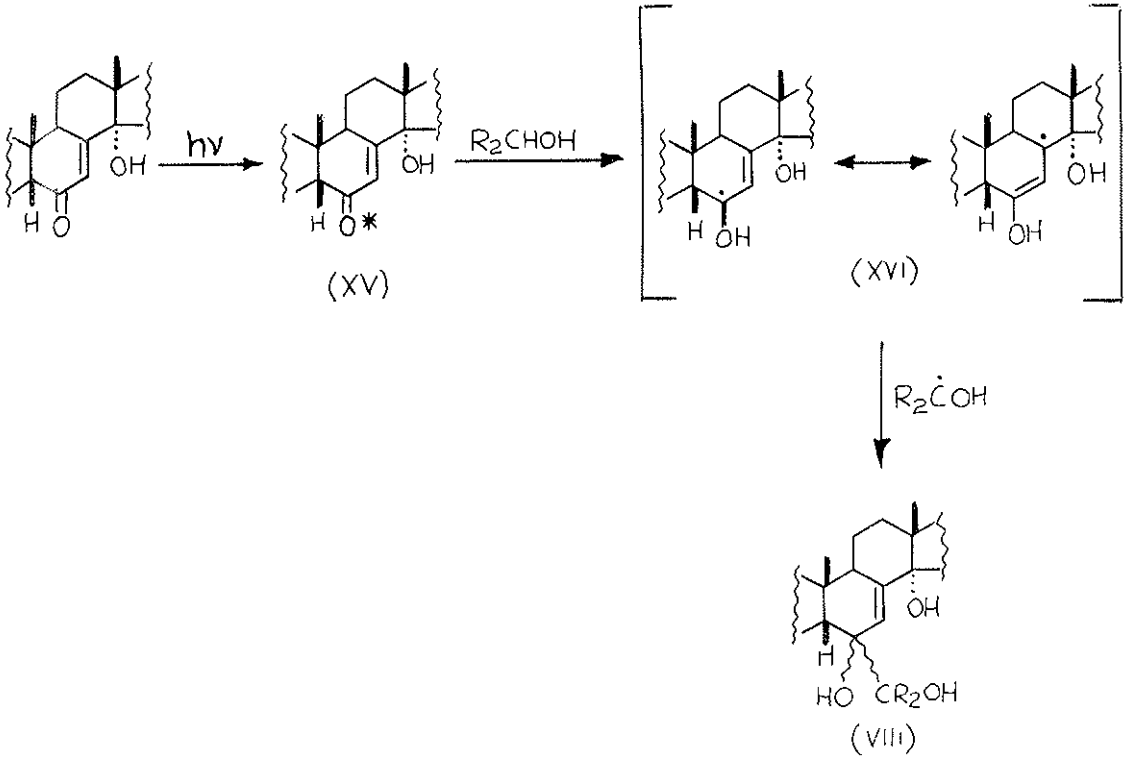


(X) (XI)

In water solution the transformation of ecdysterone is much slower. In this case the main product, obtained in good yields, is (XII), together with small amounts of (XIII); in addition, the isomer (XIV) of the starting compound and of IX is formed.

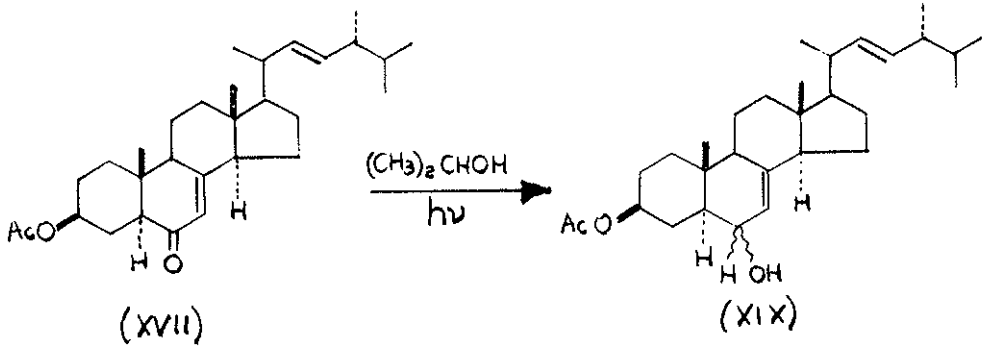


Now it is probably more interesting to discuss briefly some aspects of the formation mechanism of these compounds. The activated electron transfer and succeeding proton transfer from alcohol to ketone (XV) furnish the carbonyl carbon atom and the α-carbon atom of the alcohol radical. The intermediary formed radical (XVI) might couple with the alcohol radical to give the adducts (VIII).

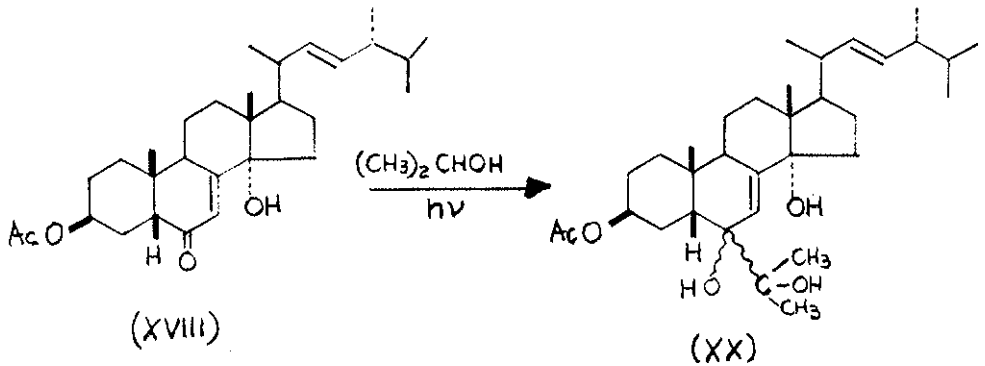


If the radical arising from the alcohol, is strongly hindered, as in the case of diisopropylcarbinol, its approach becomes more difficult and partly the radical (XVI) prefers to abstract one hydrogen atom from a different molecule of tbe alcohol.

The molecule of alcohol involved in this process and the corresponding $R_2\dot{C}OH$ radical probably are fixed through a hydrogen bond to hydroxyl group in position 14α. In fact, testing under the same conditions the phototransformations in isopropanol of two ergostane derivatives (XVII) and (XVIII) prepared as model compounds, we have found that (XVIII) repeats the behaviour of ecdysterone giving a mixture of the 6-adducts (XX), whereas (XVII) does not give adducts, but it is only reduced to the epimeric alcohols (XIX).



(XVII) (XIX)

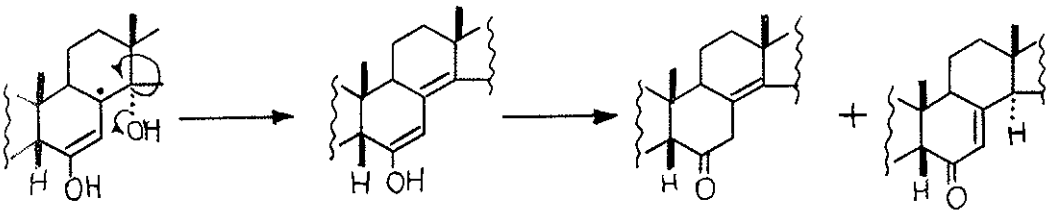


(XVIII) (XX)

In water solution, a similar pathway of solvent addition to the 6-keto group might lead to the formation of the hydrated ketone; it means the final product of the process is the unchanged starting compound. Such a process can give account of the slow transformation of ecdysterone in water. This hypothesis can be supported by irradiation experiments carried out in $H_2{}^{18}O$.

The formation of (XII) can be explained through postulating a β-bond fission of the radical (XVI) which involves tbe C-OH bond.

The small amounts of the 14-hydroperoxide (IX) can be formed from (XII) by the transformation products of the hydroxiradicals originated during the formation of (XII). The formation of such



(XVI) (XII) (XIII)

14-hydroperoxides in the photooxygenation of 6-keto-$\Delta^8(14)$ steroids has already been observed [8].

Compound (XIII) derives from a double bond migration of compound (XII), as confirmed by irradiating of the latter. Analogously, compounds (XIV) and (XV) are very probably formed by a double bond photoequilibration of (II) and (IX) respectively, according to already known processes. The biological activity in insects and mammals of phototransformation products of ecdysterone is now under investigation: but the general ideas on the relationships between structure and biological activity of steroids indicate they are probably deprived of any special activity.

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DISCUSSION

KARLSON

I happen to be familiar with work in Butenandt's laboratory on the radiation products of cholestenone.

In non-polar solvents dimers containing a cyclo-butane ring are formed by 1,2 addition at the double bond.

I wonder of you have come across similar dimers after irradiation of ecdysteroids?

CANONICA

Knowing the work you have mentioned, we have carefully looked for the dimeric products, but we have never been able to find them. Probably, in the case we have investigated, the formation of dimeric products is forbidden by stereochemical factors.

SOMERVILLE

I find the results of your micro-biological experiments interesting in two respects I think. First of all, it's a little bit surprising that whole cells of micro-organisms do not attack these compounds and secondly, I wonder if you ever observe ring cleavage? What I would like to ask is did you use selected species of micro-organisms and did you under the conditions of the experiment try other compounds such as cholesterol?

CANONICA

It is not so surprising the whole cells are not able to degradate the ecdysteroids while the lysed cells are able to do it: as I have mentioned, this behaviour can be due to the impermeability of the cell membranes

to the products: after the lysis of the membranes, the products can come in touch with the endoenzymes of the microorganism.

Concerning a possible further degradation of rubrosterone, I agree with you assuming that in the environment rubrosterone is probably degradated to simple compounds. In our experimental conditions, the activity time of the lysed cells was of a few hours and this fact can avoid a further degradation of rubrosterone; it is also possible that during the lysis of the cells enzymes responsible for this further degradation are inactivated.

MARINI-BETTÒLO

I should like to add some comments to the reaction above mentioned, in order to suggest a possible mechanism. Quinones in the presence of a proton donor are reduced by the action of light directly to the corresponding hydroquinones.

I think, although this is not exactly the same case, that a similar mechanism may take place in the degradation of ecdysteroids.

CANONICA

This process is well known and it is practically the same we have assumed in our pattern. Besides the alcohol, toluene and chloroform are good hydrogen donors, and we are investigating in this direction.

NAKANISHI

We were working on the antheridia-forming factor of the fern called *Anemia phyllitides*. The factor was collected by growing the spores on agar culture medium. An unexpected thing which we found in the cultivating medium is that it contained ponasterone A. Mc Morris and coworkers at the New York Botanical Garden have discovered the same phenomenon. I just recall this because the other day I was having a chat with Carrol Williams on the bus, and he said: Could you make radio-active ponasterone A? I think at least theoretically that this could work if one were to add labelled precursors to the medium. Now about the photochemistry. Just one brief question. I think the reason that you just get an alcohol adduct in the case when you have a β-hydroxyl is

that the allylic radical could be stabilized by the 14-hydroxyl through some sort of hydrogen bond or something like that. When the 14-hydroxyl-group 13 is not present, the stabilization is also absent. And so only in the case that you have 14-α- hydroxyl group do you get this adduct. I think you can explain it this way.

CANONICA

Clearly the cell membranes of the spores of the fern you have mentioned are permeable to ponasterone and so they are able to be crossed by this compound and to release it into the solution. On the contrary, the cell membranes of the microorganism we have tested seem to be impermeable to the ecdysteroids: in fact, they are not transformed by the whole cells but only by their lysates, that is when they can come in touch with the enzymes of the internal part of the cell.

Concerning the influence of the 14-hydroxyl group on the photo-transformation pattern of ecdysteroids and model compound, I agree with you: the formation of hydrogen bridges with alcohol molecules seems to be the more probable explanation.

KNÜSLI

I would like to come back to the question which has already been raised by Dr. Somerville. In case such a compound — let's say, such a steroid compound — would find a major use, what would in the end happen with such a molecule and what would be the prognosis as to the terminal residues? Would it degradate in the environment, in the soil to small fractions, to CO_2, to smaller fatty acids or would it stop somewhere around the phenantrene ring system? What would be your prognosis or the prognosis of some of the experts here around the table?

CANONICA

My prognosis is very probably the final degradation products in the environment are water and C_1 or C_2 compounds. This one seems to be a quite general rule which is observed for example even in the microbiological degradation of complicated aromatic compounds. In most cases,

the first attack of the molecule of the starting compound is the slower step of the oxidation pattern, while the following steps are faster. Only in well selected conditions, for example in the conditions used in the commercial production of corticoids or in the condition we have used in our researches, the degradation pattern can stop at an intermediate step in good yields.

NATURAL PRODUCT DERIVATIVES IN TROPICAL INSECT AND PARASITE CONTROL

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Introduction

There is little doubt that living organisms in the tropics face a more vigorous competition from their fellows than do temperate zone denizens. Higher plants and animals, in particular suffer from multitudinous parasites which include viruses, bacteria, protozoans, fungi, helminths and insects, to mention only a few of the smaller predators. It would therefore not be surprising if we found a richer variety of defence substances and defence mechanisms in tropical organisms than in temperate ones. In the case of the mammals, elaborate immunological defences contribute heavily towards the protection of the individual against invasion, but with plants, fungi and invertebrate animals particularly, chemical defences appear to play an important role.

It is a pity that so little careful work has been done to elucidate this role in tropical plants of Brazil, and still less in invertebrate animals. There is a tendency among chemists to establish structures but concern themselves little with biological activity. Interest generally centres on finding new structural types without concern for the function that justifies the presence of the substance in the plant. At the same time pharmacologists sometimes concentrate

efforts on tests (hypotensive action, CNS effects, etc.), which may not be immediately related to the disease of a plant whose invaders may not suffer these effects.

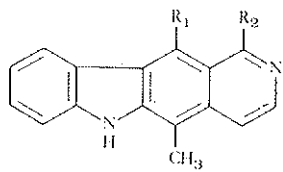
We ourselves in Brazil are particularly guilty of these omissions — I worked myself for eight years on indole alkaloids without investigating their biological properties — and where we have measured biological activity of plant-derived natural products we have usually done so with animal rather than plant parasites. However, biochemical studies have shown that there is often much in common at the molecular level between the most diverse organisms, and it is felt therefore that results that we have obtained in animal parasite systems may reflect similar properties in plant parasites. We also know that viruses, trypanosomatids [1] and nematodes, to name three examples, invade plants as they do animals. Man of course, has taken advantage of what we may suppose to be plant defence substances, for the combat of his own diseases and these still represent a major portion of all commercial pharmaceuticals [2]. There is no doubt that natural products often embody much more subtle structure-activity relationships than do the common synthetic products of the medicinal and pesticide chemist — the living world was designed by the Creator to function harmoniously, and in balance. It is to be expected therefore that natural defence substances will often either be specifically directed against probable predators or, if broad spectrum toxins, will be relatively mild and easily degraded, giving local short-lived protection against competitors but not causing widespread disturbance in the habitat. It is felt that a large number of mild natural bioactive agents have been overlooked or rejected by the pharmaceutical and agrochemical trades, which prefer one shot cures for all ills, rapid action easily synthesized chemicals that give quick financial returns, but which may do considerable damage outside their intended targets. It is our wish that this situation may be reversed and that natural products and methods, such as were used in the past, may again become more important than artificial ones in medicine and particularly in agriculture. There would be no simpler way to achieve this, than greatly to facilitate the licensing of natural products for medicinal and agricultural use. Presently it is said to cost several million dollars to carry out toxicity trials to meet EPA (United States Environmental Protection Agency)

requirements. It would seem absurd to make the same requirements for natural products that have been with us since the world began, as for products of the chemical industry whose natural existence is unlikely. Small industries are often no longer able to launch new products over the EPA barrier and it would be in the interest of natural product usage that pesticide control legislators reconsidered criteria which seemingly exclude some of these desirable substitutes for environmentally hazardous chemicals.

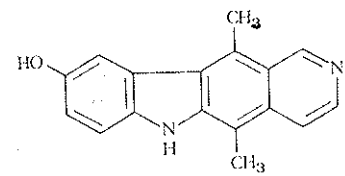
Alkaloids

It would be interesting to run briefly over some natural products which we have isolated and which have definite biological activity. Going back to alkaloids isolated some years ago, the most interesting one biologically was olivacine [3, 4, 5] and we have recently had occasion to compare the growth inhibitory activity of this and related alkaloids in cultures of *Crithidia fasciculata* (a non-pathogenic flagellate) and the related *Trypanosoma cruzi*, causative agent of Chagas' disease.

Alkaloids of this class are inhibitors of DNA synthesis by intercalation [6] and are antileukemic agents at a level of 5 mg/kg every 48h in mice [7]. Results with *C. fasciculata* indicate that olivacine (*1*) is more active than ellipticine (*2*) and 9-hydroxy-ellipticine (*3*) in this system. The last two alkaloids were supplied by Dr. N. DAT-XUONG (Gif) and the French group with whom he is associated has shown the reverse order of activity to be true in anti-cancer tests [8]. The activity was shown to be related to the curved planar structure of these alkaloids and in a synthesis of

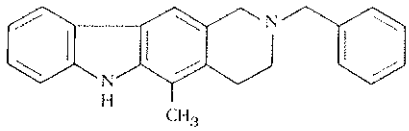


1 Olivacine $R_1 = H, H_2 = He$
2 Ellipticine $R_1 = Me, R_2 = H$



3 9-Hydroxy ellipticine

1-demethylolivacine by GOFFIC's method [9] activity against the flagellate *C. fasciculata* first appears with closure of the tetracyclic system in *4*.



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The thick bark of the tree *Aspidosperma nigricans* Handro, is yellow with olivacine, as its popular name "pereiro amarelo" testifies, and it is interesting to speculate whether this alkaloid is a first line of defence for a species widely distributed between 5° and 18° S in areas of heavy rainfall.

TABLE 1 — *Activity of olivacine* (1), *ellipticine* (2), *9-hydroxellipticine* (3) *and 2-benzyl-5-methyl-1,2,3,4-tetrahydro-6H-pyrido* [3, 4-b] *carbazole* (4) *against the flagellates* Crithidia fasciculata *and* Trypanosoma cruzi *in culture media.* (+)*-disappearance of live flagellates;* (-)*-inactive.*

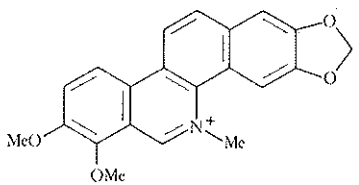
Alkaloid	*Crithidia fasciculata* [a] Concentration μg/ml				*Trypanosoma cruzi* [b] Concentration μg/ml			
	1	2.5	5	10	1	2.5	5	10
Olivacine *1*	+[c]	+	+	+	—	+	+	+
Ellipticine *2*	—	+	+	+	—	—	+	+
9-Hydroxyellipticine *3*	—	—	—	—	—	—	—	—
Intermediate *4*	—	—	—	+	—	—	—	+

[a] *C. fasciculata* Anopheles strain ATCC 11.745 in the medium described by BACCHI *et al*, J. Bacteriol, *98*, 23-28 (1969). Incubation time 48th, temperature 28° C. This and *T. cruzi* test were carried out by Drs. F. STEELE DA CRUZ, WILSON LEON and CONCEIÇÃO AGUIAR, of the Instituto de Microbiologia, Universidade Federal do Rio de Janeiro.

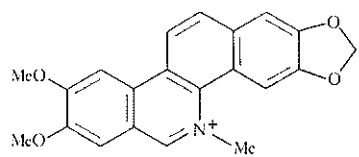
[b] *T. cruzi* Y strain (SILVA and NUSSENZWEIG, 1953 - Minas Gerais) in modified Boné-Parent medium.

[c] 1.5 μg/ml.

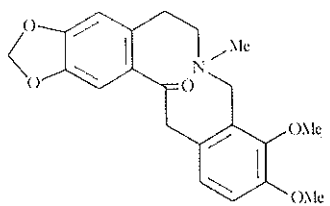
Other alkaloids of a similar spatial type that have shown antiviral [10] and anti-tumor [11, 12] activity are certain benzophenthridines, such as chelerythrine (*5*) and nitidine (*6*) which occur in *Fagara arenara* Engl [13]. Possibly the mode of action is similar to that of the preceding crescent-shaped planar alkaloids. The facility with which nitidine adds nucleophiles at position *6* may help fixation to a biological substrate. The yellow wood of *Fagara arenara* is used in popular medicine and appears to be resistant to decay. Other alkaloids which we have isolated from the same wood, allocryptopine (*7*) and, (+)-platydesmine (*8*) [13] have not yet been related to any specific biological activity.



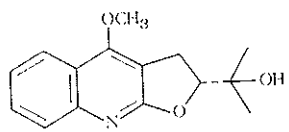
5 Chelerythrine



6 Nitidine



7 Allocryptopine

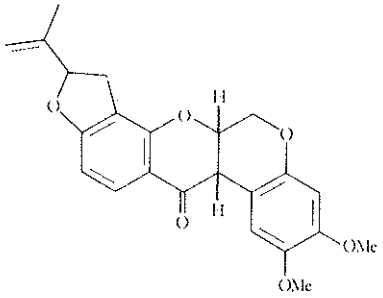


8 (+)-platydesmine

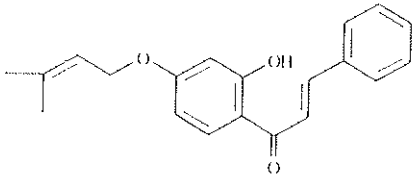
Derris

A group of plants which enjoyed a brief period of commercial application as insecticides is represented by the genus *Derris* (*Lonchocarpus*). An ethanolic extract of the roots of *Derris urucu* (KILLIP and SMITH) MCBRIDE kills fish (*Lebistes reticulata*) at 1 ppm or less, and this was man's first use for the plant. The difference in

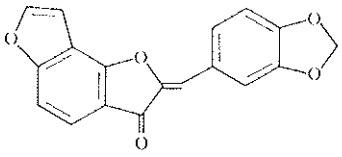
toxicity for different fish species has led to the use of thousands of tons of *Derris urucu* root dust in the specific removal of piranhas from Brazilian river and reservoir systems. According to RIZZINI and MORS [14] 48000 km² of water were treated at 3 ppm *Derris* during the years 1957-1961, for this purpose. The toxicity is attributed to rotenone (*9*)



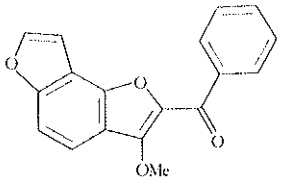
9 Rotenone
Derris urucu



10 Derricidin, cordoin
Derris sericea



11 Derris obtusa



12 Derris obtusine A

but extracts containing only 25 % rotenone were as active against mosquito larvae as pure rotenone and extracts essentially deprived of rotenone were still active [15]. For this reason MORS and collaborators [15-17] have reinvestigated several ichthyotoxic species and MARINI-BETTÒLO and GONÇALVES DE LIMA [18, 19, 20] yet another species. However the compounds isolated which are represented by typical examples (*10-12*), do not seem to account for the toxicity observed. Recent studies which are still incomplete seem to indicate that a saponin or saponins also present and only recently isolated by MORS *et al* play an important part in the bio-

logical action of these root extracts. Although the saponin itself possesses low toxicity to fish it enables the dispersion of the water insoluble rotenone and perhaps other potentially toxic principles and presumably aids penetration into the animal organism. This case is typical of numerous plant products, often used in popular medicine and whose activity is not related to single components present but to natural combinations which should not be separated. Such products may not be exploited by man if he insists on relating biological activity to a single characterized component. Here again Environmental Protection legislators should consider how to regulate the licensing of such mixtures, whose composition will necessarily be variable.

Nematodes and thir Control by Terpenoids

Both plants and animals are parasitized by nematodes and although I cannot give figures to show the economic importance of these, it is believed that some 25 million of Brazil's human population suffer from *Ancylostoma, necator* or *strongyloides* infections, genera whose early larval stages may be found in the soil. GOULART and collaborators [21] have shown that these larval stages may be combatted by natural products, and 119 extracts, essential oils or natural products with larvicidal action have been identified. Several *Eucalyptus* species, Labiates such as *Mentha spicata,* Compositae of the genus *Chrysanthemum,* and grasses such as lemon grass and vetiver, are among the better known species which eliminate nematode larvae of the cited genera from their immediate vicinity. The few responsible components identified include terpenes with α,β-unsaturated carbonyl groups such as l-carvone, citral and perillaldehyde, some simple alcohols like l-menthol and α-terpineol, and a phenol such as thymol. However it is likely that oxidation products of more abundant terpenes present may be more important contributors. Limonene is inactive but peroxidized limonene is active and the cyclic peroxide ascaridole, is of course, a well known nematocide. When lemon grass (*Cymbopogon citratus* L) was planted in a shanty-town in the Rio de Janeiro suburbs, human reinfection by nematodes of tbe Ancylostomidae occurred in

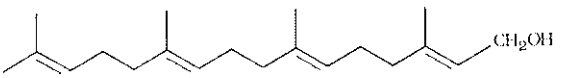
only 12 % of the population in one year and a half compared with 50 % reinfection observed in a similar period in an adjacent unplanted area [22]. The technique has been applied in agriculture using *Tagetes minuta* L. but seems to have been little explored. A serious study of the intercropping of lemon grass, vetiver or one of the mints in potato, tomato and other nematode-susceptible cultivation would seem to be justified as an alternative to the use of synthetic nematocides. The intercrop plants mentioned would themselves be commercially exploitable.

Other Terpenoids

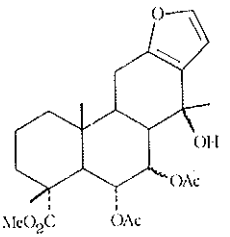
The above considerations lead one to speculate on the role of terpenes in plants. Some, such as limonene, may be stored as relatively inert compounds, which, on exposure to air, bacteria and fungi may be converted into toxic derivatives. The fruit oil of the leguminous tree *Pterodon pubescens* Benth. common in the central Brazilian plateau contains terpenoids in both their inert and oxidized forms. For example, geranylgeraniol (*13*) and its terminal epoxy-derivative (*14*) are present [23], as are the diterpenoid furans *15* and *16* [24]. *14* is highly lethal to *Schistosoma mansoni* cercariae, *13* is not, *16* inhibits growth of *Crithidia fasciculata*, *15* does not.

We thought years ago that the terminal epoxide (*14*) might have some juvenile-hormone-like function. However, this is not the case, although the corresponding methyl ester at C-1 (*17*) does in *Rhodnius neglectus* [25]. The ester *17* no longer possesses activity against *S. mansoni* cercariae.

Also highly active as cercarial penetration inhibitors are many unsaturated terpene acids. One may cite dehydroabietic acid (*18*), an artefact but derived from highly active extracts of many conifers) [26, 27] agathic acid (*19*, responsible for the antiparasitic activity of *Araucaria angustifolia*) [28], copalic (*20*) and related acids in *Copaifera species* [29], and as yet structurally unidentified sesquiterpene acids in cedarwood (*Juniperus virginiana*) [30] and in *Barbacenia bicolor* Mart. and other Velloziaceae [31]. The bactericidal effects of pine needles and exudates from Coniferae are known and only the chemical defences of such very slow growing



13 Geranylgeraniol



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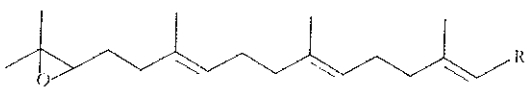
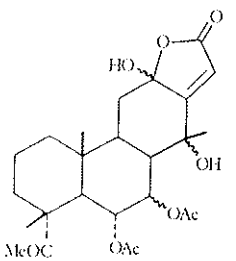


14 R = CH_2OH
17 R = CO_2Me



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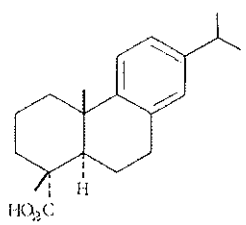

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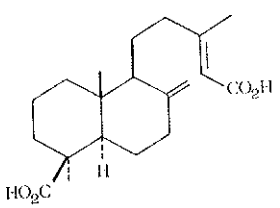

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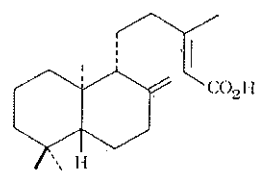

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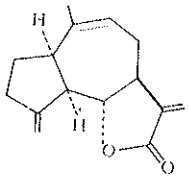
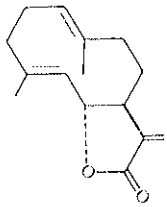
plants as are the *Velloziaceae* can explain their survival in the tropical biotope. It seems that a more detailed investigation of the role of terpene acids in these defences is called for.

Among the Compositae there are a number of insect and fungus resistant woods of which *Vanillosmopsis erythropappa* Sch. Bip. is one of the most commonly used in fencing. In this plant and

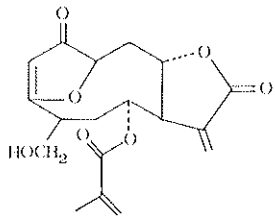
related *Eremanthus* and *Moquinea* species, sesquiterpene lactones have proved to be the principal active compounds, often accompanied by bisabolol and isovaleric acid. The lactones which are exemplified by the three types, *21-23* [32-35], are biological alkylating agents, probably assisted in penetration by the accompanying liquid isoprenoids. Their activity was measured by us in *Schistosoma mansoni* [32] but is probably broad spectrum.



21 Eremanthine
V. erythropappa
E. elaeagnus



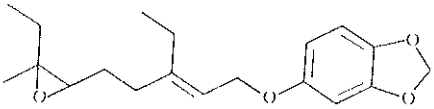
22 Costunolide
V. erythropappa



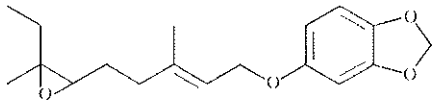
23 Goyazensolide
E. goyazensis

Juvenile Hormones

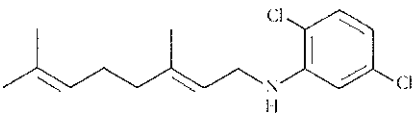
A search extending over some years for juvenile hormones in plants has not resulted in any potent compounds in the test insect, *Panstrongylus megistus*, (Hemiptera) used by us. This model, dictated for us by its importance as a CHAGAS' disease vector, is really unsuitable for screening, since it is unaffected by the great majority of synthetic hormones [36], due to its year-long cycle. However BOWERS' synthetic products [37] based on natural plant synergists, *24* and *25* were highly active in this species, and we have only equalled their activity with the citral derivative *27* [38], developed from SCHWARZ' aniline derivative, *26* [39].



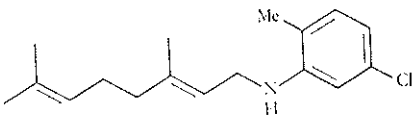
24



25



26



27

BOWERS' recent results with the precocenes [40] justify further and more careful search among plant extracts and we have recently established other models (Lepidoptera and Coleoptera) for such a screen.

Conclusion

It is felt that a wide range of plant defence substances is presently unknown, ignored or forgotten by industry, medicine and agriculture. Many more Brazilian natural products could be added to the above described examples. We can foresee three methods of supply or use — extractive industry, based on natural occurrence, cultivation of appropriate plants for extraction, and use of active plants in intercropping. As for the first of these, could not the Amazonian forest covering some 4 million km^2 supply the world with natural insecticides and pest control agents? Even if we cite only known ones — *Derris roots, Quassia amara* wood, *Ryania* spp. — an extractive industry could supply biodegradable crude extracts almost immediately for uses where synthetic products are already causing environmental problems [41]. The promotion of natural products could come, as has been indicated, from official incentives for their use, and particularly in special facilities for their licensing, which would simplify or omit many of the present expensive toxicological tests.

ACKNOWLEDGEMENT

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DISCUSSION

KNÜSLI

I liked the start of the paper of Dr. Gilbert very much when he described particularly these possibilities in the field of this low technology agriculture. I think that is a very interesting approach (a), to study what should be grown best under the local conditions, then (b), in the case it is effective, to give advice for this intercropping: to plant a row of garlic or a row of *Tagetes* or a row of Lemon-grass together with the respective crop. I say that I am very positively impressed by this even though it is the opposite of the industrial approach. But I think just in this field we need competition, and I think competition is the promoter of progress, so I would say completely yes to the approach. But then, in the second part of his paper Dr. Gilbert shows, by the way, that he, too, has two souls in his breast. You said that you identified in a plant interesting activities. You mentioned the possibility and opportunity to make out of that a commercial operation, namely to purposely grow plants and to prepare out of them crude extracts which may serve the purpose. In this case you have, too, to produce something and you have to sell it to somebody, and in such a way you have to subordinate your self to some basic economic rules. So I think the polarity in which we may be came out very clearly also from your paper. Now, with respect to the question you asked on the licensing I suppose that you did not envisage the commercial definition of licensing but rather the registration of such a preparation. This is a wide field for reflecting. I think what would be needed is to have constant quality, a constant composition in such a preparation, this also from the performance viewpoint. Then I would think that a certain documentation on the safety or the possible risk would also be required. With respect to further requirements the respective authorities should speak out.

GILBERT

I mentioned licensing exactly in the sense of permission to use the

materials. It has been common in the past not to ask permission of anyone, at least in Brazil; people just went ahead and used the product, but this can't go on forever. For example, I mentioned that the Brazilian Government itself had to clear out the piranhas from a large system of reservoirs that had been built — and they used rotenone extracts — they could do this because these were available; I calculate that at 3 parts per million, they must have used about 10,000 tons of root. This shows just how available these natural products can be. I do not know whether previous experiments were made to see what would happen to human beings that drank the water — but experiments were perfomed to find out that the piranha was much more susceptible than were the other fish — or most of the other species of fish — so they were able to eliminate the piranha completely and they did not have to repopulate the area with useful fish, which survived in sufficient numbers to repopulate it themselves. Now, licensing is very, very expensive in the sense of working out the toxicology, and these natural products I am talking about will never be, in my opinion, exploited by industry that has the necessary money. They will be exploited by small industries that do not have the money. So, what are we going to do — and I think that we have to come to a decision on this point. We have heard that the United Nations — and among other bodies, the Rockefeller Foundation — were supporting the ICIPE in Africa, from which we have seen such good work along these lines and I think it will have to be such an effort supported perhaps by a group of foundations or by the United Nations, that will have to pay for the toxicological work that is necessary on natural products that are deemed to be sufficiently good for large scale use, because I am sure industry will not do this. It is wishful thinking if we think that any industry is going to take this burden on themselves. So I think that we should recommend to those foundations that are interested in this area — and we have already heard some of them mentioned — that they should support this toxicological work, and thus make it possible for governments such as that of Brazil to exploit these products in a responsible manner.

BRADER

First of all, when you were talking about all these products you were looking for and, mainly for pesticidal, nematicidal action, I was

reminded of the talk of Nakanishi of yesterday, and I wonder what sort of collaboration there is — you are looking for a certain type of product, Dr. Nakanishi is looking for a certain type of product — and certainly with a little bit more work there could be much more outcome from such work, I was wondering. You already mentioned somewhere a little work done, that with that quassia you supposed you had an antifeedent found, but how was this — how did this come out, did you do any specific test? It is just a general remark: how can we get the maximum benefit from this type of work, that — not just looking for one type of action, but maybe for a broader group of actions you might have much more benefit. I have the same type of remark and I can only support what Dr. Knüsli said: personally I have the feeling that there is absolutely no reason not to think that natural products should not be submitted to the same type of registration requirements as are synthetic products. I think with natural products, as you have described them, also there are the same problems — it is often the dosage which makes the toxicity — and registration is in a large part — to some people it might look like a big nuisance, but in a sense registration is meant to make the best use of pesticides and there is the whole safety aspect which is involved for which registration is also developed. So I think it certainly is so that they all have to go through the same registration requirement and maybe even more — I think Dr. Shorey could tell us something about the problem of getting pheromones registered which apparently would seem less toxic than the type of products you are talking about, but just the fact that they are completely new products makes them — I would say — suspect for the registration people. You were making the suggestion that maybe some external funding agency could help in getting this sort of registration requirement activities done — I do not have the exact figures in my mind but I believe that the last figure I heard was that of the total production cost of a new chemical, about 16% is now needed for the toxicological requirements that have to be brought up for registration. This would probably mean that each product we are talking about would go — as far as these registration requirements — to a cost of about $500,000 to $1,000,000. So you are talking about quite an amount of money if you think that some fund should come in, and I am wondering if you could ever find a sufficiently big grant to get this sort of thing done. I'm a little bit pessimistic on this.

GILBERT

To the first remarks we shall undoubtedly as a result of this meeting be collaborating with Dr. Nakanishi because I am certainly very interested in his compounds and I think we have a number of natural products that would probably be interesting to test. It is a question really of exchanging materials for biological testing and knowing exactly what tests we should carry out too — we are mostly chemists, we have one entomologist, but we would like some more information really on how we should carry out the testing, the antifeeding testing for example. The second one about the toxicological testing, I am aware that costs are a million dollars or more — round about that sum — and I do not think there is any question of obtaining such a sum from a granting body. I think it has got to be put to perhaps a group of foundations, that they themselves should through some existing organization have these products tested in the interests of mankind. After all, Rockefeller has a motto: "Science in the interest of mankind". And in the case of the United Nations organizations, they are there for this purpose; and I do not think it is a question of their giving a grant to any private person — I think it is a question of taking on the responsibility for trying to introduce useful products for the tropical area — specifically for the tropical area I would say.

JACOBSON

I can certainly support what both Dr. Knüsli and Dr. Brader have said insofar as licensing and registration are concerned. In fact I can go even farther — Dr. Knüsli mentioned that certain documentation is necessary in order to apply for a license or registration. Now there are probably some of you who are not aware of the amount of documentation which is necessary for this sort of thing. I have all of this information here in some sheets that I intended to hand out when I speak later this morning but perhaps this may be a good time to distribute it so that you can look at it at your leisure. Dr. Brader mentioned also the difficulty in connection with applying for registration of a natural pheromone. In fact, the material that I have to distribute here is entitled "Model for the Development of Data Leading Toward Registration of Attractants and Pheromones". It is a sheet — well there are about seven pages of material here giving the amount of data and the kind of data and a certain amount

of information as to the expense involved. I am speaking now certainly with regard to licensing and registration by the Environmental Protection Agency (EPA) in the United States. As I say, this might be a good time to distribute this material instead of waiting until later. Another point with regard to Dr. Gilbert's talk: he mentioned that he would like to see products such as quassin and other materials from the *Quassia* plants used as insecticides since there was so much of the raw material available in Brazil. I can certainly understand that, but here again, you see, the quassin especially and some of the other materials that are constituents of the *Quassia* plants have been shown to cause dermatitis in quite a few people and they also have been known to cause extreme sneezing fits in many people when they are used, so I do not think that they would come into very general use as insecticides.

MARINI-BETTÒLO

Thank you Dr. Jacobson for your document. I will take care that it can be distributed to the participants and I suggest to discuss it after your communication.

NAKANISHI

I fully sympathize with what Ben Gilbert just mentioned and I would just like to explain the dilemma we are stuck with at I.C.I.P.E.

As I mentioned yesterday we are starting to get several interesting compounds and in fact I have been spending during the past year or so quite a lot of effort trying to straighten out patent policies together with Professor J. Pringle (Oxford University) and Dr. T. Odhiambo (Director of I.C.I.P.E.). The reason we are doing this — and I would like to get advice and maybe some comments from Ben Gilbert too — is that our feeling is that unless these are patented or registered, the commercial companies will never develop them. So far the only concrete experience we have is that of warburganal and its congeners. We have applied for a patent and we do see that there is a necessity for further development, like modifying the molecules exemplified by the pyrethroids, because I am pretty sure that if you play around with that molecule, then you can come out with more potent and more stable compounds, but someone has to do this type of thing and unless some exclusive license is granted to

an organization, it will never be done, and this requires many modifications which is not exciting from an academic point of view.

Another thing I wanted to mention about the so called bioactive compound. My feeling is that probably whatever compound you take, it will fall into the category of bioactivity. You can take sodium chloride, water, everything, there is no compound which has no activity; it just depends on the level of activity one is speaking of. Now if you take bioactivity, and even confining this to antifeedants, we have been trying during the past year to get some simple system set up at Columbia University in the Chemistry Department. Finally with the help of several people we are going to have two small insect colonies, but that is only pertaining to antifeedants. If we go to all the other fields: anti tumor, anti-fertility and so on, it is almost impossible. And my feeling now is that maybe we should use commercial organizations which just specialize in carrying out these biossays.

I happen to know two of these organizations — you send them a minimum of 50 to 100 mgs and they will carry out, say 30 bioassays in the medicinal field. We get an initial clue and then from there on we can ask the proper body to go into more extensive studies. You pay a fee, about 400 dollars per sample to be bioassayed. However once you do find these bioactive compounds we don't know how to develop them. And this is a feeling of frustration one gets.

Suppose we do isolate a potent compound then what should we do and then how? The universities are not that much interested in money; we just want to see if something is worthwhile of development, to have it properly developed. But there is no way because the commercial companies will not touch it unless they have an exclusive license and how can we get around this?

Another thing is: most of us are supported by NIH, NSF and other neutral bodies. Now that's another dilemma, because usually it has to go — in the case of USA — to NIH etc. and once it goes to NIH then again the companies will drop it. This is a dilemma.

GILBERT

As far as patenting is concerned, I have inquired in Britain to know what is the NRDC policy. The NRDC patents scheme seemed to me the best solution.

NAKANISHI

Can I just comment on that particular case? We have had several meetings with NRDC people and we finally, as far as the (I.C.I.P.E.) in Africa is concerned, dropped it. The reason is NRDC is national — the I.C.I.P.E. in Africa is an international organization. NRDC, although it is a fine organization, has one stipulation: namely the patent has to go to British industry. An international organization cannot do this, so as a consequence we are going through Research Corporation which is an international and neutral organization located in New York.

GILBERT

I am not referring to using NRDC in England but setting up an exactly similar organization in Brazil.

BOWERS

I am also very sympathetic with your desire to use somehow these crude extracts in agriculture and on the protection of public health, and I know that the problems of getting permission — that is to say licensing and registration and so on — to use these kinds of mixtures, the active ingredients of which are unidentified and whose toxicological properties are biologically uninvestigated. To use them in the U. S. or northern European countries is just virtually impossible as the situation stands now. And if Brazil eventually enacts the same sort of laws that we enjoy now, of course they will not be able to be used in Brazil, — which brings me to the point that I hear people raise time and again, especially commercial people of course, and other people that are concerned with bringing new methods of insect control into practice, i.e. this difficulty of getting something through registration and the fantastic expenses that have to be borne. I think these very rules and regulations which were passed out this morning give some idea of what a compound has to go through. These regulations, I think, are becoming an inflexible rubric; they have obviously been designed and established by US, especially the EPA, and also by a very few, I believe, northern European countries, perhaps Japan has contributed as well. These countries have all accepted the same sort of criteria. I am not sure whether these series of tests are

adequate either to protect the public or whether it is adequate to bring into commerce useful agricultural chemicals. I think perhaps it forbids the development of many potentially useful agricultural chemicals just by the sheer ponderous amount of data required and the expense involved. You pointed out that small industries, let us say research groups and so forth might be able to bring some of these natural products up to a useful product — I am simply intimidated totally by the time and expense involved in all this data acquisition in defense of the compounds and, I am sympathetic — I think we need careful screening of all agricultural chemicals to protect the public. But I wonder if all these tests are really necessary for all compounds, for all extracts, for all potentially commercially useful compounds. Just because a very few bureaucracies have established certain tests does not make them perfect or immutable — any law that has ever been passed can be changed — I think public pressure, public need should be able to change these things. I do not think any government should be so inflexible or any bureaucracy so petrified that it is not susceptible to change by public opinion and public need. The bureaucrat has an important job to do but the bureaucrat's response to a challenge is always "No" unless or until there is enough public pressure because it is always easier for a bureaucrat to say "No" than "Yes, but under these conditions", because those conditions then have to be described by the bureaucrat and it means more work. I think that if there is pressure, if the collocation of knowledge and ideas here can make any public stir, and if we, not only with this meeting but in other meetings bring pressure to bear, perhaps we can get a re-examination of some of these tests and expenses that are required and shorten the time for development of new agricultural chemicals. I am not saying that the tests are bad, we must protect the public, but I am not sure that these very expensive and long term tests are serving the public's need for more reasonable and cheaper methods of insect control in these areas which you specify that could use low technology. Perhaps there should be a difference in the laws that apply to let us say the highly developed nations who can afford to pay for very specific insecticides.

GILBERT

I thoroughly agree with that. We are dealing with the Amazon — a region where there is probably a lot less than one inhabitant per square

mile — that is, when a farmer sprays around he does not spray on many other people. And as you say, it is more important for him to have food and perhaps have some... a little personal discomfort if that is the only way that he can have food, but we have to be very careful.

The system in Brazil up till the present has been of considerable flexibility. There is a lot of bureaucracy but it is not inflexible. But this comparatively liberal policy probably can not be continued indefinitely. I mean we have obviously to have the data and to be able to advise as to what should be done even though people may not afterwards do it. There is the serious problem of the variability of crude extracts. The Brazilian government uses natural pyrethrum, for example, in large quantities and everyone who uses natural pyrethrum knows that this is a very variable product. The amount of actual pyrethrum in it goes up and down and I do not know how you license such a product. Anyone who has worked on natural products knows that if you extract one plant you get so much of a particular compound and then you go and extract the same species even from the same area another time and you get quite a different quantity of the same compound and sometimes find another compound there so that you cannot guarantee the composition at all. All you could do is measure by a bio-assay — in some way define a natural product by a bio-assay — but you cannot license it in the sense that you can license a pure compound; and I suppose the content of toxic and irritating compounds will also go up and down as well as the insecticidal ones. So it is quite a difficult problem but it is one that we have to tackle because I think these products on the whole — in the end — will be relatively safe in areas like the Tropics. I would mention that almost any compound that is released — organic compound — will be degraded very fast in the Tropics in comparison with its rate of degradation in temperate areas. I am saying that as a sort of general statement without much evidence but I do recall having fungus growing very well on a medium containing an organo-tin anti-fungal agent, which is supposed to be absolutely wide-spectrum, tributyltin oxide and which is yet apparently very good for growing a certain fungus species in Brazil. I do not know what the fungus is but it grows very thickly on this material, thus you do get micro-organisms — or means of degradation in the Tropics that you do not have in the temperate area.

BELL

Crotalaria species have been found to reduce the concentrations of nematodes in the soil.

ELLIOTT

One comment on Professor Nakanishi's suggestion about sending compounds to a central testing agency. I think it is a good procedure to discover the nature of the biological activity but I believe that to refine the potency of a series of compounds close contact with the workers doing the bioassays is preferable because without continually interchanging information with them, many of the subtle aspects of the activity of a compound are missed. So sending the compounds to a central testing agency is very much a second best procedure but better than nothing.

BRADER

To a certain extent I can fully understand the remarks of Dr. Bowers. I must say, however, that I disagree with 95% of it. I think it is too easy to say that it is just a bureaucratic action. Some of these registration procedures are developed in view of difficulties that have arisen and it should be realized that even now — even with these rather strict procedures — we are in a situation that sometimes products have to be even withdrawn again from the market which were already registered because only after very extensive use some difficulties were recognized. However, I agree that the registering procedures might be reviewed particularly for a special group of compounds. I am just making this remark to bring to your attention that in the National Academy of Sciences of the U.S. there was just very recently — in August, it was — a meeting on this whole aspect of pesticides for the future. I would almost say that the total trend of that meeting was more or less the same as brought up here. A review of some of the registration might be needed. It is the idea that this working group of the National Academy of Science with a working document will work closely together with the EPA to reconsider the situations. There is, however, one difficult point — one dangerous point, I think — and maybe that is the fact that I am now

related to FAO — to which I would strongly oppose saying: Let us make two sorts of strategies — one for the low — how did you call it — low technologies and one for the high technologies. That is one of the things we are absolutely opposed to. I would even — could even — make a plea for saying it the other way around: Let us make some stricter rules for the low technology where the danger of misuse of pesticides — and I have experienced that myself for eleven years in Africa — can create more difficulties than in developed countries where the knowledge about how to use and how to apply pesticides is much better developed than in the low technology. So I am certainly not in favour of this sort of dual proposition. And the last point I want to make — a somewhat more positive point which came to my mind during this discussion — we have just very recently received at FAO requests from donor countries that they would like in their own countries to undertake activities which could be supportive to work in underdeveloped countries and I was thinking particularly when this point was brought up, can we not subcontract some of this toxicological work to other institutes in the developed world? I think this might be an opportunity of developing this further and it could be good to maybe follow up later.

SIDDAL

I feel obliged to reply to Dr. Heimpel. With all due respects, I find it impossible to believe that complete toxicology on any substance of Bacillus can be carried out for $60,000. I think one should therefore ask a question: What happened to the other 10 or 20 million dollars in the USDA budget for this kind of activity? I think it simply is the difference in accounting procedures. Accepting that the overhead in industry is high, one of the reasons it is high is that industry keeps records and necessarily has to account for the expenditure of its money. If the same type of accounting were applied to universities, to funding agencies, and to USDA, I do not think there would be such a difference in costs and this can be documented. The National Academy of Science has recently collected and referenced data to this end.

MARINI-BETTÒLO

I think that the discussion on this point is very interesting but that

it should be postponed until after Dr. Jacobson's presentation because it involves not only the natural products but all the policies of registration of the pesticides. Prof. Gilbert will answer the other questions now.

GILBERT

I will only answer very very briefly about Crotalaria. Yes, I have seen this used in Brazil. I think it is a rather toxic plant though, and there are some better ones like lemon grass which are not toxic. With Dr. Elliott I entirely agree. I spend a large part of my time that should be spent in the chemistry lab circulating in the biology testing labs because I like to see how everything is being done because otherwise you just get back the answer "inactive" and you know perfectly well this is often wrong. It is the way it has been tested that is wrong very often. You just can not farm out substances without very careful instructions about formulation, otherwise you get back the wrong answer. So I do like to see what is being done. With regard to Dr. Heimpel, I think the Brazilian government certainly will be willing to pay for the toxicology tests. We can probably get that done, by government biological laboratories — perhaps not as well as it should be done but it would be done. Probably the results would not be accepted by the rest of the world. We have had that experience in the past. In reply to Dr. Brader with regard to the term low technology — my definition is completely different for low technology — it is not misuse of products that have come from high technology. That is, it is not misuse of products that have come from industrial nations. It is use of local techniques and local products (in which nothing has been imported from outside) by the people themselves. I think that is all. Thank you.

THE INSECTICIDAL ENDOTOXIN OF *BACILLUS THURINGIENSIS*

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Numerous attempts have been made to use micro-organisms in the control of insect pests by the introduction of pathogens. The most notable success has been the total control of Japanese beetle in North America by introduction of *Bacillus popilliae,* the micro-organism associated with milky disease of that pest. Varied results have been obtained with other micro-organisms and these can perhaps best be exemplified by the many studies with *Beauveria bassiana.* Although well characterised as an insect pathogen in the laboratory it has not proved easy to predict the effectiveness of preparations of this fungus when applied in the field. It is possibly because of the variability in control that general interest in microbial pathogens as control agents has subsided to some extent. Pathogenicity alone is insufficient for a micro-organism to act as a control agent — an understanding of the factors that influence infection and effectiveness in the field is clearly needed.

Bacillus thuringiensis is the only micro-organism that has been successfully developed as a commercial insecticide. While the pathogenicity of this micro-organism to a selected range of insects (lepidopterous larvae) has played a significant part in its devolpment, much of its success lies in the ways in which it can be considered as similar to chemical insecticides. The activity of *B. thuringiensis* stems from production of a toxin, β-endotoxin,

which is in several ways similar to chemical toxins. Thus, preparations of *B. thuringiensis* can be formulated, stored and applied by methods similar to those used for chemical insecticides. The endotoxin is distinguished by its selective activity to lepidopterous larvae which is associated with a crystalline parasporal inclusion body formed concomitant with sporulation. While a great deal of information has appeared on the efficacy of different preparations against a wide range of target insects, comparatively little is known at the molecular level about the nature of the toxin, or its mode of action. It is on these aspects that the present contribution will be focussed. By and large only recent literature is cited; for details of earlier studies reference should be made to relevant reviews [1 - 4].

Biologically active products of B. thuringiensis

The endotoxin is only one of several interesting biological activities associated with *B. thuringiensis* (Table 1). Three toxins with activity towards insects have been clearly identified: the heat-labile endotoxin, a related polypeptide from spores which is discussed below, and the β-exotoxin, a heat-stable nucleotide which is a structural analogue of ATP and inhibits DNA-dependent RNA polymerase from a wide range of organisms [5, 6]. Although some studies have been carried out on the exploitation of exotoxin, for example to control flies in chicken faeces, it is unlikely to be used in insect control because of its high mammalian toxicity [7]. Not all strains of *B. thuringiensis* produce exotoxin and its absence does not appear to detract from activity against Lepidoptera.

Recent studies by BOMAN and colleagues [8, 9] have shown that *B. thuringiensis* inactivates the humoral immune system of *Hyalophora cecropia.* Two separate soluble fractions have been obtained one of which is heat-labile, and the other stable to heat for 5 min at 90°C. Mutants lacking β-exotoxin and endotoxin were compared for their virulence in this system and results imply that neither of these previously characterised toxins is of importance for activity against the immune system. Both inhibitors of the immune system are believed to be involved in the pathogenicity of *B. thuringiensis,* although no direct evidence has so far been

obtained. These inhibitors remain to be characterised but may well represent new types of biologically active molecule.

Paradoxically *B. thuringiensis* appears to have an entirely different effect on mammalian immune systems [10, 11]. Preparations of *B. thuringiensis* cultures have been found to enhance the humoral immune response in rats and guinea-pigs. It has been suggested that this activity is associated with an antitumour protein component of the crystal of *B. thuringiensis*. Crystal preparations have high antitumour activity against Yoshida ascites sarcoma and a purified fraction has been obtained which shows both antitumour activity as well as toxicity to *Bombyx mori*.

TABLE 1 - *Inhibitors and toxins of Bacillus thuringiensis.*

Activity	Species affected	Nature of active component	References
Endotoxin	Lepidoptera (larvae)	Polypeptide	3
Spore toxin	Lepidoptera (larvae)	Polypeptide	15,17
Exotoxin	Widespread	Nucleotide	1,5
Immune inhibitor A	Insects	Heat labile	8
Immune inhibitor B	Insects	Heat stable	8
Antitumour	Yoshida ascites sarcoma	Polypeptide	10,11
Hemolysin	Mammalian erythrocytes	Polypeptide	12

From this brief survey it is evident that several interesting biological activities are associated with *B. thuringiensis*. The best characterised of these are undoubtedly the β-endotoxin and exotoxin; further studies are required to confirm the identity and nature of the other activities listed in Table 1. Besides these, other components of *B. thuringiensis* cultures, such as hemolysins [12], proteases and lipases may have secondary roles in the pathogenicity of the micro-organism.

Microbiological aspects of B. thuringiensis and endotoxin activity

B. thuringiensis belongs to group I of the aerobic spore-forming genus *Bacillus* [13]. Biochemical and genetic approaches [14] to taxonomy suggest that it forms an identifiable species, closely related to *Bacillus cereus* and *Bacillus megaterium*. Acrystaliferous mutants (*cr*) of *B. thuringiensis* are, morphologically, virtually indistinguishable from *B. cereus*. Within the *B. thuringiensis* group some 10-12 serotypes have been identified all of which form a parasporal inclusion, although serotype 2 can be clearly distinguished by microscopic examination as the crystal is located inside the exosporium rather than in the sporangium as is the case in all other strains. Both the toxicity and the effectiveness against a range of lepidoptera clearly vary from strain to strain of *B. thuringiensis* although no comprehensive reports have appeared of comparative studies under standardised conditions.

The endotoxin activity is associated with the parasporal inclusion which is formed during the development of the spore in non-proliferating, stationary-phase cells of *B. thuringiensis*. Examination of serial sections of cells during sporulation has shown that the crystal is assembled on the exosporium membrane during stages II-III of sporulation. As soluble crystal antigens have not been found during sporulation it is probable that the polypeptides of the crystal are synthesised at the same location. When sporulation is complete the cell lyses to release a single spore and, with the exception of serotype 2, a free crystal. More than one crystal can be formed in each cell. Careful studies with separated crystals and spores have shown that the crystal is of about one-third the dry weight of the spore and is composed largely, if not entirely, of polypeptides. There is no clear evidence to indicate any physiological function for the crystal although it has been suggested that formation results from unregulated production of spore coat protein. Most, if not all, cultures of *B. thuringiensis* have been isolated from infected insects. However, an experienced eye is needed to distinguish cultures microscopically from *B. cereus* and it may be that *B. thuringiensis* occupies a role in Nature as a soil micro-organism as well as an insect pathogen.

Early studies with *B. thuringiensis* demonstrated that toxicity

was associated with the crystal and purified preparations of crystals have been shown to be lethal. Recently, however, it has been shown that spores contain a comparable amount of toxicity activity to that in crystals [15]. Both the spore and the crystal seem to be essential for optimal activity against *Galleria mellonella*. Burges and co-workers [16] have shown clearly that the activity of a mixture of spores and crystals is much greater than would be expected from either alone or from the sum of their separate activities. Commercial preparations are derived from whole cultures and consequently contain spores and crystals.

Treatment of spores with a reagent that solubilises crystal protein, followed by centrifugation, results in a supernatant solution that is toxic and is inactivated by antiserum to crystals. This toxin can be partly solubilised from spores by incubation with larval gut juice, suggesting that it plays some part in the entomocidal activity of *B. thuringiensis*. In addition to the common property of toxicity, much evidence has accumulated to indicate that some, at least, of the components of the crystal are related to polypeptides in the outer layers of the spore. Recently this relationship has been demonstrated cytologically by immune labelling of thin sections of spores with antiserum coupled to ferritin and by the peroxidase-antiperoxidase technique [18]. It would be tempting to conclude that the toxin is a normal spore component and that deposition of the crystal results from unregulated synthesis of the toxin along with other insoluble polypeptides whose formation is controlled by related mechanisms. In support of this hypothesis, *cr* mutants which can be easily isolated following conventional mutagenic treatments lose almost all of their toxicity, leaving activity several orders of magnitude less than that of the wild type and similar to that found in spores of *B. cereus*. The toxicity in the spore has been shown to be located in two fractions of characteristic morphology one of which has been indentified as exosporium and the other, tentatively, as spore coat [15, 18]. The toxic fraction from *B. cereus* spores is associated with a protein with a molecular weight of 30,000 [14], lower than that ascribed (67,000) to the crystal toxin by the same workers. Spore preparations do not dissociate to give electrophoretic patterns similar to those resulting from SDS-gel electrophoresis. Despite the immunological and toxicological similarities between spore and crystal

toxins, it is clear that important discrepancies remain to be resolved. In the *cr* strain, in which toxicity is virtually absent, there is no concomitant alteration in the morphology or amino acid composition of the isolated spore fraction with which toxicity is associated in the wild type. As the toxin appears to be a major component of the crystal it would seem unlikely that loss of toxicity would not be accompanied by alteration in spore morphology if the hypothesis outlined above was true. Although derepression of structural protein synthesis appears to play some role in crystal formation, without more detailed knowledge it is possible only to speculate on the precise nature of the regulatory steps involved.

δ-*Endotoxin*

Although endotoxin activity appears to reside in both spores and crystals insufficient evidence is available to conclude that the activities are identical and the endotoxin, for present purposes, can be taken as the insecticidal activity residing in the crystal. The crystal is a protoxin in that dissolution, and possibly some degradation by proteolytic enzymes, is an essential step before crystal preparations demonstrate activity when injected into the hemocoel of susceptible insects and it is assumed that a similar process occurs following ingestion of crystals.

Several methods have been developed for purification of crystals of *B. thuringiensis* and results, from several laboratories, of amino acid analysis of preparations from different strains are in such close agreement as to suggest that the number and composition of polypeptide components of the crystal cannot vary greatly from strain to strain. Although there is general agreement on the overall composition of the crystal there is a variety of differing reports on the number and molecular weight of the polypeptide components and on the nature of the toxic moiety. Reports on the overall composition of the crystal have been previously summarised [3] and range from a single component (molecular weight 1400) to several components all with molecular weight greater than 55,000. Studies on the crystal polypeptides have been hampered by the use in some cases of impure preparations, by the need for extreme conditions

to effect dissolution and by the tendency for aggregation to occur when the solvent is replaced by dialysis against milder buffer.

Examination of the published evidence indicates that the crystal is composed of several components of high molecular weight (Table 2). HERBERT *et al.* [19] purified, by ion-exchange chromatography, a component of 55,000 molecular weight which corresponded to the smallest component of the crystal when examined by gel electrophoresis under dissociating conditions; the crystal contained several other components of greater molecular weight. Recent work in our laboratory [26] has established a simple procedure for purification of crystal toxin in which crystal solution is incubated *in vitro* with gut juice or proteolytic enzymes under conditions which resemble those in the insect gut. Examination of the crystal solution by SDS-gel electrophoresis during proteolysis shows that all but one of the components are degraded. The component remaining corresponds to that isolated by HERBERT *et al.* although the molecular weight is now estimated at 67,000. This protein has been further purified by gel permeation and ion-exchange chromatography and, within the limits of the bioassay used, retains all of the original toxicity of the crystal solution. This purification suggests that the role of the alkaline gut juice may be to release the toxin from hydrophodic and ionic bonding to other polypeptides, rather than to activate it by cleavage of covalent bonds.

A number of reports suggest that the toxin is of much lower molecular weight (500-15,000). In several of these rather loose criteria have been used for the association of toxicity and molecular weight. Perhaps the strongest evidence for a low molecular weight toxin comes from two recent studies. FAUST *et al* [24] found that digestion of whole crystals followed by separation by gel permeation chromatography gave a number of peaks whose polypeptide profile corresponded to the toxicity of the eluate. The lowest molecular weight assigned was 1500; although this is obviously beyond the scope of such a chromatographic system their published evidence suggests strongly that several toxic polypeptides exist with molecular weights below 67,000. These workers also found that digestion with trypsin alone resulted in a single toxic polypeptide with a molecular weight of about 67,000.

After initial observation of antitumour activity associated with

TABLE 2 - *Reported values of molecular weight of active components of crystalline endotoxin.*

Strain of *Bacillus thuringiensis*	Nature of treatment	Estimated molecular weight (daltons × 10^3)	Methods used in estimation	Reference
berliner	*Digestion,* proteases *P. brassicae*	5-10	Gel filtration	20
berliner	*P. brassicae* proteases	5-10 > 200	Gel filtration	21
sotto	Chymotrypsin	0.5-1.0	Membrane ultra filtration	22
tolworth	Dissolution, no digestion	55	SDS-gel electrophorsis	19
entomocidus	*P. ricini* proteases	50 > 200	Gel filtration	23
kurstaki	*B. mori* proteases	1.5, 30, 62, 235	Gel filtration	24
tolworth	Trypsin	67	Gel filtration SDS-gel electrophorsis	26

crystals, PRASAD and SHETHNA [10] isolated a polypeptide of molecular weight 13,000, estimated by gel chromatography, following dissolution of crystals in 1 M NaOH and ion-exchange chromatography. This fraction showed both antitumour activity and toxicity to *Bombyx mori*.

The accumulated evidence indicates that the crystal contains several polypeptides. At least one of these, that of lowest molecular weight (67,000), is toxic. A single toxic component of this approximate size has been obtained by three procedures: direct chromatography of crystal solutions and digestion by proteases followed by chromatography, which have been discussed above, and continuous electrophoresis (J. R. NORRIS and H. J. SOMERVILLE, unpublished). This polypeptide is toxic both by injection and ingestion. *In vivo* this toxin is released by digestion of other polypeptides and may itself be degraded to toxic fragments of lower molecular weight.

There is no clear explanation for strain to strain variation in toxicity of *B. thuringiensis*. Clearly several relevant factors do vary between strains including levels of the several identified insect toxin and, as evidenced by immunological studies, small but probably significant differences both in the quantitative distribution of the crystal components and in their primary structure.

Mode of action of endotoxin

As pointed out above the endotoxin is highly selective for larvae of *Lepidoptera*, with an effectiveness that varies both between strains of *B. thuringiensis* and across the range of host species for a given endotoxin-producing bacterial strain. The same symptoms appear to be exhibited by each susceptible species as the dose is varied but the dose required, e.g. for gut paralysis, varies widely between insect species [25]. The toxin normally enters susceptible insects by ingestion; dissolved toxin, but not whole crystals, is effective when injected into the hemocoel. It remains to be seen whether purified toxin is effective against a wider range of insects, outside *Lepidoptera*, when injected directly.

Although several hypotheses have been explored on the mode of action of the crystal (Table 3) there is no convincing evidence to

TABLE 3 - *Some suggested modes of action of endotoxin.*

	Activity	Experimental system	Reference
1.	Neurotoxin	Cercal nerve synapse *Periplanata americana*	27
2.	Acetylcholinesterase	Cytological examination *Pieris brassicae* larvae	28
3.	Glucose transport	Glucose uptake *Bombyx mori*	29
4.	Ion transport	*Bombyx mori*	30
5.	Respiratory uncoupling	Mid-gut mitochondria	31
6.	Phospholipase	Direct assay	32

indicate the nature of the primary effect on susceptible insects. The toxin has an effect within minutes of ingestion as evidenced by almost immediate cessation of feeding by larvae. Most cytological studies have been carried out at much later times and these generally show disruption of the cells bordering the mid-gut. However, this effect could well be secondary and the rapidity of the intoxication is more consistent with a neurotoxic or hormonal effect.

Many successful commercial insecticides are neurotoxins and some tentative evidence has been published indicating that the endotoxin affects insect nervous systems in two ways. COOKSEY *et al* [27] suggested that endotoxin blocked transmission between the cercal nerve and the abdominal ganglia of the cockroach *Periplanata americana.* Recent attempts, in the Shell laboratories, to confirm this activity have been unsuccessful (R. J. DOWSON, R. FLATTUM, personal communications). On the basis of a cytological study KOENIGSTEDT and GROTH [28] suggested that acetylcholinesterase of *Pieris brassicae* is inhibited by endotoxin. No direct enzyme assays were carried out and attempts to confirm the inhibition with an acetylcholinesterase preparation from housefly heads have been unsuccessful (LILLEY and SOMERVILLE, unpublished evidence).

Several authors have suggested that the endotoxin acts directly on transport processes across the mid-gut membrane and effects on

glucose and ion transport have been noted by several workers. However, FAST and MORRISON [30] concluded that the rate of accumulation of potassium in mid-gut tissue of *B. Mori*, following ingestion of endotoxin, was not sufficient to account for other effects such as that on glucose transport which were more rapidly elicited. In a previous paper, FAST and DONAGHUE [29] concluded that glucose transport into the hemocoel was stimulated within one minute of endotoxin ingestion. However, examination of the published evidence suggests that this conclusion must be regarded as equivocal.

Recently, the possibility of endotoxin acting as a phospholipase has been investigated [32]. Although no phospholipase activity was found to be associated with crystal preparations, gut juice from *P. brassicae*, on the other hand, had a high level of activity. This activity, as well as other activities mentioned earlier, may well be implicated in some of the secondary symptoms of intoxication with endotoxin.

Examination in the electron microscope of thin sections of of larval mid-guts 30 minutes after ingestion of a lethal dose of endotoxin shows a marked effect on the microvilli of epithelial cells. The microvilli of goblet cells are not affected. TANADA *et al* [33], working with a granulosis virus of *Pseudaletia unipuncta*, have identified a synergistic factor from the virus inclusion body which promotes the infection. The virus apparently penetrates through the microvilli of mid-gut epithelial cells. It has been previously pointed out [3] that similarities exist in structure, composition and mode of penetration between endotoxin and inclusion viruses and it may be that a similar penetration mechanism operates for the endotoxin.

Despite considerable experimental effort the mode of action of the toxin remains unclear. To some extent, studies have been hindered by the relatively rudimentary knowledge of insect physiology and metabolism. Although it is clear that the mid-gut is affected there is no evidence to confirm the nature or location of the primary effect. While it is tempting to assume that the mid-gut is the primary site of action, because the toxin penetrates through the mid-gut and morphological damage to the mid-gut clearly ensues, more detailed evidence is required to support any of the current hypo-

theses. Further studies should concentrate on the use of purified toxin and should be directed initially at localising the site of action. A useful start might well be made by a careful study of the toxicological and cytological effects of purified toxin when compared by injection and ingestion.

Production and formulation of B. thuringiensis

B. thuringiensis can be readily cultivated on a wide variety of media. In the laboratory, the best preparations of spores and crystals are obtained on solid media. However, economic considerations point to liquid media for production purposes although suitable semi-solid media can be devised. The selection of an appropriate strain is of great importance because of the wide variation in the toxicity exhibited by different varieties. Precautions should be taken during growth to exclude other micro-organisms in order to avoid any possible contamination with plant or mammalian pathogens. In the absence of exotoxin, such preparations of *Bacillus thuringiensis* are without known hazard to man and other living species excepting susceptible insects.

Although details of commercial processes are not available it is probably safe to assume that commercially available preparations are produced by batch fermentation. As demand increases it may prove desirable to develop continuous processes for production. Recently a two-stage process in which vegetative cells are grown in one vessel and then allowed to sporulate and produce crystals in another has been shown to be feasible [34]. Cultures can be dried, following separation by centrifugation, using a variety of procedures without loss of activity. As has been pointed out, formulation of preparations presents no particular difficulties and concentrated suspensions, emulsions and wettable powders are available. Shelf storage must, of course, take into account the heat lability of the toxin. One particular problem is the standardisation of activity: although several methods have been tried, with varying success, it appears that some variation must be expected in results even when standard preparations are used under controlled conditions.

Future developments

B. thuringiensis is firmly established as a viable commercial pesticide and its use appears to be increasing both in quantity and in the range of target insects. An international program has been commenced on the development of new strains under central organisation by Dr. H. T. Dulmage of the Agricultural Research Service (USDA) at Brownsville, Texas. Preliminary results (H. D. BURGES, personal communication) indicate that toxicity, to some twenty-six host species, fits into the serological system of classification of strains of *B. thuringiensis*. Several of the isolates tested were active against a more useful range of host insects than strains in present commercial use and many isolates had more activity against individual pests or small groups of pests than commercial strains. It seems likely that particular strains will be developed for use against selected pests.

Future outlets of *B. thuringiensis* will probably include use in mixtures with chemical insecticides, particularly when it is felt desirable to use low dosage rates of the latter.

It is not clear whether synergism can be exhibited between chemicals and *B. thuringiensis* preparation: however such a result might be expected when improved penetration of the chemical could occur through the mid-gut as a result of the effect of endotoxin.

Clearly there is a need for a greater understanding of the pathogenicity of *B. thuringiensis*. Not only is understanding needed of the nature and mode of action of the endotoxin but also an explanation is required of the variability in the effect. There is a need to relate the action of endotoxin to the contribution of other active products of the bacterium such as the inhibitors of the immune system. In the present climate of environmental concern it is somewhat paradoxical that *B. thuringiensis* should be in such widespread manufacture and use when so little is known about the detail of its effect on insects.

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DISCUSSION

WIGGLESWORTH

I notice that you dismissed the idea of ion transport as being " in limbo " — I think that was the expression. The midgut epithelium of Lepidoptera plays a very important part in ionic regulation, particularly in potassium uptake, and it seemed to me that if that control were broken down it could rapidly result in toxic effects on the insect. That was one point I wanted to ask you about; the other was that in Lepidoptera, which as you say are a very specialized group, the larvae are characterized by having highly alkaline gut contents, and digestive enzymes, including proteases, which are active in extremely alkaline conditions. I wondered if that is a factor which should be considered in the activity and in the specificity of toxic action in Lepidoptera.

SOMERVILLE

These are both very good points: In fact, I am not dismissing the idea of ion transport — I am just suggesting that there is no evidence which indicates clearly the mode of action at this time. I think it is a distinct possibility. With regard to the second question: yes, it is very important. I think what I would like to see done as a first move is to compare the injection and ingestion toxicity of the purified toxin to see if it has the same effect. It is worth commenting that dissolved crystals are toxic when injected, so that once it is degraded, or solubilized, it does appear to be toxic. It is very difficult to comment on the ion transport hypothesis because, as you are probably well aware, the knowledge of these processes in insects is fairly rudimentary compared with either bacteria or mammals.

KARLSON

I have three questions — let me take the one pertaining to the

mechanism first. You are certainly aware of the work on the mechanism of action of cholera toxin and related toxins. Are there any resemblances in the toxins and in the possible mechanisms of action?

SOMERVILLE

Certainly I am aware of that and that is one thing that we are hoping to test. We are developing an assay for adenylcyclase and hope to test that directly.

KARLSON

I was very much interested in the exotoxin. You mentioned it was an analogue of ATP and that it would inhibit DNA dependent RNA polymerase. Does it work on all polymerases or does it have specificity towards one of the three major polymerases (A, B and C) now detected everywhere?

SOMERVILLE

To be frank, I don't know the details. I do know that it inhibits those polymerases against which it has been tested and no exceptions have been found.

KARLSON

Would a sample be available for testing? Dr. Seifart in our laboratory is working on polymerases — he would be glad to do so.

SOMERVILLE

My colleague, at Shell Research Ltd. Dr. Bond, has some and limited samples are available from him.

KARLSON

A last question: I was very much surprised that trypsin would not digest this toxin. There is certainly arginine present and presum-

ably also lysine. Why does it not attack this protein? Trypsin normally cleaves all lysyl and arginyl bonds.

GRANITI

I wonder if bacteria infections can limit the practical application of *Bacillus thuringiensis* in nature and also I would like to know what is the effect of phages on toxins released from bacteria and *Bacillus thuringiensis.*

SOMERVILLE

To answer Professor Karlson's question, we do not know what stops digestion — it looks as though it should but it seems to be peculiarly resistant to proteolytic digestion — in our hands, I should add.

On the question of phage — well — I think it is safe to say that all production of *Bacillus thuringiensis* is in batch culture — and it is unlikely that phage would be a major problem under these conditions. You might lose an occasional run if you have bad husbandry of your microbes. Recently there has been some work in the Soviet Union on continuous production; at Shell we looked at continuous production a long time ago. It is a general comment, I think, that phage does not seem to be a problem in continuous processes, largely because you have a continuous selection operating in the micro-organisms, so that you may get small oscillations in bacterial densities caused by phage, but where people have tried deliberately to infect cultures, they have always found a resistant population comes up. It is not in the phage's own interest, is it?

CANONICA

The idea to utilize microorganisms against other microorganisms or the other living organisms appears often for different purposes, but in the field experiments, for instance in chemical decontamination experiments, the results have always been bad. In fact, the strains of allogenous microorganisms introduced in a natural environment generally quickly disappear because they are not able to survive in those environmental conditions. The idea to utilize their toxins instead of the living cells

exhibits more chances of success: but in the case of proteic toxins there is the danger that their complicated and sensitive molecules can be unstable in field conditions.

SOMERVILLE

I do not know what the lifetime of the insecticide is once it is applied, but it is comparable to chemicals in the sense that chemicals disappear by photo-oxidation and solubilization. It is basically a problem of formulation. *Bacillus thuringiensis* can be applied just as a chemical, as wettable powder or in any of the classical ways of applying pesticides.

KNUSLI

You mentioned these very favorable toxicological pictures of such preparations. Now, just before I left for this meeting, somebody put on my desk, knowing that I would leave for this meeting, a Russian publication which appeared in '76 which says that persons using these preparations sometimes show symptoms of dermatitis, conjunctivitis and chronic inflammation of respiratory organs. Could you eventually comment on this point?

SOMERVILLE

I think everybody will be aware that when enzyme detergents were brought in which were based — some of them — on *Bacillus subilis* which is another spore-forming bacteria, there were some problems. With dermatitis now I suspect that expose any person to large amounts of bacteria, of any sort and they don't handle it with care, you might get dermatitis. Given reasonable precaution there is no risk involved.

ABO-KHATWA

You mentioned that endotoxins are less likely to affect mitochondrial coupling and oxidative phosphorylation and I think I agree with this because there is no incidence in literature where polypeptides can affect oxidative phosphorylation of mitochondria but on the other hand you mentioned that exotoxins do possess an enzyme which may be an analog

of ATP. Have you examined the influence of exotoxins on mitochondrial coupling? because it could be very likely that these nucleotides could interfere with the nucleotide transport across the membrane. And then you can get an uncoupling effect.

SOMERVILLE

It has been looked at but not by myself. All the evidence with exotoxin is consistent with its mode of action, being through polymerase?

SIDDAL

Could you say what are the results of digestion of the crystal solution or crystal using protease from insect gut preparation? Are there any more toxic compounds?

SOMERVILLE

When we have done these experiments with crystal solutions digested with gut juice from *Pieris brassicae* and *Trichoplusia ni* we have found a very similar pattern to that which I showed you. It is confused somewhat by the presence of proteins and pigments in the gut-juice. But it appears that the only product — major product — that is toxic is the same one.

BELL

Could I follow up Professor Karlson's question about the stability of this toxin? Again, have you done any end-group analysis? Is the compound possibly a cyclic polypeptide?

SOMERVILLE

We have done end-group analysis, and the N-terminal — I am quoting from memory — is serine. With carboxipeptidase we have results consistent with a normal sequence at the other end. There the exact sequence I cannot remember.

[*11*] II, 10 - *Somerville* - p. 21

BALLIO

Always related to the question of stability — Is it possible that toxin is an inhibitor of proteolytic enzymes? is one question; the second one: has it been tried if the molecule is attacked by proteolytic enzymes after denaturation, say, with urea 6N?

SOMERVILLE

There has been some work done on the inhibition of trypsin by binding of fairly crude preparations of the toxin to trypsin. I myself don't think this is likely to be sufficient to indicate that as a mode of action — this was work done by Faust and coworkers, some time ago. As to proteolytic digestion after denaturation, if you dissolve and treat with urea and then remove the urea, you still have the toxin — and we have not tried it after heating, or more rigorous treatment.

SHOREY

An essential reason for our emphasis on selective natural products in the protection of plants is the protection of naturally occurring parasites and predators which should be fostered by all possible means to enhance their capability to keep most potential pests at non economic levels.

THE USE OF VIRUSES IN PLANT PROTECTION

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INTRODUCTION

After thirty years in pursuit of knowledge in the fields of Insect Pathology and Microbial Control, I am continually reminded of the fact that there have existed scientists whose thoughts and goals in their work still are so original and so far reaching that now, in 1976, in Rome, those men should be here helping us with our deliberations.

I refer to men like Rucijka, in Austria, who proved that insect viruses could survive in the environment, by adapting obsolete World War I mortars to blast virus-contaminated trash and litter from the forest floor into the crown of trees infested by the Nun moth *Lymantria monacha*. The resulting viral epizootic decimated the population of insects and saved the trees.

I refer to Metchinkov, in Russia, who first used a fungus to combat the weevil, *Cleonis puntiventris*, and Forbes and Snow in the United States and to Steinhaus the North American father of Microbial Control.

The concept of using pathogens of destructive insects to control their numbers and prevent them from harming our food and fiber crops and stored produce is obviously not new. It is only in recent years that we have acquired the knowledge and the technology to exploit them to our advantage.

Actually, man is the creator of his own misery, trouble and tribulations. As you are all aware, as mankind increased in numbers he required more and more food. Soon he could not "live off the land" as it were. The result was the rapid creation of monoculture of food plants and plants that we harvest fiber from that make paper and clothing. These relatively enormous acreages of one type of plant encouraged insect species to propagate and build up into enormous numbers.

During the 1930's and 40's chemicals toxic for insects came into being and entomologists began to relax. Here was the answer to our self created problems. Then gradually, instead of the narrow minded concentration on our difficulties with one harmful species, we realized what desecration we were imposing on our environment. It became important to any thinking individual that we must devise some means other than poisoning the land where crops are grown.

Insect Viruses

All of the insect viruses isolated to date are of the same types as those that cause disease in vertebrates and in plants. Only the Baculoviruses (BV) are unique in that they are rodshaped, enveloped, double-stranded DNA viruses. These will be discussed later.

About 603 viruses of all types have been isolated and the preponderance of them are BV from Lepidoptera [1]. Table I describes the various types of insect viruses known today.

Some years ago Dr. Angus, the director of the Insect Pathology Research Program in Sault St. Marie, Ontario, Canada, and I discussed the attributes one should wish for in the "perfect" pathogen for insect control. We arrived at a set of criteria that are so stringent that to date none of the developed control agents, nor those under development, satisfy them all. A great deal of research has gone into supplementing these missing attributes in the formulations now available. These requirements are as follows:

1) The pathogen should be virulent and should retain that virulence. It should kill quickly in order to give maximum protec-

TABLE I — *Types of Insect Viruses.*

Virus Type By Genera	Symmetry of Nucleocapsid	Size of Nucleocapsid	Type of Nucleic Acid	Inclusion Body
Baculoviruses	Rod shaped	(40-70) X (250-400nm)	DNA 2**	+
Cytoplasmic Polyhedrosis (Is*) (Reovirus)	Spherical (Is*)	60 nm	RNA 2	+
Entomopoxvirus	Brick shaped	(170-250) X (300-325nm)	DNA 2	+
Iridovirus	Spherical (Is)	130-180 nm	DNA 2	—
Parvovirus	Spherical (Is)	60 nm	DNA 1	—
Enterovirus	Spherical	16-40 nm	RNA 1	—
Rhabdovirus	Bullet shaped hollow	70 X (130-220)	RNA	

* Is = Icosohedral symmetry ** Number of strands of nucleic acid.

TABLE II — *Viruses Used to Control Hymenopterous Insects Feeding on Trees.*

Insect Species	Virus Type	Results	Location
Diprionidae			
Gilpina hercyniae Eur. spruce sawfly	NPV	Excellent	Europe N. America
Neodiprion lecontei Red-headed pine sawfly	NPV	Excellent	N. America
Neodiprion pratti Banksianae Jackpine sawfly	NPV	Fair to poor	N. America
Neodiprion pratti Pratti Virginia pine sawfly	NPV	Good	N. America
Neodiprion sertifer European pine sawfly	NPV	Excellent	Europe N. America
Neodiprion Swainei Swaine's jack pine sawfly	NPV	Good	N. America

tion of the plant. It should demonstrate adequate efficacy in the field.

2) It should be resistant to harmful factors in the environment such as solar radiation, drying, changes in pH, heat, etc.

3) It should have a resistant or dormant stage in its life cycle, that permits production and storage, without loss of viability, for relatively long periods of time (at least nine months to a year).

4) It should be relatively inexpensive to produce in large quantities.

5) The organism must be safe and innocuous for all other living forms, plant and animal.

Considering the virus types that have just been reviewed, it is obvious that those viruses that best fulfil requirement, three, are the Baculoviruses, comprised of the nuclear polyhedrosis viruses (NPV) the granulosis viruses (GV) and the entomopox viruses (EPV).

Naked, non-occluded virions are treated very harshly in the environment, but the protein crystal, that surrounds the particles of these occluded viruses, protects them since they remain viable in soil and in cadavers above the ground for months to years [2, 3, 4].

Since the Baculoviruses have been the most extensively used, throughout the world, I will deal with them almost exclusively herein.

Production

Since insect viruses are obligate parasites they can only grow in a living cell, thus the intact insect or tissue culture cells derived from intact insects are used for virus production. In the past, the viruses used in test and control programs has been obtained in two ways. A large number of investigators have collected diseased larvae, in large numbers, in the field. The viral preparations, thus obtained, were "dirty" preparations and contained other microorganisms that could confuse the results of tests. It is not uncommon

TABLE III — *Viruses Used to Control Lepidopterous Insects Feeding on Trees.*

Insect Species	Virus Type	Results	Location
Dendrolimus sibericus Siberian pine caterpillar	GV	Good	Russia
Dendrolimus spectabilis Pine caterpillar	CPV	Good	Asia
Malacosoma fragile Great basin tent caterpillar	NPV	Fair	N. America
Malacosoma disstria Forest tent caterpillar	NPV	Good	N. America
Hemerocampa pseudosugata Douglas fir tussock moth	NPV	Excellent	N. America
Lymantria dispar Gypsy moth	NPV	Good	Europe N. America
Thaumetopoea pityocampa Pine processionary moth	CPV	Excellent	Europe
Kotochalia junodi Wattle bagworm	NPV	Good	S. Africa

to find a protozoan or a virus co-existing with yet another virus in the same host larva.

Another method is to mass rear larvae of a species, under controlled conditions, on artificial media. The amount of virus collected from these animals, after infection is quite astounding and is quite economical to produce. For example a single larva of the cotton boll worm *Heliothis zea* produces, on the average, 6 billion polyhedra and may produce as high as 13 billion [5].

This was the basis for the establishment of an international unit called the Larval Equivalent (L.E.) which was to be equal to 6×10^9 polyhedra. This unit was agreed upon by an international committee at the Colloquium on Insect Pathology at Wageningen, The Netherlands, in 1966. It is startling to realize how many species, particularly in the Lepidoptera, can be reared on semi-artificial or artificial media. Both viruses, registered for use in the

TABLE IV — *Selected Examples of Viruses Used to Control Lepidopterous Insects Feeding on Food and Fiber Crops.*

Insect Species	Virus Type	Results	Location
Gelechiidae			
Phthorimaea operculella Potato tuber worm	GV	Excellent	Pacific
Noctuidae			
Agrotis segetum Cotton cutworm	GV	Good	Russia
Autographa sp.	NPV	Excellent	Russia
Hadena sordida Cereal noctuid	GV	Excellent	Russia
Heliothis sp. Bollworm, tobacco budworm	NPV	Excellent	N. America Russia S. America
Prodenia praefica Western yellow-striped armyworm	NPV	Good	N. America
Spodoptera exigua Beet armyworm	NPV	Excellent	N. America S. America
Spodoptera litura Cotton leafworm	NPV	Excellent	Africa Middle East
Trichoplusia ni Cabbage looper	NPV	Excellent	N. America S. America
Colias eurytheme Alfalfa caterpillar	NPV	Excellent	N. America

United States, are produced in mass-reared insects. They are the Heliothis virus, produced by Sandoz Inc. under the trade name Elcar and the NPV from the Douglas fir Tussock moth, *Hemerocampa pseudsugata*, produced by the Forestry Service of the United States Department of Agriculture.

Perhaps the most sophisticated approach to viral production is the development of highly specialized insect cell lines, from various species, to propagate the nuclear polyhedrosis viruses. It would now appear that the technology to accomplish this is very close to hand.

FIG. 1 — Baculovirus
(Nuclear polyhedrosis virus-MEV)
a. Carbon replica of *Trichoplusia ni*
b. Section of *Autographa californica*

FIG. 2 — Baculovirus
(Nuclear polyhedrosis virus - SEV)
a. Shadowed polyhedra of *Heliothis zea*
b. Section of *Heliothis zea*

Micron scale on all « a » plates = 1nm; on 6a and all « b » and « c » plates = 0.1nm

Over the past two decades, data on the physiological and nutritional requirements of cultured insect cells has slowly been acquired, and several investigators are now moving to the use of pilot-plant-scale fermentors to produce cells and subsequently the viruses in the cells.

Within this time, specialized cell lines from many species have been isolated, selected and adapted to media that contain inexpensive components such as yeastolate, lactalbumin and tryptose broth that replace costly pure chemicals that were originally used.

Certainly the systems and technology developed to produce vaccines for human and animal viral diseases can feasibly be adapted to production of insect cells. Insect cells in suspended culture have been grown in 2.5 liter volumes of media, although aeration must be carried out in volumes over 250 ml.

It is the concensus that the current yields of cells (1×10^7 cells/ml) is not sufficient; however, if 1×10^8 cells/ml can be obtained, the technique will become commercially feasible and a Larval Equivalent would cost *circa* 4.5 cents, a competitive figure. This goal, I have been assured, is ammenable to research (personal communication Vaughn). There are companies with costly equipment used to produce viral vaccines, that could change over to insect virus production in periods when they are not producing animal viruses, thus making maximum use of their plant. Never-the-less it is certain that insect viruses will cost more to produce than chemical insecticides.

Viral survival in the environment

Most viruses now under development are indigenous to the country where the studies are carried out. Indeed, many of these viruses cause massive epizootics on an annual or a cyclic basis. Unfortunately, these epizootics usually occur too late in the larval life to prevent damage to the host plant. The epizootics usually originate from virus persisting in the soil. In two studies the *T. ni* NPV was found to survive for 6 years [4] and for nine years [3].

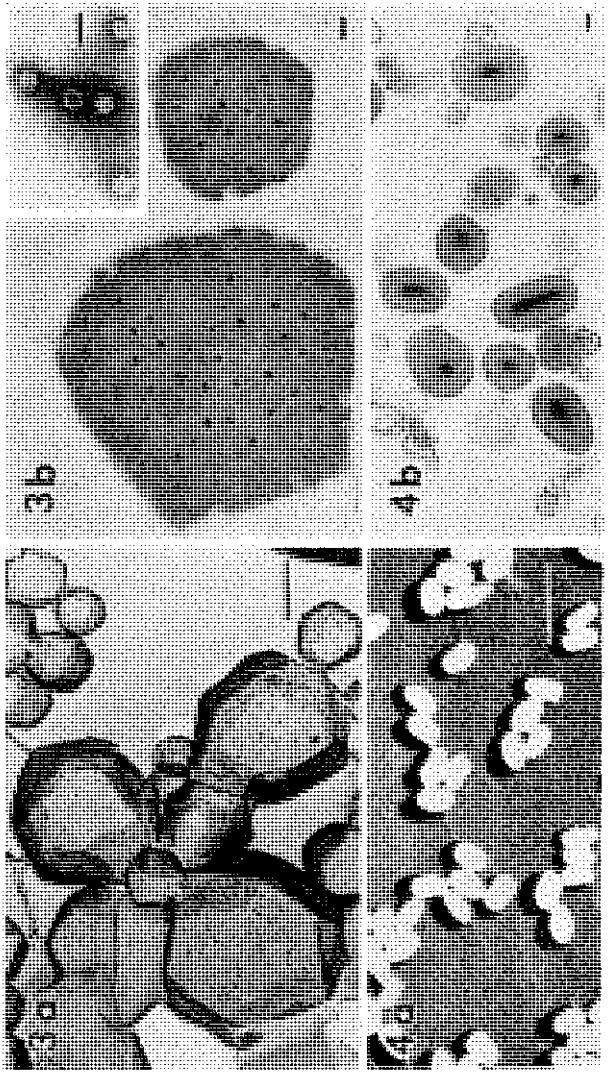

FIG. 3 — Cytoplasmic polyhedrosis virus
a. Carbon replica of *Pectinophora gossypiella* - CPV
b. Section of *Ceramica picta* CPV - insert - negatively stained virions of *Pectinophora gossypiella* CPV

FIG. 4 — Granulosis virus
a. Shadowed capsules of *Estigmene acrea* GV
b. Sections of capsules of *Lespeyresia pomonella*

Micron scale on all « a » plates = 1nm; on 6a and all « b » and « c » plates = 0.1nm

DAVID and GARDINER [6] showed that the granulosis of *Pieris brassicae* is not seriously reduced in soil and sand after two years.

Splashing of virus-containing soil on young plants or blown on by the wind likely are the most common ways in which the epizootics begin. In forest stands the gypsy moth larvae frequently crawl down to the trunk of the tree before dying from virus infection and these sites protect the virus over winter and are sources of infection the next year [2].

Once the larvae begin to die on a plant they contribute vast amounts of fresh virus to the plant surface. A virus killed larva is very fragile and breaks open even at the touch of a raindrop and spreads out on the leaves while invariably some washed off into the soil. In a classical study of the spread of virus in a population of the spruce sawfly, *Gilpinia hercyniae*, EVANS and ENTWISTLE [7, 8], showed that predators and parasites as well as some birds pass unharmed virus through their guts and depending on the range of their activity can spread virus from yards to kilometers away from the original feeding site.

It is the experience of most investigators that most of the Baculoviruses do not survive long in nature except in the soil. Several adverse parameters have been identified and studied. The most obvious and possibly the most destructive single agent is sunlight. According to MORRIS the atmospheric ozone layer filters out some of the germicidal wavelengths except those between 290 nm and 380 nm [16, 12]. This range of wave length comprises about 7% of the total sunlight reaching the earth's surface. Less active germicidal effects on pathogenic bacteria and viruses occur from light between the longer wave lengths 264-366 nm [17, 12].

A great deal of effort has gone into research on screening substances that reflect or absorb critical wave lengths of sunlight, [9, 10, 11]. Industry has also developed adjuvants that protect viruses after application. The content of these materials are unknown due to the necessity of obtaining patents of the formulation. It is interesting that the body contents of the virus killed larvae is an efficient protectant. The dark color and the protein content are involved [1].

Molasses has been used for some time as a protectant and as a

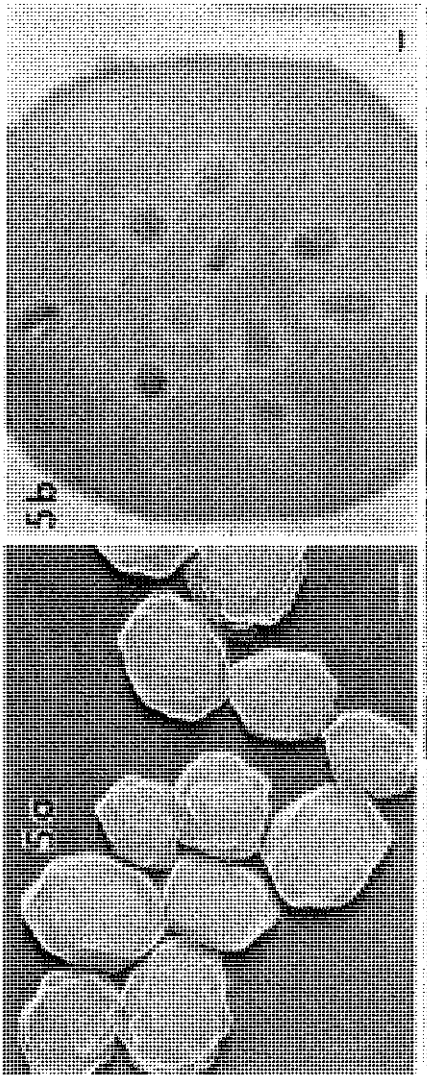

FIG. 5 — Entomopoxvirus
a. SEM of *Euxoe auxiliaris* entomopoxvirus
b. Section of *Melanoplus sanguinipes*

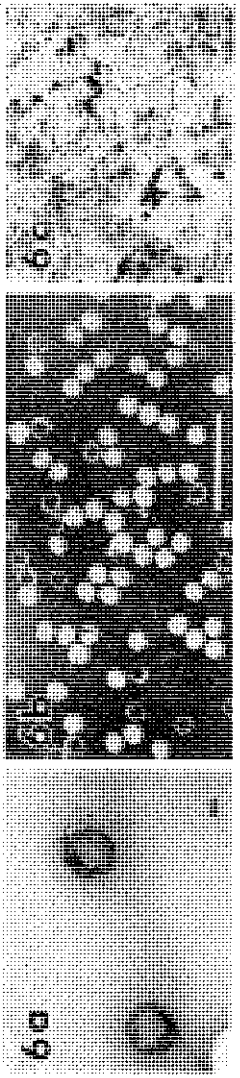

FIG. 6 — Nonoccluded viruses
a. Mosquito iridescent virus
b. Densonucleosis virus (Courtesy Kurstak)
c. Sigma Virus (Courtesy Teninges)

Micron scale on all « a » plates = 1nm; on 6a and all « b » and « c » plates = 0.1nm

sticker. Although its choice was likely the result of an empirical screening investigation MORRIS discovered that molasses absorbed wavelengths below 360 nm and transmitted above 380 nm, where beneficial energy is most effective [21]. It is nice to have scientific proof to explain its action.

Heat is also implicated in reducing viability of virus preparations both before application, in storage and in the field close to the Equator.

It is interesting that plants may excrete or secrete substances harmful to Baculoviruses. The protective polyhedral protein crystal is normally dissolved by treatment in alkaline-carbonate solutions. The same process occurs in the lepidopterous larval gut where the pH is frequently over pH 9 and the contents are highly reducing [20]. Epstein and colleagues [18, 19] found that part of polyhedral protein is an alkaline-protease capable of breaking down the crystalline polyhedral protein. Leaves of the cotton plant secrete alkaline salts that, when wet by dew, activate the enzyme in the polyhedron causing dissolution of the protein crystal and rapid death of the exposed virions. This explains the previously incomprehensible loss of virus when applied at dusk in the presence of a heavy dew. Accordingly, buffers have been added to virus preparations to counteract this effect on cotton. T.R. Shieh, of Sandoz Incorporated, has conducted much research on protective formulations. One of the interesting results is that a formulation that works well on one crop plant may not do well on another host plant. This may mean that formulations may have to be tailored to a host plant in some cases (*).

Safety of viruses

It is becoming generally accepted that insect *Baculoviruses* are innocuous for living forms other than insects. This is based on extensive testing on several insect viruses. For those that are interested in this subject, the latest review was written in 1973 by

(*) Personal communication.

C.M. IGNOFFO [13]. Generally the following tests have been conducted, in part or in total on rodents, game, animals, fish, wild birds, wild ungulates, estuarine animals (shrimp and oysters), monkeys and bees. Acute oral feeding tests, acute dermal toxicity, inhalation, eye sensitivity, dermal sensitivity, subacute oral toxicity (90 day), carcinogenicity tests, teratological tests, intraperitoneal injection, intracerebral injection, intradermal injection, subdermal injection, intramuscular injection and intravenous injection have been carried out. In addition, free viruses have been inoculated in tissue cultures of a number of cell lines from humans and other animals. The results of all these tests have been negative, with the following rather puzzling exceptions. HIMENO *et al.* [14] extracted active DNA from the silkworm NPV virions and introduced it into a culture of FL cells. He detected the formation of polyhedra in the nuclei of the mammalian cells. To my knowledge, these scientists were unable to repeat this experiment and several other competent scientists in the world were unable to reproduce these results.

In another instance, MCINTOSH and SHAMY [15] treated a viper snake cell line culture (VSW) with virions of the alfalfa looper (*Autographa californica*) virus from insect tissue culture. The virus was labelled with (^{3}H) thymidine. Apparently the virus entered the cells of the VSW line and congregated in or around the nucleus as granules. Some viral proteins were apparently produced by the cells, but no replication of the virus was detected [16].

Application of viruses

In the rush "to get ahead with the job" of developing and testing insect viruses, most investigators had borrowed application equipment designed for the dissemination of toxic chemicals, both for ground and aerial applications. This is in many ways unfortunate although there is a certain awareness developing now, that in some cases, equipment may have to be adapted radically or specifically designed in order to obtain maximum results.

Viruses must be ingested in certain numbers in order to infect the insect. This means that absolute coverage of the plant surface

must be achieved. It is then clear why equipment designed to apply a contact insecticide might be totally inadequate for application of a virus. Most chemical insecticides are so toxic that only a very small amount is required for total success; however, the insect must consume a minimal lethal dose of virus before infection and death occur. In many food and fiber crops the leaves grow so rapidly that the plant literally grows away from the virus contaminated area. This coupled with the fact that the virus is dying steadily, over time, requires more applications than do chemicals.

The importance of formulation and specialized application equipment that is as inexpensive as possible cannot be over-emphasized.

Recently Falcon (*) recommended the use of a spray unit that can deliver droplet sizes of from 1-20 nm. Using ultra-low volume sprays with oil emulsion he achieved good results using bacteria and a virus.

This problem of coverage is particularly acute in forest treatments. Using aeroplanes, good coverage is difficult to achieve and the results are not as good as the protection of trees in park areas using ground application equipment permitting excellent coverage. Helicopters will often do a better job than possible from an aeroplane, over forest stands due to the roiling action of air from the blades.

Conclusions

Except in the rare cases where viruses are capable of decimating insect populations and then holding them below the economic threshold, the insect viruses will be used most effectively in integrated control programs. They will, however, be important tools because of their specificity. It is important to make available as many virus species as possible since the trend today is to manage pest populations. Eradication of a pest is a word that is rarely used now by enlightened entomologists. I can remember Carroll Smith

(*) Personal communication.

once saying to me « Eradication is really quite easy — why we have eradicated the Mexican fruit fly from Florida three times! ».

The introduction of viruses into integrated control programs will require a great amount of work in the field so that they may be used with knowledge. It is my concern that there are far too few competent people working on this aspect of microbial control. Perhaps this is a normal development of a new field and one phase of research must precede another.

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DISCUSSION

SHOREY

I am interested in this question of specificity of virus attack against hybrid species, and particularly — although you only alluded to it in your talk — I am curious about this lack of specificity of *Autographa californica* virus, and as to whether there are any general rules as to degree of specificity, and furthermore, is it true that the *Autographa californica* virus is indeed more virulent against some species other than *Autographa californica* than it is for its own species, and how does this relate to the question of specificity?

HEIMPEL

It would appear that the multiple embedded viruses are less specific for Lepidoptera than are the single embedded viruses such as the *Baculovirus heliothidis*. The *Autographa californica* virus bas the broadest spectrum of activity that I have ever encountered; to date this virus has been shown to infect and kill lepidopterous larvae from eight families. There is every reason to predict that far more species are susceptible. Generally, the noctuids are infected by this virus, to one degree or another. Oddly enough the tobbaco budworm, *Heliothis virescens* is very susceptible but *H. zea* is somewhat resistant. We are investigating this anomaly.

BRADER

I was a little bit astonished about your remark — you said that the viruses could maintain that activity for a very long period. As far as my information goes, one of the worries about the viruses is still that they are pretty easily decomposed after they are sprayed on the plants and it is for that reason that you have to spray them pretty often to guarantee success.

HEIMPEL

Yes, you are right and you are wrong. The viruses are very susceptible to ultraviolet radiation. I had to jump the whole section on that in my talk due to the lack of time before lunch; however, you will be able to read what I said about it in my paper. Most of the virus survives in the soil; David and his colleagues found that the granulosis virus of *Pieris brassicae* was not reduced in viability after two years in soil and sand. Thomas found that cabbage looper virus survived in fields after nine years. Another report cites survival after 6 years. So they do remain for a long time in the soil depending on the type of soil. For example, alkaline soil is very likely harmful to the virus.

BRADER

You talked about the cost of production and the possibility that it might really come to commercial use — that it might become acceptable for commercial use once the cost is no longer restrictive. Are you referring there only to those viruses which are related to insects which are among the most important insects in agriculture, or do you feel that also goes for insects of little economic importance for the whole of agricultural production but which may still be very important for certain crop growers?

HEIMPEL

The production of viruses for insect control is going to be as expensive as the production of chemical insecticides in those countries that have extensive regulations regarding the registration of pesticides. In these countries the cost can only be justified by the seriousness of the depredations by the pest insect and the size of the market. Since there is convincing evidence that the Baculoviruses are safe, the production and use of insect viruses for pest control in developing countries should be less expensive and should be seriously considered.

SOMERVILLE

You commented on three possible ways of producing these viruses: first of all, from dirty preparation in the field, and then by bucket pro-

duction in caterpillars, and thirdly you mentioned tissue cultures. Don't you think that the first two methods are likely to be ruled out because of the difficulties in excluding the possibility of having other viruses which infect mammals?

HEIMPEL

Well, you are quite right, the best way to produce them would be in tissue culture because the product is virus and just a very small amount of insect tissue. But most viruses that are produced now commercially in the United States are grown in insects reared on artificial media. I think that this is the method that will be used for some time to come, especially in developing countries, until the technology of the tissue culture cell production has been fully developed.

SHOREY

I am curious as to the major factors that have held back the demonstration of efficacy of viruses, and specifically, I can still remember 20 years ago a report by McEwen in Canada, in which he in a very gross fashion ground up a little more, I think, than about five to six virus infected larvae. McEwen ground up just a few larvae of *Trichoplusia ni* and sprayed the material onto cabbage fields and reported almost complete epizootic of the caterpillars in the field. That was a really exciting finding and seemed to indicate that this sort of approach, despite problems with environmental breakdown and such, might very rapidly be developed; yet that was 20 years ago, and I wonder what have been the major influences in causing this exciting concept not to develop into something very important.

HEIMPEL

Well, the first virus to be developed commercially did not show up too well as far as efficacy is concerned until proper formulation was developed. The reason for poor efficacy was that cotton bollworm, *Heliothis zea* feeds on the terminals of the plant as a young larva. Not only do the terminals grow very fast, growing away from the virus sprayed area but they fully expose the virus to harmful ultraviolet

radiation. Furthermore, the cotton plant produces an alkaline material which, when wet by dew, destroys the polyhedra very rapidly and dissolves them, and the exposed virions are killed very rapidly. On the other hand, on cabbage and on cole crops the cabbage looper, *T. ni*, is a negatively geotropic feeding insect, and the virus survives far longer underneath the leaves where the animal feeds; consequently it survives longer and gives adequate protection.

GRANITI

I wanted to know if you have some idea how high is the probability that natural mutation — occurs in the population of viruses — this is a general question including ? beetles virus that can originate strains which can be pathogens to other animals or insects of plants.

HEIMPEL

Yes, that is a question that is always asked at these meetings, and I think that I could quote Dr. Steinhaus in saying that when you have an obligate parasite like the virus, that grows in such a radically different environment in the insect as compared to a plant or in a vertebrate, the possibilities of the numerous and possibly sequential mutations that would have to occur to make it pathogenic for another type of host are very, very rare, and a very low possibility.

GRANITI

Yes, but you know that there are viruses which are transmitted from insects to plants, and vice versa. In such a case they affect both hosts: an animal and a plant. This is the danger, I think.

HEIMPEL

No, as I pointed out, the Baculoviruses are unique and it is highly doubtful that they would be able to adapt to another group of animals or plants.

KNUSLI

Could you please give me some additional help for the understanding of the answer you gave to Dr. Brader. You pointed out that in principle the big problem for producing the material is that for the time being you still need insects. Now, on the other side you said that in the environment these viruses persist for a substantial time. Now, if this is true, which are the conditions which allow them to survive and eventually to multiply under this non-specific condition, when no insects are in the game?

HEIMPEL

I think their survival depends upon this polyhedral protein crystal. This is an extremely inert protein that surrounds them and protects them in the soil. As I have said, the viruses cause mass epizootics of insects in nature and in this way constantly replenish the pool of virus in the environment; however, in most cases the insect larva is killed after major damage is done to the plant. The use of virus early in the insect's development insures kill and control before plant damage occurs.

III

ARTHROPOD CONTROL THROUGH NATURAL PRODUCTS

Juvenile hormones - Pheromones
Natural products and the environment

THE JUVENILE HORMONE AS AN AGENT FOR PEST CONTROL

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I shall begin by saying something about the physiological effects of the juvenile hormone. The activities of this hormone were described long after the existence of the "moulting hormone" (whose action was first described by KOPEČ in 1917) was known. It was at once recognized as a "morphogenetic hormone". I recall that on seeing the experiments in about 1935 Boris Ephrussi expressed his interest on the grounds that this was the first clear example of a morphogenetic hormone.

It was described as inhibiting the differentiation of adult characters — and thus preventing metamorphosis. I called it the "inhibitory hormone" (WIGGLESWORTH, 1934). Adopting the same interpretation more recently Carroll Williams has called it a "status quo" hormone. But by 1940, for various reasons, I had come to think of it rather as actively evoking larval characters — presumably by action very close to the gene system in the chromosomes. It was therefore named the "juvenile hormone".

The juvenile hormone has another morphogenetic function: as shown by MARTIN LÜSCHER (1973), different dosages, perhaps different chemical species of juvenile hormone, are responsible for the changes in form which lead to the different castes of many termites. There is some evidence that polymorphism in Aphids and the phase changes in locusts may be similarly controlled. It is likewise invol-

ved in another polymorphic change, the development of the queen honey bee.

But the juvenile hormone has other effects which are more readily thought of as being effects on metabolism. In the adult of *Rhodnius* and many other insects, the same juvenile hormone is necessary for yolk formation in the oöcytes of the female, and for the activity of the accessory glands of the male, which produce the spermatophores (WIGGLESWORTH, 1936). The nature of these effects is uncertain; perhaps the more probable mode of action is by controlling the synthesis of specific enzymes in the fat body and in the reproductive system. But effects on membrane permeability cannot be excluded. Arrest in secretion of the juvenile hormone is the immediate cause of adult diapause (that is, reproductive diapause) in the potato beetle *Leptinotarsa* (DE WILDE and BOER, 1961). That is probably true in some other insects also; but in many insects neurosecretory cells in the brain are even more important in controlling reproduction.

The extensive use of juvenile hormone by the male accessory glands is of interest because it was from the adult male cecropia silkmoth that CARROL WILLIAMS (1956) was able to obtain active extracts of juvenile hormone. The female silkmoth matures her eggs while still within the pupa, and the female after emergence contains much less juvenile hormone than the male which, like other Lepidoptera, secretes large numbers of spermatophores. Indeed the juvenile hormone seems actually to be stored in the male accessory glands (DAHM *et al.*, 1976).

It is a fact of great historic interest that when, for the first time, he had obtained active extracts of juvenile hormone, Williams put forward the view that once we knew its chemical composition we should have the perfect insecticide. At that time Carroll Williams was on sabbatical leave in my laboratory in Cambridge and he showed me the draft of his letter to *Nature* with this suggestion in it. I said that I did not believe it. And I still do not believe it. But what an effect that innocent remark has had on the activities of chemists during the past 20 years!

Why am I not attracted to this idea? For the same reason that I am not attracted to the idea of using thyroid or pituitary hor-

mones for the control of rabbits. Hormones are potent biological agents; but they are subtle in their activity. And because they are potent and potentially dangerous, the animal is well equipped to deal with them and to inactivate any excess.

Experiments on the application of juvenile hormone to the 5th-stage larva of *Rhodnius* were illuminating. The obvious procedure was to inject a strong emulsion of the oily extract from cecropia. It was completely inactive (WIGGLESWORTH, 1958). The hormone was presumably metabolized and inactivated by the insect. (GILBERT and SCHNEIDERMAN (1960) got the same results on other insects). The oily extract was effective if applied to the intact cuticle: it was then slowly but continuously absorbed in small amounts.

More recently I have made quantitative assays along these lines (WIGGLESWORTH, 1973). I have found that, if, as the result of starvation the cuticle of the 5th-stage larva of *Rhodnius* is very thin, large applications of purified hormone to the cuticle surface are without effect: the hormone is absorbed too rapidly and is quickly inactivated. If larvae with cuticle of normal thickness are used a pure solution of juvenile hormone (JH-1) in octane applied to the surface is quite active (a dose of 330 ng per insect gave a standard 50 per cent effect). But if the hormone was diluted 1:1000 times in olive oil the effect was greatly increased (a dose of 15 ng gave the same standard 50 per cent effect). The triglyceride protects the hormone by inducing very slow absorption (cf. GILBERT and SCHNEIDERMAN, 1960).

Williams' original idea was that exposure to excess juvenile hormone would lead to the production of non-viable intermediates between pupae and adults. RIDDIFORD, AJAMI and BOAKE (1975) exposed a confined population of *Blattella* to a food bait containing 0.01 per cent of a wide range of the most active JH mimics. But even with the most effective compounds the population had not been completely exterminated by the end of seven months. EDWARDS (1975) has described successful control of a natural population of the ant *Monomorium* by means of a bait containing 0.5 per cent of Altosid (isopropyl-11-methoxy-3,7,11-trimethyl-dodeca-2,4-dienoate).

But an entirely new slant was given to the problem by the ob-

servations of SLAMA and WILLIAMS (1966*a*) on the so-called "paper factor", which occurs in paper made from balsam pulp and which causes abnormalities of metamorphosis in larvae of the linden bug *Pyrrhocoris apterus* when this insect rests on such paper. The active substance, which turned out to be the methyl ester of todomatuic acid (BOWERS, 1966) is not closely related chemically with the juvenile hormone. It proved exceedingly active in reproducing juvenile hormone effects in bugs of the family Pyrrhocoridae, but it is completely inactive in other insects.

It was these observations which have led to the search for so-called juvenile hormone mimics: chemicals, often not closely related to the natural juvenile hormone, which have more or less disruptive effects on insect development.

It was soon found that the "paper factor" had very damaging effects on reproduction: small doses applied to the surface of the cuticle of adult *Pyrrhocoris* either sterilized the female or caused her to lay sterile eggs or eggs giving rise to non-viable offspring (WILLIAMS and SLAMA, 1966*b*).

Similar effects were obtained by RIDDIFORD (1970) after applying juvenile hormone to the eggs of the cecropia silkmoth; and by PATTERSON (1974) on the mosquito *Aedes*. A popular material for use in such experiments has been a chlorinated mixture derived from farnesoic acid, produced originally by LAW, YUAN and WILLIAMS (1966). Farnesyl methyl ether has heen used, and the highly sophisticated products of the Zoecon Corporation, notahly "Altosid", which are more distantly related to the natural hormone.

In spite of this chemical diversity the effects are often described as juvenile hormone effects, and are often thought of as reproducing the physiological action of the juvenile hormone. A widespread effect of juvenile hormone and its mimics, occurring in almost every phylum of the animal kingdom, as has been emphasized by DAVEY (personal communication), is that it induces neurosecretory cells in the central nervous system to discharge their secretion. That is an effect also of many insecticides, which cause the discharge of diuretic hormones (MADDRELL and CASIDA, 1971).

It is not known to what extent the juvenile hormone is involved in the regulation of embryonic development. Large doses applied

to the eggs produce abnormalities of growth — but that does not necessarily illuminate the nature of juvenile hormone activity in normal emhryonic development.

RIDDIFORD (1972) following SLAMA and WILLIAMS (1966), has argued that the juvenile hormone (and JH-mimics) inhibit the transformation from egg to larva just as the juvenile hormone inhibits the change from larva to pupa. But I am not aware of any physiological evidence for this idea.

What action could one expect the juvenile hormone to have on the emhryo? The adult pattern is already latent in the epidermis of the *Rhodnius* larva at the time of eclosion from the egg: moulting induced in the ahsence of juvenile hormone reveals this latent pattern (WIGGLESWORTH, 1934).

Shortly before eclosion the maturing embryo lays down a thin smooth cuticle which invests the individual appendages. The surface of this cuticle shows no cuticular specialization, apart from the spicules on the front of the head which probably aid in displacing the egg cap. Ecdysis, with shedding of this embryonic cuticle to reveal the definitive first stage larva, occurs during the act of eclosion (SIKES and WIGGLESWORTH, 1931).

During the hours which precede eclosion, the epidermis presumably becomes competent to lay down the alternatives of larval or adult integument. At this time the juvenile hormone must be responsible for maintaining the larval pattern. If that is so, the application of the "anti-juvenile hormone" agent, "precocene" (BOWERS *et al.*, 1976) during this final critical phase in the egg would be expected to result in the appearance of adult characters in the newly hatched larva.

Dr. Micciarelli-Sbrenna working with Prof. Colombo at Ferrara has recently found that farnesyl methyl ether applied to eggs of the locust *Schistocerca* can induce the development of bristles in the embryonic cuticle, which is normally devoid of all setae. This could be interpreted as a "premature larvalization". It looks like a morphogenetic effect. But in addition there are effects leading to completely ahnormal embryonic development and these are clearly toxic effects. Thus we seem to have teratological effects due to toxic action on the cells early in development on the one hand, and to

disturbances in development more or less related to the normal effects of the juvenile hormone on the other.

A new juvenile hormone mimic with trade name "epofenonane" (VOGEL *et al.*, 1976), which has proved equal in effectiveness with standard insecticides against various mealy bugs, has shown some physiological effects of interest. The anal plate of pronymphs in mealy bugs is smooth; but it shows characteristic pores in the nymph; and their number increases still more in the adult male. Nymphs exposed to epofenonane will moult several times and the pores disappear progressively from the cuticle. This seems to be another example of a partial reversal of metamorphosis, as first described in the intact adult *Rhodnius* (WIGGLESWORTH, 1939) and in implanted fragments of the integument in Lepidoptera (PIEPHO, 1939), and since observed in a number of other insects. In addition many treated mealy bug larvae die during ecdysis and females are sterilized by suppression of development of the ovary.

It was found by RIDDIFORD (1972) that in some cases, where eggs treated with juvenile hormone mimics have produced apparently normal larvae, these have suffered a delayed effect: the corpus allatum secretion has not been turned off at the appropriate time and giant larvae or intermediates have been produced.

A converse delayed effect was described in *Rhodnius* (WIGGLESWORTH, 1948). Transplantation of corpora allata from moulting 5th-stage larvae or young adults into young larvae of 2nd or 3rd instar, caused these to show a precocious development of adult characters when they reached the 5th instar. I was inclined at the time, to ascribe this effect to absorption or breakdown of juvenile hormone by the implanted adult corpus allatum; but later I preferred the idea that the implanted corpus allatum had weakened the activity of the corpus allatum in the host insect. These results were the converse of those of Riddiford: in her experiments the corpus allatum appeared to have shown persistent secretion of juvenile hormone. In my experiments the corpus allatum secretion appeared to have been partially suppressed. However, when my 5th-stage larvae moulted to become adults they still retained partially larval characters: the corpus allatum was still secreting some juvenile hormone.

When HUNT and SHAPPIRIO (1973) applied juvenile hormone analogues to developing embryos of *Lygaeus*, they found that the colour pattern of the early larval instars was retained in later instars. These authors point out that there are many parallels to these phenomena in vertebrates: "treatment of mammalian embryos with androgen or oestrogen, prior to the onset of endogenous secretion of these hormones, causes major changes in the quantity and pattern of their secretion later in development".

Turning to the application of these ideas to pest control: what we are doing, as I see it, is looking for new insecticides, the chemical basis of which was originally given by the natural juvenile hormone, but which have been subjected to all kinds of empirical variations. The result is a new family of insecticides. The hormonal source of these materials is not particularly relevant — but since "insecticide" is sometimes regarded as a dirty word it may well be good politics to refer to these materials as "hormones" or "bioregulators".

The potential toxicity of "third generation insecticides" was discussed by ANDRE S. MEYER (1972) who pointed out, for example, that natural juvenile hormones and many JH-mimics are epoxides, and that epoxides are alkylating agents with carcinogenetic properties. But epoxide formation is not an essential element in the structure of JH-mimics.

I do not, of course, venture to predict the future of those insecticides based (often rather remotely based) on the chemical structure of natural hormones — which I imagine is what we mean by "third generation insecticides". But to my mind the important thing is that biologists are now once more fully involved in the devising of methods for pest control. The era of the chlorinated hydrocarhon insecticides which followed the initial triumphs of DDT was characterized by the domination of the chemist. The modest entomologist accepted his inferior status and ahandoned his biological principles when faced by the wizardry of the chemist. As we all know that proved disastrous. To-day the entomologist has regained his self-confidence; he is once more coming into his own, and in co-operation with the chemist is looking afresh at the operation of pests as living organisms. That is the really promising

revolution: the return to "economic entomology", to "integrated control", to "pest management" as they were practised in the past — but now fortified by increasing knowledge of chemistry, physiology and ecology.

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DISCUSSION

MARINI-BETTÒLO

I thank you very much for your stimulating presentation. I think that the solution can be given in the future by the efforts of both chemists and biologists in this field.

STAAL

The effects on locust embryos you have described were obtained with farnesyl methylether which I consider to be a rather poorly active juvenile hormone analog. I would not entirely exclude the possibility that it is so poor in this respect that it may be behaving as a juvenile hormone antagonist similar to what I found in some other compounds that I will describe later. It would be very helpful to our understanding if the described effects could also be produced by the natural juvenile hormones or very active analogs.

WIGGLESWORTH

The only results of this kind that I know of are those obtained by Micciarelli-Sbrenna. I might point out that farnesyl methylether is very active in *Tenebrio*; in *Rhodnius* it has one quarter of the activity of JH-1.

NAKANISHI

Before any serious discussion takes place, since you have attacked chemists I would like to criticise the entomologists now. One thing is not directly pertaining to your talk, but about two years ago we analyzed with Dr. B. Lanzrein (from Prof. M. Luscher's lab at Bern) the JH's of a cockroach. I think it's rather dangerous when you speak of juvenile

hormone titres because according to the galeria wax test, the most common JH bioassay, there is a difference of a few orders of magnitude in the response of those insects to the three JH's. In spite of this, the mixture of JH's is analyzed collectively, and entomologists start saying all sorts of things just based on the galeria wax test.

I would just like to add — an extension of this work has led to a rather rich source of JH and that is the termite, the *Macro subhyalinus*.

This was done in collaboration with Martin Luscher and Dietrich Mayer, by Beatrice Lanzrein at our place (unpublished).

One queen termite contains 0.2 milligrams of JH-III. The reason we did this was to get some chemical basis for the role the queen plays in caste determination of the colony. We found that most of the JH was 0.2 milligrams of JH-III and a trace of JH-I. At least in our hands we could not detect any JH-II. But I can ask you a question, that is, I would like to know whether you think any other juvenile hormones are existing and if so, how would you go around this dilemma? Unless you use the particular insect itself for bio-assaying it for its juvenile hormone, there seems to be no other way.

WIGGLESWORTH

I thought I criticised the entomologists for not standing up to the chemists! My quotations about juvenile hormone assays in *Rhodnius* referred to pure recemic C_{18} JH (JH-I). I agree that we need to know far more about the possibly changing titer of different species of JH in different living insects. I am afraid I do not know Martin Lüscher's most recent conclusions about the role of JH in caste determination in termites, which may well differ from the statement given in my lecture.

BOWERS

Professor Wigglesworth warned me beforehand that he was going to propose an experiment, and he did propose to use the anti-juvenile hormones just now to treat the bedbug and see if it inhibits this nymph as it emerges from the egg. Well, in having proposed this I will say that we found out that the anti-juvenile hormones have some ovicidal activities against a number of insects and I did not report it here; but

when we fumigated things like the Mexican bean beetle eggs or milkweed bug eggs with precocene 2, we frequently found that the embryos developed all the way up into mature embryos and just before hatching we could see the embryos through the chorion but then they died. A very few of them did hatch but they died shortly afterwards. I was reluctant to say very much about this other than the fact the precocenes were ovicidal because I simply did not know what went on in the egg, but apparently Professor Wigglesworth does and anticipated this. I certainly want you to do this experiment and I am going back and look at a few of these unhatched embroys myself.

WIGGLESWORTH

Perhaps if you take your embryos out you will find that you have already done the experiment!

BOWERS

It *is* possible - I did not consider that as a possibility and that just might explain this interesting effect. I thought: well, perhaps it is just simply poisoning the older embryos and that is the end of it.

STAAL

It appears that the precocenes exhibit a very peculiar selectivity spectrum. They seem to affect several species from the family Pyrrhocoridae. This includes the genus *Dysdercus*, but *Pyrrhocoris apterus* (which belongs to the same family, Pyrrhocoridae) turned out to be insensitive to precocenes in our hands. One can always find toxicity at high dose levels as in other insects but nothing that could be interpreted as premature metamorphosis. Species belonging to the family Lygaeidae such as *Oncopeltus fasciatus* are also sensitive to precocenes. On the other hand, the JH analog juvabione is very active on *Pyrrhocoris apterus* and has little, or no activity, on *Oncopeltus fasciatus*. It is also known that some other very highly active JH analogs such as the 7,11 dihydrochloride of methyl farnesoate are generally active on pyrrhocorid genera and *Oncopeltus*. We may therefore conclude that the selectivity of JH mimetics and compounds with JH antagonistic activity

towards pyrrhocorid and lygaeid bugs is quite unpredictable. The true juvenile hormones of neither family of bugs is known.

WIGGLESWORTH

It is interesting that in the case both of juvabione and of precocene it is members of the Hemiptera which show the unusual effects.

WILLIAMS

I have always viewed Sir Vincent as my mentor — and I still do. Therefore, his views on recent and current goings-on in insect endocrinology are always noteworthy. It is true that down through the years from time to time we have taken a few playful pokes at each other's theories. Clearly, that practice continues and is likely to do so.

I think I will just wait for that chance.

SIDDAL

I would like to ask Sir Vincent if he thinks that the Hemipterous use any of the known juvenile hormones in their normal development.

WIGGLESWORTH

I would sincerely hope so. We should very much like to know what is the juvenile hormone in *Rhodnius*, if you can tell us. I suppose we might expect it to be JH-III (what we used to call « Bowers' compound ») — although as you pointed out JH-I and JH-II might well he present also in the more primitive groups of insects.

ANTI-JUVENILE HORMONE EFFECTS OF A DIFFUSIBLE AGENT THAT INHIBITS THE CORPORA ALLATA

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« THE BUGS ARE COMING » exclaims the banner headline of a recent cover story in *Time* magazine. In the article itself we learn how the human race is presently losing ground in its age-old battle with the insects. The scenario involves two intractable difficulties. The first is that conventional chemical pesticides which are being used throughout the world on an ever increasing scale are too broad in their effects. They are toxic not only to the pests at which they are aimed but also to other animals. Moreover, by persisting in the environment — and sometimes even increasing in concentration as they are passed along the food chain — they present a hazard to other organisms including man. The second difficulty is that insects have shown a remarkable ability to evolve a resistance to conventional chemical pesticides. For this reason the conventional pesticides — our first line of defense against insect pests and vectors of disease — are losing much of their former effectiveness.

There is nevertheless reason to believe that the realities of the situation may not be quite so gloomy as that presented by *Time* magazine. Since World War II, basic research on insects has been greatly accelerated throughout the world. For example, in the United States, governmental agencies such as the NSF, the NIH, and the USDA have fostered basic research on the biology, physio-

logy, and endocrinology of insects. The same is true for certain private organizations such as The Rockefeller Foundation and, more recently, for certain branches of the chemical industry. A byproduct of these investigations has been the identification of chinks in the physiological and ecological armor of insects — Achilles' heels that can be attacked by what have been termed « biorational » techniques (DJERASSI *et al.*, 1974).

One class of biorational agents are the so-called « third generation pesticides » — hormonally active substances which can be used to derail the metamorphosis of insects. Up to the present time nearly all of these agents have been synthetic analogs of juvenile hormone (JH).

The JH mimics have proved to be most promising in the case of those species whose deleterious effects are limited to the adult stage. This happens to be true for most medically important species that transmit human or animal diseases. Manifestly, such pests as mosquitoes, fleas, or tsetse flies pose no problem as long as one can block their metamorphosis.

The situation is quite otherwise in the case of the vast majority of agricultural pests that prey upon man's food and fiber. Here the larval stages often do most of the damage. It would be of little comfort to a beleaguered farmer to learn that the larvae which are consuming his crop will be done in days or weeks later at the time of metamorphosis. Clearly, the need is for new and better strategies for controlling larval insects.

POTENTIAL OF ANTI-HORMONES AS PESTICIDES

A way out of this dilemma would be provided by third generation pesticides possessing anti-hormone activities. On the basis of present knowledge one can predict that an effective anti-ecdysone would block larval growth, molting, and metamorphosis thereby enforcing a developmental standstill reminiscent of diapause. An anti-JH would oppose the production or action of endogenous JH; in this manner an anti-JH would provoke precocious lethal metamorphosis of immature larvae. Moreover, in species requiring JH

for egg maturation, we may anticipate that an anti-JH would sterilize females that had already attained the adult condition.

ANTI-ECDYSONES

The USDA's Insect Physiology Laboratory at Beltsville, Maryland, has been most energetic in the study of agents blocking the synthesis or action of ecdysone (for review see THOMPSON *et al.*, 1975). A large number of ecdysone analogs and intermediates in ecdysone synthesis have been assayed by incorporating them into the diet of larval insects. Particularly active have been certain azasteroids as well as steroids containing the ecdysone nucleus but lacking the side-chain hydroxyls. Certain of these materials are reported to be active in the ppm or ppb range in blocking molting and metamorphosis.

Until recently, all materials with anti-ecdysone activities have been steroids. This is a serious disability since molecules of such complexity are exceedingly difficult to synthesize and in most cases must be derivatized from natural products. The real need is for anti-ecdysones which are not steroids and which are active after topical application. Particularly exciting, therefore, is a recent report from the Beltsville Laboratory (ROBBINS *et al.*, 1975) of anti-ecdysone activity for a series of simpler, non-steroidal secondary and tertiary amines. Though the compounds in question bear a chemical resemblance to JH, they are reported to be lethal to all larval stages. And, unlike JH, they are usually more active during early larval instars than later on.

ANTI-JUVENILE HORMONES

Because of their great theoretical interest and practical concern, there is reason to believe that many laboratories throughout the world are engaged in the search for anti-JHs. In one of the few reports in the "open" literature, SLÁMA *et al.* (1974) describe the screening of about 200 different compounds which were structurally related to JH while showing little or no JH activity. All tests

were conducted on freshly molted penultimate instar larvae of the bug, *Pyrrhocoris*. In not a single case was any trace of anti-JH activity observed.

Greater success has apparently attended the efforts of the Zoecon Corporation, as described in an unpublished lecture presented by Geraldus Staal in November, 1975. A number of synthetic compounds showing traces of JH activity were found to have anti-JH activity when administered to immature larvae of the tobacco hornworm, *Manduca sexta*. The agents in question had no effect on JH synthesis. Moreover, their effects could be completely eliminated by simultaneous treatment with active JH analogs. Consequently, it seems likely that the anti-JH effects were due to competitive inhibition of endogenous JH in at least certain of the target tissues of the larval insect.

Last November at the same International Symposium at which Staal spoke, Dr. William Bowers startled the audience by announcing the discovery of two natural products possessing anti-JH activity. These findings have subsequently been summarized in two publications (BOWERS, 1976; BOWERS *et al.*, 1976). In brief, ether-acetone extracts prepared from the ornamental plant, the so-called « bachelor's button, » *Ageratum houstonianum* (Compositae), were found to show anti-JH activity when placed in contact with immature larvae of the milkweed bug, *Oncopeltus fasciatus*. A typical result was molting to give one additional larval stage followed by precocious metamorphosis to form diminutive non-viable adults. The active principles in the extract proved to be two closely related chromene derivatives which, because of their ability to provoke precocious metamorphosis, were termed « Precocenes I and II. » Thus far the two agents have shown substantial anti-JH activity for only two genera of true bugs, *Oncopeltus* and *Dysdercus*. Nevertheless, the stage is obviously set for the synthesis of more potent chromene derivatives with a broader spectrum of activities.

Present indications are that the Precocenes somehow shut off the synthesis or secretions of JH by the larval corpora allata. In this sense they mimic or bring into play — albeit in an untimely manner — the normal mechanisms which final instar larvae use for turning off their corpora allata as a necessary prelude to metamorphosis.

During the past few years it has been this normal mechanism that my co-workers and I have sought to comprehend in studies carried out on larvae of the tobacco hornworm, *Manduca sexta.* In the final moments at my disposal I propose to summarize our findings.

Brain-centered mechanism for inactivation of the CA

When the final (5th) stage larva of the tobacco hornworm attains the weight of about 5 g, a diffusible agent that we call the « inhibitor » is released from some unknown source in the abdomen. The inhibitor acts on the brain. Then, *via* its nerves to the CA, the brain turns off further secretion of JH. We have evidence that the brain does so by curtailing the secretion of a CA-stimulating factor (« allatotropin », Scharrer, 1958) and simultaneously secreting a CA-inhibiting factor; for the latter we suggest the name « allatohibin ». We suspect that both are neurosecretions which, in *Manduca,* are axonally transported to the CA.

The need to postulate two rather than just one controlling factor is made necessary by the behavior of « loose » *Manduca* CA when separated from the brain and bioassayed by implantation. Under that circumstance, active CA lost most but not all of their activity presumably because they were deprived of allatotropin. In similar tests of inactive CA, the glands reacquired a certain low level of activity because they were no longer acted upon by allatohibin. At the present time nothing is known about the molecular nature of allatotropin or allatohibin.

The inhibitor

What can we say about the diffusible inhibitor which begins to be released when the larva attains the critical size of about 5 g? Sivasubramanian and Williams (1976) used an *in vitro* system for detecting its presence in the hemolymph. Each culture consisted of 20 active brain+CC+CA complexes obtained from early fifth

instar hornworms. The presence of the inhibitor was recognized by its ability to turn off the active complexes. Control cultures were prepared in Grace's medium; experimental cultures, in 1 part Grace's medium and 1 part hemolymph or subfraction thereof. After 48 hr the complexes were rinsed and recultured in fresh Grace's medium. After 24 hr this fresh medium was extracted for JH, the extract partially purified, and subjected to bioassay.

In this manner we were able to determine that an agent capable of turning off the active complexes begins to appear in the blood after about 48 hr of the fifth instar. It peaks on day 4 — i.e., in hemolymph derived from larvae weighing 7-8 g; it then declines on day 5 and becomes undetectable on day 6.

Further studies (SIVASUBRAMANIAN and WILLIAMS, 1976) suggest that the inhibitor is a heat-insensitive, trypsin-sensitive substance whose molecular weight is similar to that of bacitracin (ca. 1400 daltons). It may be noted that these are properties identical to those reported for an « antigonadotropin » extracted from the ovaries of *Rhodnius* (LIU and DAVEY, 1974). If, as seems likely, the inhibitor proves to be a polypeptide, it could contain about a dozen ordinary-size amino acids. Once the inhibitor is obtained in pure form, its sequencing and synthesis should be straightforward. But obtaining it in pure form is likely to be a formidable undertaking.

By virtue of its ability to activate the brain-centered mechanism that turns off JH secretion by the corpora allata, the inhibitor thus indirectly serves as an anti-JH. Perhaps the Precocenes have leverage on this system.

Though a new industry has come into being for making and marketing biorational insecticides, I do not wish to give the impression that any of the presently known agents will prove to be a panacea. Nor do they promise a final and permanent solution to the control of insect pests and vectors of disease. Presumably given

sufficient time, insects can evolve resistance to them. Manifestly, what is required is a continuation of the pure and applied research effort. Though we can't outbreed the insects, we are well equipped to outthink them. And if we keep at it, I venture to think that the Sounds of Spring will continue to enrich our lives.

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DISCUSSION

STAAL

I would like to question how essential for pupation the period after the first secretion of ecdysone is. There is documentation in the work of Bückmann clearly indicating that the prepupal phase, encompassing such behavioral phenomena as spinning, migration, and several integumental color transformations, is induced by a low level or a low surge of ecdysone. This is then followed by a higher surge of ecdysone that induces the pupal molt proper. Bückmann showed that one could experimentally overshoot the entire first phase of prepupal behavior and color change by just one high surge of ecdysone. So it seems to me that the first phase is not really that essential for the pupal molt, although it is probably a critical phase in the life cycle of the insect in which the cocoon spinning, the migration to a pupation site, etc., take place.

WILLIAMS

Yes, I recall that by the injection of high doses of ecdysone into mature larvae of the lepidopteran, *Cerula vincela*, Detlef Bückmann was able to induce pupation without provoking the usual prodromal color change seen after the injection of lower doses. Perhaps this is an example of hyperecdysonism. Nevertheless, I would not underestimate the importance of the first of the two pulses of ecdysone that take place prior to pupation. Thus, as Lynn Riddiford has shown, it is exposure to the first pulse that causes a switchover in the commitment of the integumentary tissues to secrete a pupal rather than a larval cuticle. The actual implementation of this commitment then depends on the epidermis being exposed to the second and much larger surge of ecdysone some two days later. All this is of course extremely interesting and of vast concern to developmental biology. We need to know the cellular and molecular basis of the switchover. An ancillary question is whether the two periods

of high ecdysone titer must necessarily be separated by a period in which ecdysone is very low or even absent. This indeed appears to be the case in the pupation of *Drosophila*. Thus, as Geoffrey Richards has shown in his *in vitro* studies of *Drosophila* salivaries, the normal sequential pattern of puffing is seen only when exposure to ecdysone is interrupted by at least 3 hours of culture *without* ecdysone (*).

HEIMPEL

I would normally have asked two questions, but the use of the word normal insect by Dr. Williams stimulated me to make a comment. I don't want to be offensive, hut I think that 60%-70% of the physiology that has been done on insects has been done on chronically infected insects, insects that are not healthy. Either by cunning or by high intelligence Dr. Williams has picked an insect that doesn't have many diseases. But I would point out that a lot of the colonies of *Manduca* in this country are chronically infected with cytoplasmic virus disease. As an example, Dr. Bowers knows that there are microsporidia that produce juvenile hormone; experiments in this work on insects containing microsporidia wouldn't be valid. At this point in time I am normal, Dr. Williams, but unhealthy, and Dr. Bowers is young and healthy and I would like the term healthy rather than normal. Entomologists are often not aware of diseases and tolerate them — throw out the dead animals and take the semi-infected animals and work with them. Frankly, I think rhat physiological studies should be carried out with insects that are reared on sterile media and that are sterile. Now I would like to ask two questions. Do you have cytoplasmic disease in your *Manduca* rearings? and the second one is: Have you ever used *Manduca* from the field to compare to the animals that were reared on the artificial or semi-artificial media?

WILLIAMS

Unlike saturniid silkworms which almost invariably succumb to pandemic disease when reared under laboratory conditions, *Manduca* is quite

(*) *Devel. Biol.*, *54*, 274, 1976.

resistant to infection. Our culture was started about 7 years ago with eggs obtained from Dr. Robert A. Bell, then of the U.S.D.A. Laboratory in Fargo, North Dakota. Since Dr. Bell had maintained his cultures for a number of years, the strain had already become well adapted to laboratory conditions.

As recommended by Dr. Bell, the larvae are reared in individual capped plastic cups to prevent cannibalism and oppose infection. Streptomycin is routinely included in the synthetic diet and so are the mold and yeast inhibitors, sorbic acid and methylhydroxybenzoate. Nevertheless, I must admit that from time to time, especially during the more humid months of summer, we are troubled by mold and, more rarely, an outbreak of bacteria that form pink colonies on the surface of the diet. Difficulties of this sort are nearly always synchronized with a breakdown of hygienic practices as after a change in personnel. Such occasional outbreaks are treated ruthlessly by discarding all preparations showing any trace of infection.

Dr. Heimpel has suggested that physiological experiments should, in principle, be carried out on germ-free or virus-free animals. I can well believe that this may be desirable from time to time under very special conditions. However, I venture to think that an aseptic animal is far more abnormal than is an apparently healthy individual carrying around his, her, or its fair share of bacterial flora, not to mention latent viruses and so on. Indeed, I think it fair to say that such a healthy individual is Dr. Heimpel himself.

One must of course keep one's eyes open. It is indeed true that certain strains of the sporozoan, *Nosema*, can produce materials with JH activity, as Dr. L. Linlayson first demonstrated while working in my laboratory over 20 years ago (*). Obviously, it would be quite impossible to carry out endocrine studies on larvae infected with *Nosema*.

Let me try to answer the two questions posed by Dr. Heimpel. Whether the Harvard strain of *Manduca* has « cytoplasmic disease » depends on how one defines disease. According to Dorland's *Medical Dictionary*, disease is « any departure from a state of health; an illness or a sickness. More specifically, a definite morbid process having a characteristic train of symptoms ». Our *Manduca* seem too happy to qualify under that definition.

(*) *Nature 180*, 713, 1957.

Dr. Heimpel also inquires as to whether our findings have been examined against wild *Manduca* collected out-of-doors. The answer is no. Nearly all wild *Manduca* spawn a host of braconid parasites to which they succumb late in larval life. They would be quite unsuitable for the study of anything except the host-parasite relationship.

HEIMPEL

Dr. Williams, have you had your eggs sectioned for electron microscopy — have you ever examined your animals for the presence of low level virus or other diseases?

WILLIAMS

No.

WIGGLESWORTH

There are many points in this communication which I have found extremely interesting. We have long supposed that the brain normally controls the number of instars by the perception, in some way, of the size of the body; it is most interesting to hear that Professor Williams and his colleagues have now discovered how it is done. With regard to the cerebral control of corpus allatum activity: we have generally believed that activation was brought about by neurosecretion (which is visible in the axons from the brain to the corpus allatum) but that the inhibition of activity was a nervous effect. I shall look forward to seeing the new evidence that both effects are due to neurohormones.

As regards the corpus allatum and ecdysone secretion: for a good many years Fukaya's group in Japan have been claiming that diapause in the rice stem borer was due to corpus allatum secretion — but I did not find the experiments convincing. During the past year or so, however, Yagi and Fukaya have described results which give convincing support to this belief. At just about the same time Nijhout and Williams made the fascinating discovery that in the last larval instar in Lepidoptera the arrest of juvenile hormone secretion produces a surge of secretory activity in the prothoracic gland; and, again around the same time, Chippendale and Yin showed that diapause in the stem borer in California results

from a failure in the mechanism which inactivates the corpora allata. All this fits together into a consistent story.

Another story which has come together very nicely is the double surge of ecdysone secretion. Many years ago Buchmann described in *Cerura* the change in colour and the wandering behaviour initiated by the first small surge, followed later by the large surge, which induces moulting. This has been confirmed in *Pieris*, in *Leptinotarsa*, and now in *Manduca*.

What I am not happy about yet, I must admit, is the inhibitory hormone coming from some unknown source in the abdomen. I shall look forward to seeing the evidence in black and white.

WILLIAMS

I must say that I appreciate those remarks, Sir Vincent. Obviously, you continue to do your homework. And once again you demonstrate your remarkable grasp of the very oldest as well as the very latest happenings in insect endocrinology.

CANONICA

The molecule of the juvenile hormone is the final result of a long chain of individual biosynthetic reaction. It can be interesting to wonder which step of this chain is stopped by the inhibitor you have discovered. I think such an investigation is possible and really interesting because it can disclose many opportunities. As everybody knows, the first steps of the biosynthesis of the natural juvenoids are the same or like the steps which are involved in the biosynthesis of cholesterol and of all terpenoids. So from this work it will be possible perhaps to get new ideas for the preparation of cholesterol and other compounds which are biosynthesis inhibitors.

WILLIAMS

I agree.

KARLSON

I would like to comment on the titers of ecdysone during the last

larval instar. We have done similar measurements in *Bombyx* (*) and found the same two peaks, but we don't find two peaks of ecdysone secretion in the development of *Calliphora* larvae. Yet. *Calliphora* larvae show the same change in behaviour as the lepidopteran larvae. Obviously, here is a point where the lepidoptera differ from diptera in regulation of ecdysone tite.

Secondly, I was quite impressed to learn now that juvenile hormone prevents production and/or secretion of ecdysone by the prothoracic gland. A couple of years ago, preliminary evidence was presented (and, as far as I recall, it came also from your laboratory) that the juvenile hormones should *promote* the synthesis and secretion of ecdysone. This I always found hard to swallow, and I would rather go along with the new version of the day.

WILLIAMS

Dr. Karlson, I have already referred to the recent studies of *Drosophila* salivaries which G. Richards has carried out in Ashburner's laboratory at Cambridge University. Thus in the case of mature *Drosophila* larvae, pupation becomes possible only when the two pulses of ecdysone are separated by an ecdysone-free period. I venture to think that two pulses may be required for the pupation of most insects — certainly for those that display a pronounced change in behavior prior to the actual onset of pupation.

With respect to your other remarks on the control of ecdysone secretion hy the prothoracic glands, it is important to emphasize that JH blocks the synthesis and secretion of ecdysone by acting, not on the corpora allata, but on the brain. Its action is to block the secretion of the brain's prothoracicotropic hormone. The other class of phenomenon to which you refer is JH's ability, under certain conditions and circumstances, to act directly on the prothoracic glands to provoke the synthesis and secretion of ecdysone. As far as I know this action of JH — or for that matter of implanted active corpora allata — has been fully documented only for diapausing or brainless diapausing saturnid pupae.

BOWERS

Several years ago when I was working on *Manduca* we fed larvae

(*) SHAAYA E. and KARLSON P., « Developm. Biol. », *11*, 424-432 (1965).

some of the methylenedioxy hormone analogs and they would cause even the last instar larvae of *Manduca* to continue molting many times. They would eventually die. They formed several additional larval cuticles but they did not ecdyse. I would find these enormous larvae that were quite dead and turning black and I wondered why they died. I found that I could take off the old cuticle and there was another one underneath it. I stripped it off and there was another one underneath that. I think I actually stripped one down about 7 cuticles, all overlapping one another with a little molting fluid in between. I don't know if this is particularly characteristic of the methylene dioxy compounds or not, but it was an interesting thing to find anyway. The second thing I wanted to ask was: have you tried your diffusable factor — your inhibitor — against the adult brain-corpora cardiaca-corpora allata complex.

WILLIAMS

I recall those remarkable experiments on *Manduca* which I first heard you describe at a Gordon Conference in 1968. Perhaps your methylene dioxide JH analog has the just-mentioned ability to act directly on the prothoracic glands to turn them on. This would bypass the entire brain-centered controls and might cause repeated molts.

I am of course very interested in your remarks concerning the control of the corpora allata in Homoptera versus other Orders of insects. However, the lesson of « juvabione » documents that remarkable specificities can characterize the biological actions of hormonally active molecules.

In experiments which I hope to continue in the very near future, Dr. Sivasubramanian obtained evidence that hemolymph fractions containing the putative inhibitor could oppose the brain's activation of the corpora allata in pharate adults.

BOWERS

So that means, very likely, the immature and mature stages control the corpora allata in the same way.

NAKANISHI

Since the neurosecretory hormones are rather difficult would the

following experiment make any sense, especially since you have obtained an estimate molecular weight? In other words could add by injection or orally tritiated five or six, amino acids, because, in order to know the precursors.

WILLIAMS

What we need is a less tedious bioassay for the putative inhibitor than is provided by the *in vitro* system. We need to get on with its purification. I am just now waiting for the arrival of a new brood of postdocs.

SIDDAL

Is it possible to relate the timing of appearance of your inhibitory factor to the timing of appearance of the specific juvenile hormone esterase which has been described by Kramer and Law and the group in Chicago? Is there a relationship between these two things?

WILLIAMS

Yes, you bet there is. Fred Nijhout has evidence that the JH-specific esterase is what erases receptor-bound JH in the target cells.

SIDDAL

Do you know if the esterase appears before your inhibitory factor or after it? I just wonder, because the esterase could accomplish all the things which your inhibitor indirectly accomplishes.

WILLIAMS

The inhibitor begins to appear a day or two before the surge of JH-specific esterase. The latter comes along and cleans up cell-bound JH that is otherwise very persistent. The esterase is of course a large protein. By contrast, the inhibitor seems to be a modest size polypeptide of ca. 1400 daltons.

ABO-KHATWA

Professor Williams, I have noticed that bovine serum albumin (BSA) at 1% was present in Grace's medium. Is it possible that BSA binds JH thus influencing the active titre of the hormone?

WILLIAMS

That is exactly why we put it into bind and protect JH during the 24 hours of culture. Then we used organic solvents to strip off the JH for the bioassay. Even if we left the serum albumin attached, I doubt whether it would interfere with its action in a bioassay. Do you find BSA-bound JH to be inert?

ABO-KHATWA

We have noticed that you can reverse the influence of some juvenile hormone analogues on mitochondrial respiration by the inclusion of 0.3% BSA.

WILLIAMS

Were you using sarcosomes from flight muscles?

ABO-KHATWA

We were using mitochondria isolated from the flight muscles and from the ovaries of termites.

WILLIAMS

Years ago, in one of the earliest studies of insect mitochondria (*), Watanabe and I found that the giant mitochondria (sarcosomes) isolated from the flight muscle of blowflies were happy only in media containing BSA. It's good stuff to have around.

(*) *J. Gen. Physiol.* 37, 539, 1954.

ESSENTIAL DIFFERENCES BETWEEN NATURAL JUVENILE HORMONES AND JUVENILE HORMONE ANALOGS ELUCIDATED BY USE OF A SUBSTITUTION ASSAY *

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Introduction

Bioassay methods for juvenile hormone (JH) generally use topical tests on specimens (last instar larvae or pupae) that have a critically low endogenous JH titer to ensure metamorphosis. These tests usually measure a morphogenetic response in the next molt (STAAL, 1972). The drawbacks of such methods are manifold:

1) The maximum effect, a supernumerary larval instar or supernumerary pupa, is difficult to attain and often requires exceedingly high doses. With most dose response curves obtained this way the scores near a 0 or 100% effect are asymptotic and thus not very reliable.

2) The results sometimes have a discouragingly high variability, requiring a large number of specimens. In addition to the variability at extremely high or low dose levels (asymptotic effect), a considerable source of variability is also present in the less than perfect physiological synchronization of the specimens or to intrinsic variations in the internal metabolism of the compound. It should

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* Contribution No. 151 from Zoecon Research Laboratories.

be kept in mind that the capability for metabolic degradation of JH is at a maximum at these periods of lowest JH titer levels, probably due to the activity of JH specific esterases (SANBURG *et al.*, 1975 a and b).

3) These tests may suffer from a remaining background of endogenous JH and may therefore not yield true values since the exogenous application to be evaluated is only an addition and not a substitution.

4) The test specimens can only be observed after one subsequent molt, because the morphological intermediates produced are generally unviable.

Therefore, there is good reason to design tests in which endogenous JH is entirely substituted by exogenous application(s) of a test compound at a time that JH is required for normal development. Such a test would require removal of the JH source, the corpora allata (CA), as early in larval development as possible. The ideal way to administer the substitution compound would be to imitate the normal secretion cycle, leading to titer curves with presumed peaks and valleys in every intermolt period. However, these titer curves are not established for young larvae and adequate modulation of the administration of JH for individual specimens would be virtually impossible. Therefore, the next best choice is probably continuous administration at a constant level. Many experiments have indicated that mild overdoses of JH on larvae are almost without effect on development. The physiological significance of JH titer curve fluctuations in the early larval instars, if they exist at all, is therefore probably low.

Pulsed treatments by injection and topical application are probably not the best way to approach this problem because they may produce unnatural internal fluctuations in the titer that may lack all relevancy to the normal situation. A substitution test on larvae of *Bombyx mori* using single topical or injected substitution doses was described earlier by OHTAKI *et al.* (1972). Their report does not allow for many conclusions as to the homogeneity and reproducibility of the results, and only one further molt was involved.

The alternative chosen for our experiments, administration through artifical food medium, provides continuous exposure through

feeding, skin contact, and possible vapors, so that a continuous equilibration can take place. This test system has also worked very well for the quantitative study of JH effects on pupation with intact larvae of *Heliothis virescens* (HENRICK *et al.*, 1975).

We have selected as our test insect *Manduca sexta* (the tobacco hornworm) which provides additional benefits for this type of study not present in most other Lepidoptera. The larvae of this species show a clearcut response, in the form of a pronounced black pigmentation, to deficient JH levels too high to cause premature metamorphosis, but too low to induce the normal green pigmentation. This same black pigmentation is a characteristic of the JH deficient black mutant *Manduca* strain (SAFRANEK and RIDDIFORD, 1975) and it can also be produced through ligating at the right physiological stage (TRUMAN *et al.*, 1973). The black mutant strain, as well as ligatured normal larvae have been used as subjects for JH bioassays measuring the restoration to green pigmentation (FAIN and RIDDIFORD, 1975). Although these assays are among the most sensitive and satisfactory assays developed for JH, neither one combines the ability to provide a full dose response curve with the certainty of elimination of the intrinsic JH source and with the benefit of equilibration with the exclusive exogenous JH source over an extended period including two larval/larval molts. Also, *Manduca sexta* has proven to be exceedingly well resistant against the double trauma of anesthesia and surgical allatectomy.

Materials and Methods

The principal colony of *Manduca sexta* has been maintained without diapause in our laboratories for several years on standard artificial media at 16 hrs daylight and 25°C (BELL and JOACHIM, 1976).

Freshly molted IIIrd instar larvae were collected within 30 minutes after molting and set aside for another 30 minutes to ensure full hardening of the head capsules as required for surgery. Generally, the synchronized circadian rhythm of molting allowed for the collection of several hundred of these IIIrd instar larvae within a period of a few hours during the morning. Prior to surgery the larvae

were anesthesized in ethyl ether vapors in *Drosophila* anesthetizers (in which direct contact with liquid ethyl ether is avoided) in groups of five for a period not exceeding 10 minutes. In contrast to experience with other lepidopteran species it was found that the anesthesia provided by carbon dioxide was insufficient, took very long for full effect, and was not adequately maintained during the following submersion in saline required during surgery. This ethyl ether anesthesia, if properly executed, was not a source of mortality and proved to be very satisfactory in all respects.

The surgery equipment consisted of a solid paraffin dish with a rubber holder fixed to the bottom. The holder and the larva could be entirely submersed under saline, leaving no disturbance of the liquid surface to distort the optical path. The larva holder was carved out of a piece of thick rubber tubing. It held most of the body horizontally and without damage, leaving the head free and exposed. (Most allatectomy holders described for grasshoppers and beetles grasp the head, but this is less practical with caterpillars.) Allatectomy was performed under a stereo-dissecting scope equipped with foot focusing and a strong concentrated light source with finely ground curved forceps in one hand and a curved perfusion needle in the other hand. The corpora allata were removed after penetration of the neck membrane with the forceps and under continuous perfusion of the wound with saline (STAAL, 1961). It should be stressed that only with a completely unobstructed optical path, as obtained with complete submersion, curved instruments, and continuous wound perfusion with saline, can the corpora allata be made sufficiently visible for routine allatectomy of these young larvae. After removal from the surgery dish, the larvae were allowed to dry on blotting paper for a few minutes up to a few hours, after which they were placed individually in 1 oz polystyrene cups with treated artifical medium. In all treatments and controls, series of ten larvae were routinely tested. Neck membrane wounds in this, and other species of insects, were found to heal without complications provided that the longitudinal neck muscles are not damaged, and caused no impediment to subsequent molting. The specimens were reared at the usual conditions of 25°C and 16 hrs artifical light per day.

The food medium was prepared in bulk and when still warm and liquid, acetone solutions of the test materials were thoroughly

mixed into the media. The medium was then poured into the 1 oz cups (approximately 10 ml per cup), capped with plasticized paper lids, and allowed to cool and solidify. Media thus prepared were occasionally stored for several weeks at 5°C without any apparent degradation of the test compound when compared with freshly prepared media. Before use, the medium in every cup was sliced in half by a transverse cut and one half was placed in a new cup. Thus, larvae were able to feed on a clean vertical surface while resting on the bottom of the cup.

Observations were made daily on every specimen to determine the time of molting. Immediately after the molts to the IVth and Vth instar, the specimens were scored. The scoring system (fig. 2) was developed empirically, based on the different responses that were observed during the initial phases of the experiment and what could be easily distinguished in routine observations. It claims no linearity with respect to the dose/response curve, but leaves no ambiguity as to the hierarchy of responses.

All natural juvenile hormones and analogs used (fig. 1) were of synthetic origin and of high purity (generally $\geq$ 95% of the most active geometrical isomer), but racemic with respect to the optical enantiomers, with the exception of methoprene which was an 80% enriched preparation of the *d* enantiomer.

Results

Allatectomy in early IIIrd instar larvae not followed by any JH substitution invariably led to prepupal behavior and the formation of typical pupal cuticle after a long delay compared with normal, adequately substituted larvae or sham operated larvae. The formation of pupal cuticle was unmistakable but these prepupae never actually shed their larval skin and the tanning of the underlying pupal cuticle remained deficient. Even very minimal amounts of JH or JH analogs in the food medium induced the larvae to molt at least partially (head first) and without much delay while still retaining mostly pupal cuticle but even often with traces of black, larval cuticle (score 2). Completely pupal (unmolted) specimens never showed any trace of black pigmentation.

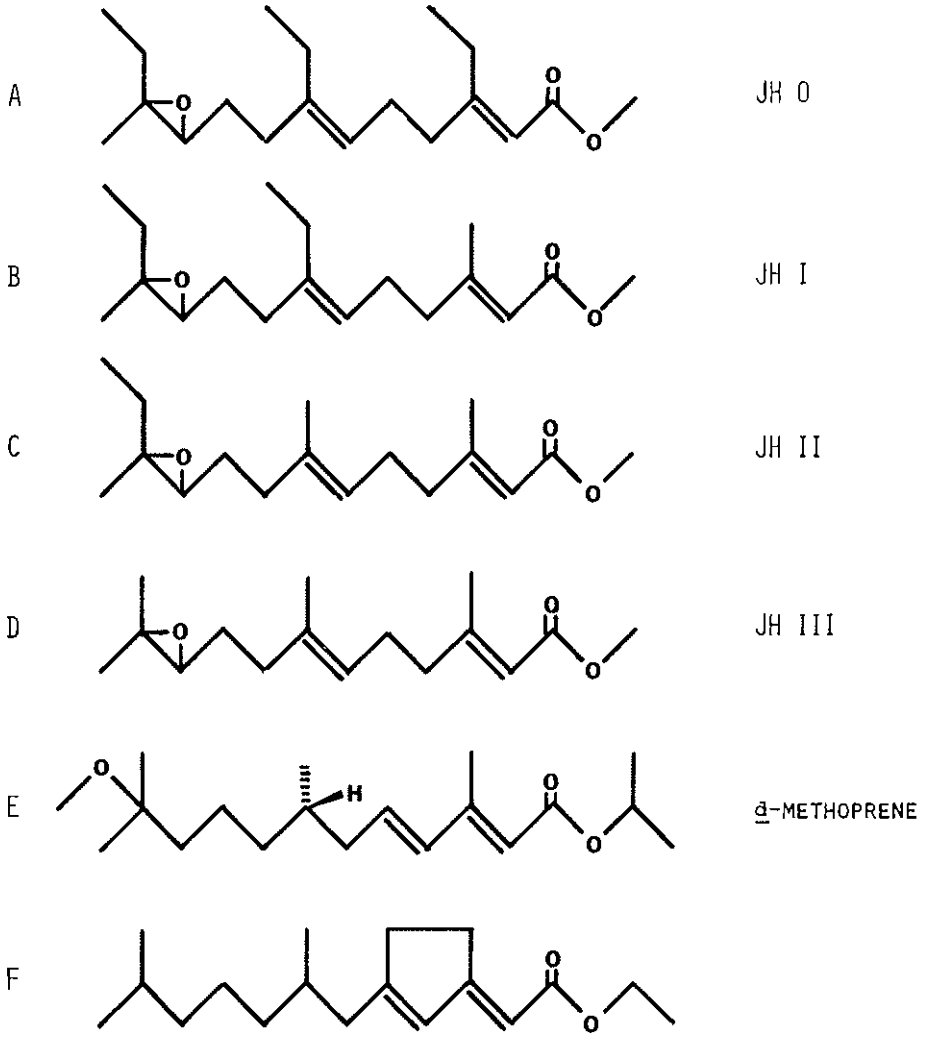

FIG. 1 — The natural juvenile hormones and some active analogs ("JH 0" is a synthetic analog).

SCORE SYSTEM FOR JH SUBSTITUTION ASSAY
ON *MANDUCA SEXTA* III-V

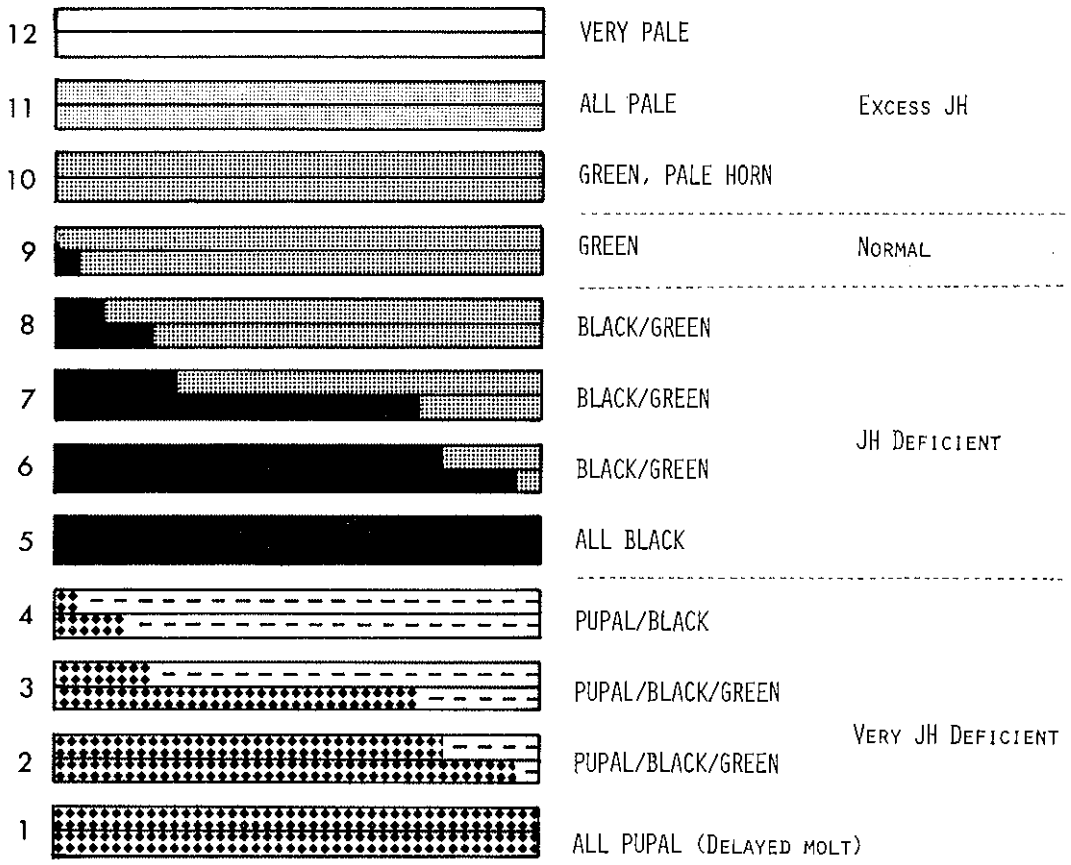

FIG. 2 — Score system for allatectomized *Manduca* larvae on JH treated medium. The score from 1 to 5 is based on the extent of pupal cuticle (complemented by black); from 5 to 9 on the extent of black pigmentation (complemented by green); and above 9, on the appearance of a pale color due to the disappearance of green pigment.

The sharp difference between score 1 and score 2 larvae in the time of molting made it possible to assign score 1 to larvae that showed considerable delay in their first postoperative molt and prepupal immobility while retaining a green color.

Increasing amounts of JH reduced the area of pupal cuticle with simultaneous increase of the area of black pigmentation (we observed that cuticle can never be black and pupal simultaneously, but these different areas were found to occur in irregular mosaic patterns), until score 5 is reached (all black, no pupal cuticle). Further increases in JH reduced the black pigment until score 10 is attained which represents a normal larva with no, or only a minimal amount of, black pattern. Further increases in JH quantity caused a pale color due to the loss of the blue pigment, first observed in the horn only (score 11), but in the higher doses also on the entire body (score 12). In score 12 (very high overdoses) the growth is usually inhibited: the larvae remain small, the molts are delayed and mortality increases throughout successive instars. We believe that this is the first description of specific overdose effects for JH as well as JH analogs in larval stages prior to the last larval instar.

The homogeneity of the test results was generally very encouraging. The occasional larvae seemingly unaffected by the treatments could always easily be identified as allatectomics that had failed, and these could be removed from the final score. Apart from other occasional casualties, a rather homogenous group of at least 5 to 7 larvae with approximately the same score usually resulted from the batch of 10, from which the median score was calculated under rejection of the occasional aberrant extremes that were clearly not part of the homogenous group.

In the experiments described in this paper, maintaining the larvae on the same treated medium until the Vth instar usually yielded a repetition of the same score as in the IVth instar with only few exceptions. Specimens with scores lower than 4 in the IVth instar were found not to be viable. These larvae were unable to feed and died before further apolysis. Specimens with score 6 and higher were morphologically entirely normal except for the black pigmentation and continued to grow and molt normally. Because of the steady state obtained with this procedure for the compounds used in this study throughout the molt to the IVth and the Vth instar,

only the scores to the IVth instar are represented in the graph (fig. 1).

The dose response curves (fig. 3) indicate an essential difference between JH I and II on one hand, and JH III and all other JH analogs investigated on the other, in that the graphs for JH I and II show a significant shoulder at the midpoint. Or, in other words, JH I and II produced the same effect (score 5-6) over a more extended range of concentrations than any other compound investigated (fig. 4). This property of JH I and II makes any statement as to their potency compared with other compounds meaningless unless it is indicated at which level of effect the comparison was made. Thus, at score 3, the relative potency of JH I is 1000 fold that of JH III, whereas at score 9 the relative potency is only 100 fold. (Topical assays such as the *Galleria* wax test which measure a low level effect, have previously indicated an approximately 200 fold higher potency.) At the higher level of complete substitution (normal larval appearance) it is obvious that the levels of any of the natural hormones required for complete substitution are relatively high.

All JH analogs tested displayed a straight and steep response curve of the JH III type or even steeper (fig. 5). Some of the most active JH analogs (e.g. cpd F) therefore are far more potent than natural JHs at the high effect level, but not at the low effect level, at which nothing was found that could outperform JH I and JH II.

In this insect species, the difference in physiological activity between JH I and JH II appeared to be insignificant or not existant.

"JH 0" (*not* a natural hormone) has yielded a response curve without a shoulder, and was rather similar in potency to JH I and II at the higher response levels. At the low response levels, however, it was not as potent as JH I and II. It also appears that the response curve is not as steep as that of analog F.

Discussion

In spite of some obvious advantages of the JH substitution technique, questions can still be raised on the possible interaction of endogenous JH remnants with the test compound on the result.

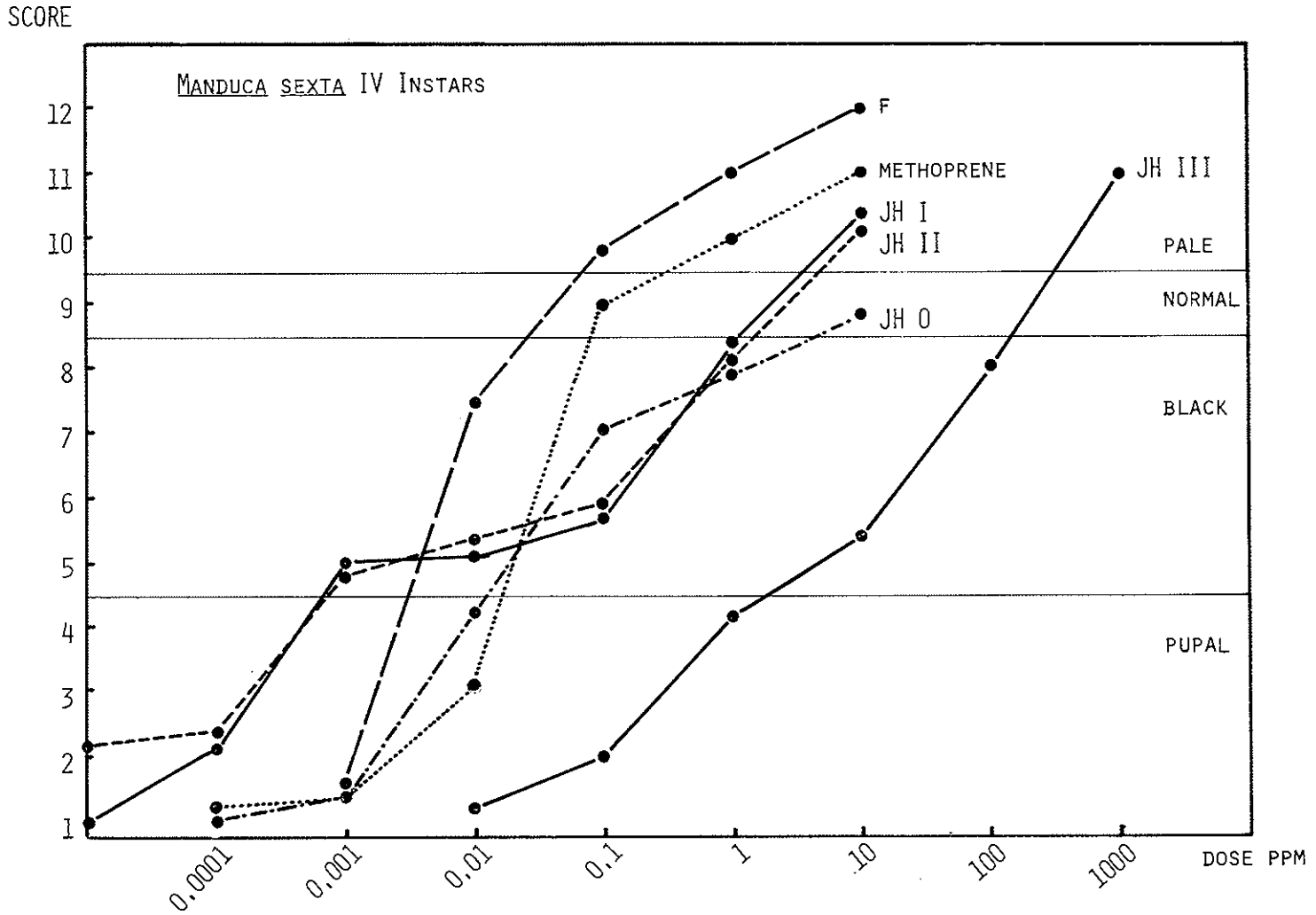

FIG. 3 — Dose response curves obtained by substitution with different JH type compounds on allatectomized IIIrd instar *Manduca* larvae as scored immediately after the molt to the IVth instar. Details on the compounds are given in Fig. 1.

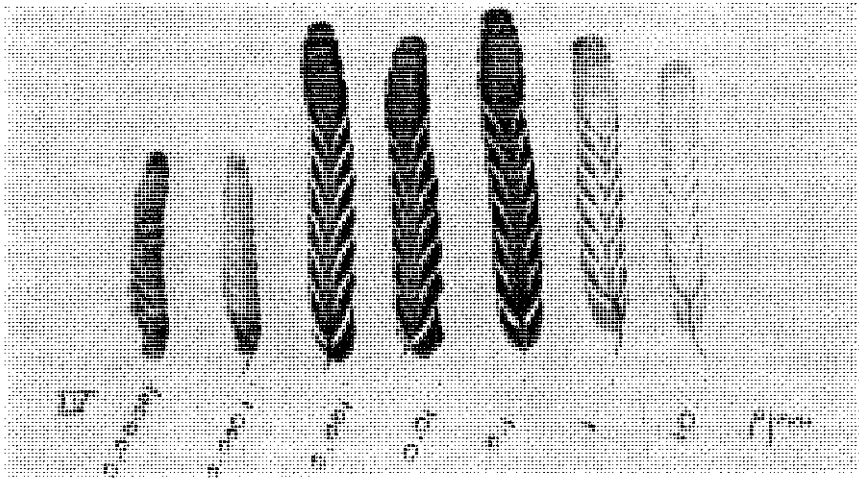

FIG. 4 — Response of IVth instar larvae of *Manduca sexta* to various doses of JH I mixed with the food medium, supplied to allatectomized IIIrd instar larvae. At a dose of 0.0001 ppm and lower, a pupal molt follows; at 0.001, 0.01 and 0.1 ppm the response is virtually identical (black larvae); at 1 ppm a normal larva is obtained; at 10 ppm overdose effects are seen.

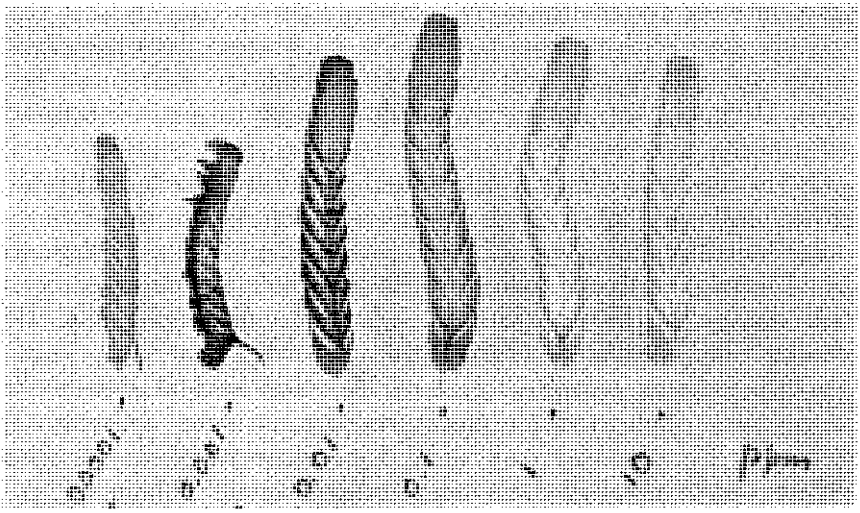

FIG. 5 — Response of IVth instar larvae of *Manduca sexta* to various doses of compound F mixed with the food medium supplied to allatectomized IIIrd instar larvae. At 0.0001 ppm a delayed pupal molt is observed (no tanning yet); at 0.001 ppm a pupal/larval intermediate results; at 0.1 ppm the normal larval appearance is restituted. At higher doses typical overdose effects are observed (pale color, growth inhibition).

Since treated food was supplied immediately following allatectomy no certainty exists that the original endogenous supply was depleted at that time. It is therefore possible that the substitution compounds could act as a protectant for the remaining small amount of natural JH. Although we have found for a few compounds that a 24 hr wait hetween allatectomy and substitution changed virtually nothing in the score obtained as compared to immediate substitution, extrapolation to other compounds may not be entirely justified and one may ask whether a 24 hr wait would not be advisable as a standard precaution. The halflife time for JH I in *Manduca* after ligation was found to be 1.5 hr (FAIN and RIDDIFORD, 1975), and this would guarantee effective removal in 24 hrs, but no certainty exists that applications of huge overdoses of certain compounds of the JH type could not change this degradation drastically. We determined a critical moment at which JH should be present in order to avoid premature pupation to be within a few hours after this first 24 hr period. Thus, a 24 hr wait before compound application might produce other errors if compounds differ in the period required for full equilibration with the larvae. It would be advantageous to study the possible compound differences obtained by substitution after different periods for every compound used separately, but this was not done in the studies reported here. Instead we selected the immediate substitution and chose to depend on differences between IVth and Vth larvae to provide clues as to the adequacy of substituting compounds.

The results have clearly indicated that the juvenile hormones, as well as the analogs used in our study, are perfectly adequate substitutes for endogenous JH at proper dose levels throughout larval development and there is little room left for the hypothesis that analogs of JH only act through protecting endogenous JH (SLADE and WILKINSON, 1973).

The relative potencies obtained with our method appear not to he very different from those reported on more conventional systems. Discrepancies may stem from many sources such as a different test species, differences in application techniques, the physiological stage used, etc. *Manduca sexta* is not a good species for bioassay of JH through inhibition of metamorphosis to the pupa since the treated Vth instar larvae tend to become unwieldingly large

and postpone further molting almost indefinitely. Even if they finally apolyse, the molting is often defective or incomplete and clearcut intermediates are rarely seen. Also, the quantities of JH active compounds required are large. More promising is a pupal wax test but the pupae of this species are relatively expensive to produce and for this reason no extensive bioassay efforts have ever been undertaken. The results obtained in our system make it very clear that most response curves are rather linear in the intermediate zones, up to the restoration of normal larval pigmentation, thus avoiding some of the pitfalls of assays aiming at prevention of metamorphosis. The clearcut exception to this rule are JH I and JH II, which display a characteristic shoulder around score 5 to 6. This shoulder has never been observed before in other JH assays *in vivo* and can perhaps he best described as a "buffering" effect related to the homeostasis of juvenile hormones. According to GILBERT *et al.* (1976), the binding capacity of JH specific proteins available in the haemolymph of *Manduca* is at least a thousand fold over capacity compared with endogenous JH levels, and this fact may well be related to the observed shoulders in the curves. Biologically it seems to be very advantageous for a developing insect to be able to "buffer" and protect its endogenous JH through binding to proteins thus preventing unwanted catabolic breakdown of a precious resource but also presenting a safeguard against fluctuating overdoses. It is perhaps meaningful that this buffering zone is located at an effect level (5-6) that does insure full viability, even while it is somewhat JH deficient. Somewhat against this explanation of curve shoulders through the protein binding buffering system is an observation made by GOODMAN (1976), who found that the synthetic analog JH 0 has an even greater affinity for specific binding proteins than JH I and JH II. In our system, however, the response curve for this compound does not show the characteristic shoulder. The relative abundance and importance of specific JH binding proteins throughout development has been insufficiently investigated, however, to make conclusive statements.

We would like to stress that potency differences obtained with this assay on a larval system may not be related to differences obtained in a system using the prevention of already induced metamorphosis. This induction may also entail the stimulation of the potent

enzymes that, in this stage, can freely attack bound JHs (SANBURG, *et al.*, 1975 a and b; GILBERT, *et al.*, 1976. Thus, the resistance against this breakdown may become the dominating factor in this stage. We have no information indicating that allatectomy combined with early substitution would stimulate this enzyme system, the subsequent rather normal larval development of adequately substituted larvae seems to rule this out.

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DISCUSSION

BELL

I wonder if you can tell us anything about the nature of the green pigment in this insect and is it a precursor of the dark pigment?

STAAL

I regret to say that we have not done biochemical work on pigments, so I have no comments on this.

WILLIAMS

I can comment on the pigmentation of larval *Manduca*, a matter which my colleague and former student, Dr. Peter Cherbas, has studied in great detail. It turns out that in those areas of the larval integument which are green, the cuticle is transparent and what one sees is the green coloration of the underlying epidermis. By contrast, in those areas of the integument which are black, melanin is deposited within the cuticle itself which is therefore black and opaque. It may be noted that the epidermal cells attached to the black cuticle lack the green coloration of the cells attached to transparent cuticle.

For those not familiar with the rather extensive literature on the subject, it will come as something of a surprise to learn that green insects almost never contain green pigment. The green coloration is due to the simultaneous presence of blue and yellow proteins. The chromophores of the yellow proteins are generally carotenoids.

Dr. Cherbas has studied in special detail the blue pigment of larval *Manduca*. From hemolymph as well as epidermis he extracted and purified and ultimately crystallized an intensely blue protein for which he has suggested the name "insecticyanin" (INS). The details of its chemistry need not concern us here. Suffice it to say that it is a water-

soluble biliprotein whose chromophore appears to be the open-chain tetrapyrrole biliverdin-IX_γ. Under most circumstances INS is a tetramer of apparently similar subunits each with a molecular weight of 23 000. There is one chromophore in each subunit. We have evidence that INS is synthesized by the larval fat body and progressively released into the blood. Synthesis ceases at the onset of the prepupal stage. It follows therefore that the gene coding for INS qualifies as a member of the larval gene-set. In first stage larva INS remains a blood pigment. At the molt to the second instar approximately 50 % is taken up by the epidermal cells presumably by special receptors or cellular transport mechanism. Throughout all subsequent larval instars there is a continuous synthesis of INS accompanied by its 1:1 partitioning between blood and epidermis.

The uptake of INS by the epidermal cells is of great interest in itself. The pigment is deposited in the cytoplasm as granules about 1.5 μ in diameter, there being on the average about 50 per cell. Uptake of INS by the epidermal cells is somehow under the control of juvenile hormone (JH)—a matter which has been studied at the Harvard Laboratory, not only by Peter Cherbas, but also by Louis Safranek, James Truman and Lynn Riddiford. In broad outlines, what seems to be going on is as follows:

At a certain stage in each larval molt the epidermal cells " read " the JH titer. If this exceeds a certain minimal value, the decision is made to remain green by continuing to take up INS throughout the ensuing instar. If this minimal JH titer is not present at the molt, the decision is made that throughout the ensuing instar INS will not be taken up by the epidermal cells and that such INS as preexists in these cells will be destroyed or dumped back into the blood. The epidermal cells, now devoid of INS, then proceed to melanize the overlying cuticle.

This phenomenon can obviously explain why *Manduca* larvae turn black when head-ligatured at a certain critical stage in a molt, as first noted by Jim Truman. It can also account for Truman's further finding that the blackening can be blocked by the topical application of JH or analogs thereof. Thus we see a rational basis for the well-known " black larva bioassay " for JH.

The latest chapter in this rapidly unfolding story is, of course, the serendipitous discovery by Lou Safranek of a mutant strain of *Manduca* which routinely turns black after the penultimate larval molt. It will

be recalled that the phenotypic expression of the mutant gene can be blocked by the topical application of as little as 1 ng of C_{18} JH.

So, in summary, we see that the study of the pigmentation of *Manduca* has provided a colorful story.

STAAL

I would like to point out that the larvae in all my experiments are bluish in color. One will never see larvae like this in the field because there they consume considerable amounts of yellow carotenoids that make them green. In the laboratory we do not provide carotenoids. This deficiency does not seem to affect their health or development.

CANONICA

My question was answered in the same way by Professor Williams.

BOWERS

I have a whole lot of questions here. Does the *Manduca* anti-JH antagonize JH? My understanding is it antagonizes JH activity at a site somewhere. Is that right? Does it antagonize JH activity in attacked animals? Does it work on insects other than *Manduca*? Does it inhibit JH in any of your *in vitro* organ cultures like silicon, and may I have a sample of it?

STAAL

Actually, at the last moment I decided to include some data on this JH antagonistic compound today because it seemed to fit into the philosophy of the substitution assay. Tomorrow I will provide more details about this compound.

NAKANISHI

Can the *Manduca* larva see? The reason I ask is because of the comment on carotenoids you mentioned.

STAAL

Actually, our larvae are in such close contact with the medium that it would not matter whether they can see or not. But the adults do need good vision for mating and oviposition, even under our laboratory conditions. They are sensitive to light and their diurnal activity rhythm is regulated by light. They are only active for mating and oviposition during the twilight and it appears that their visual acuity is not seriously impaired by the present dietary regime over a large number of generation cycles.

NAKANISHI

In that case we have recently made a retinal which binds to the protein opsin and makes a non bleachable rhodopsin and I was looking for an insect in which we could try this because you can play around with this rhodopsin in the light and nothing happens because it is non bleachable. In essence what it would do if it goes *in vivo* is to make the animal blind.

BOWERS

Yes I agree. I believe there is someone else who has done some study on diets lacking the several pigments that seem to be necessary. Karlson worked on it for some time and his conclusion was they were quite blind. In laboratory, of course, I do not think this would really matter for investigation.

NAKANISHI

Just for general information, the compound we made is a rhodopsin but nothing happens if you bring it out into the light. We are now making larger quantities, just to see what we can do with vision. This is in the negative sense, but this is to gain preliminary information on another information which would have an interesting positive meaning if worked. You simply add these to the diet instead of retinal or carotenoids and see the effect. Since there is no cis-trans isomerization, no bleaching can take place. It fortunately can bind with protein and gives a very simple compound.

It is a 11,12 dihydro-retinal. Namely it is hydrogenated across the crucial C-11/C-12 double bond which is involved in the bleaching of rhodopsin leading to recognition of vision.

INSECT CONTROL WITH INSECT GROWTH REGULATORS BASED ON INSECT HORMONES *

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Due to the characterestics of modern agriculture such as large area monoculture, the associated extensive natural habitat destruction and the demand for first grade produce, the dependence on chemical pesticides will exist for a long time to come. Integrated pest control methods, through the careful use of more selective control agents, and the more efficient utilization of available biological control elements, may reduce this dependence and avoid the pitfalls of secondary pest surges and early resurgence of the main pests, which have accompanied the use of broad spectrum insecticides. Broad spectrum pesticides usually create environmentally undesirable side effects, pose dangers for both the farmers and consumers, and tend to lose their effectiveness as the target pests develop resistance. Therefore, there is a continuous need to develop new types of pesticides with greater selectivity for insects in general (implying more safety for the user, the consumer, and the environment) and if possible, also for smaller groups of insects.

Insect growth regulators have come to be regarded as a promising new breed of pesticides. It is therefore useful to examine the progress made in light of the practical rules that apply for the development of all new pesticides. The scrutiny for undesirable side effects

* Contribution No. 152 from Zoecon Research Laboratories.

of all candidate or current pesticides is intensifying while governmental rules and regulations have not stabilized. It is especially difficult to predict how many of the present broad spectrum insecticides will survive this intense scrutiny, and it is not clear how well new compounds, each with their own series of handicaps (mostly of an economical nature), will compete with the old standbys. The trend can be recognized for an ever increasing demand for pesticides with greater selectivity for insects as a group, and, in specific cases, also for pesticides with more limited selectivity for the target pest species.

The Realities of Pest Control Development and Practice

The economic organization of most of the world indicates that large scale use of a particular pesticide cannot develop without industrial participation. For this, economic incentives must exist in the form of anticipation of adequate returns on the investments made. Since the investment for a single chemical may be in the order of $5 to $10 million (for research, toxicology, process development, chemical plant investment, etc.) and since a positive cash flow usually cannot be expected for the first eight to ten years after the beginning of the investment, the initial risks are discouragingly high (Djerassi, *et al.*, 1974; Knüsli, 1975; Green, 1976). The development of resistance, unforeseen side effects, or competitive compounds may later eliminate any prospects of profit and thus the investors are forced to adopt a most critical posture concerning each new candidate compound prior to full scale commitments.

Compounds with greater target selectivity have correspondingly smaller market expectations and are at a disadvantage from the start. So called "minor uses" for specific small markets usually can only be considered for compounds already registered for other purposes. A practical pesticide therefore, has to be a compromise in terms of selectivity between the economical incentive and the biologically most desirable. It should be recognized that many specific pest problems in the past were a direct result of the use of broad spectrum insecticides for other pests. Spider mite surges in orchards are a classical example of a pest problem promoted by the use of compounds that

eliminate predatory mites and other biological control factors. Thus, by using only selective agents, the spectrum of pest problems is likely to decrease as will the total quantity of pesticide required. Unfortunately, more selective compounds are not necessarily less expensive in their requirements for capital investment in toxicology and other development factors. Their benefits therefore can only come at a higher price for the consumer of the products and other beneficiaries of the concept.

What are Insect Growth Regulators?

Insect growth regulators can be defined as compounds that alter specific growth or formative processes in insects. Thus, they do not include compounds that elicit short term mortality through action on the nervous system (as do most current types of insecticides) or compounds that have a more general toxic activity on vital processes. This definition implies a degree of potential selectivity for insects, since most formative and growth processes are either intrinsically different or regulated in ways different from those of other groups of organisms.

Based on this definition the predominant characteristics of IGRs are: a rather slow mode of action and a high degree of selectivity for insects as a group. Selectivity for orders, families, genera, or species is not an *a priori* characteristic of IGRs but is found to occur frequently as a result of minor chemical modifications.

IGRs are a varied collection of compounds and the spectrum will probably continue to enlarge. It is therefore of value to add a further modifier to the term IGR which specifies the mode of action (e.g. IGR with JH activity). Sweeping conclusions as to the usefulness of IGRs are not meaningful without this more precise definition, and it should be strongly emphasized that in most cases the exact chemical identification of the specific chemical is equally indispensable.

Implications of the Slow Mode of Action of IGRs

Since most IGRs have a slow mode of action, it is worth examining whether this feature is acceptable in practical insect control. The

question has two sides: 1) can the user adapt to a control method where the degree of success can only be evaluated at a moment that retreatments or other curative control measures cannot be successfully applied any more, and can he do without the psychological factor of immediate satisfaction? 2) can some continuation of damage after application be sustained?

A general answer to these questions cannot be given due to the variety of IGR types and pest population characteristics. JH analogs for instance, do not curtail ongoing damage caused by immature insects and thus can only be effectively applied where preventative control in preceding generations is feasible. This is generally not practical for pest species that are highly migratory, survive on a variety of wild host plants and have few generation cycles per year. Many important lepidopteran pest species belong to this type (e.g. *Heliothis virescens* and *H. zea* in the USA and relatives of these elsewhere). On the other hand, pest species that go through several short cycles per season, that are more sedentary, live in confined environments (facilities for stored products, greenhouses, etc.), that are less polyphagous or that cause most of their damage in the adult stage rather than the larval stage make far better targets for this type of IGR.

Somewhat faster acting IGRs, such as Dimilin®, are less of a problem in this regard, since applications very early during the larval stage may still curtail most of the damage by deranging the subsequent early larval molting processes. IGRs of the hormone antagonistic type could, at least in theory, have a somewhat similar efficacy.

The psychological aspect is dominated by the competitive availability, efficacy, and acceptance of control agents with short term action which provide fast relief for situations that threaten to get out of hand. The results of more preventative control with IGRs could initially be safeguarded by standby, short term pesticides which in certain cases could also be applied simultaneously to combine the benefits of both (but then usually only with the loss of some benefits such as selectivity).

Types of IGRs

Pest control by means of natural regulators (e.g. hormones) or their synthetic mimics can only be successful if a period(s) exists in the insect's life in which the absence of the regulator is essential for normal development. Such a relationship exists very clearly for juvenile hormones (JH) during the later part of immature development. Applications of juvenile hormones or their analogs during these sensitive periods produce morphological abnormalities that are generally irreversible and in many cases lead to death at or after the metamorphic molt(s), and in any case before reproduction can take place.

For molting hormones (MH) it is known that periodic peaks in their titer are related to the initiation of molting cycles but whether the valleys in this titer curve are physiologically essential is not so well known. Applications of molting hormones can accelerate or delay subsequent molting cycles and excessive doses can produce irreversible morphological damage but, generally, only injections do this with any efficiency. Moreover, minor morphological abnormalities produced in molting are sometimes reversible, or at least not immediately lethal.

For most, if not all, hormones, any effective inhibition of their synthesis or interference with their regulatory action would be quickly deleterious to the developing insect and to a varying degree also to the adult. Several chemical antagonists for insect juvenile hormones (anti JH) and molting hormones (anti MH) are known, but these are all still in the experimental stage.

Besides these four groups of possible IGRs based on hormonal processes only one other IGR type is recognized so far, that of the compounds disturbing cuticle synthesis represented by Dimilin® (TH 6040). One may assume that other types of IGRs will be developed that interfere with other developmental processes or their regulators.

The experience presently available is still tilted towards the IGRs with JH activity, since this group has yielded the only IGRs that have come into actual use today, but practical experience with the Dimilin type compounds is now rapidly increasing. The development of JH analogs has been a very important step in the enrichment of the pesticide arsenal, but it should again be emphasized that extra-

polation from the practical experience gained with these agents to predict efficacy of other possible IGR types for insect control is not appropriate.

IGRs of the JH Type

The three natural juvenile hormones (JH I, II, and III), occurring separately or in combination in all insect species investigated (Judy *et al.*, 1976) (Fig. 1), have never been serious candidates for use as pesticides. Synthesis and evaluation of thousands of synthetic analogs has yielded compounds with higher specific activity, higher stability, lower manufacturing costs, more selectivity for insect orders, and last, but not least, proprietary protection for the manufacturers. However, with few exceptions (e.g. kinoprene), the basic mode of action of the analogs appears to be identical to that of exogenously applied natural JHs. The truly unique and gigantic collaborative effort between pure science and industrial enterprise has accelerated the evaluation of these compounds, and led to a good understanding of the mode of action on the level of the organism as a whole. The mode of action on the cellular level is still regrettably obscure and its elucidation may provide new incentives.

It should be kept in mind that effective exogenous applications of any JH to even the most sensitive insect stages requires doses that are several orders of magnitude higher than endogenous hormone titers. Obviously the insect has found ways to economize its internal hormone reserve that externally applied hormones or mimics are unable to exploit. We may suspect that the endogenously produced hormones are protected from catabolic enzymes in the blood through binding to specific "carrier" proteins.

The three natural JHs, differing only in the lengths of their side branches, may have somewhat specialized functions (Lüscher and Lanzrein, 1976). If both the branches at C-7 and C-11 are methyl groups as in JH III, the activity in most biological test systems is low; so low in fact that it is hard to believe that JH III can be an essential juvenile hormone at the quantities in which it occurs in the insect. In the 2,4 dienoate series to which methoprene and kinoprene belong (Fig. 1), ethyl side chains do not impart high insect activity for reasons not fully understood, whereas in some other

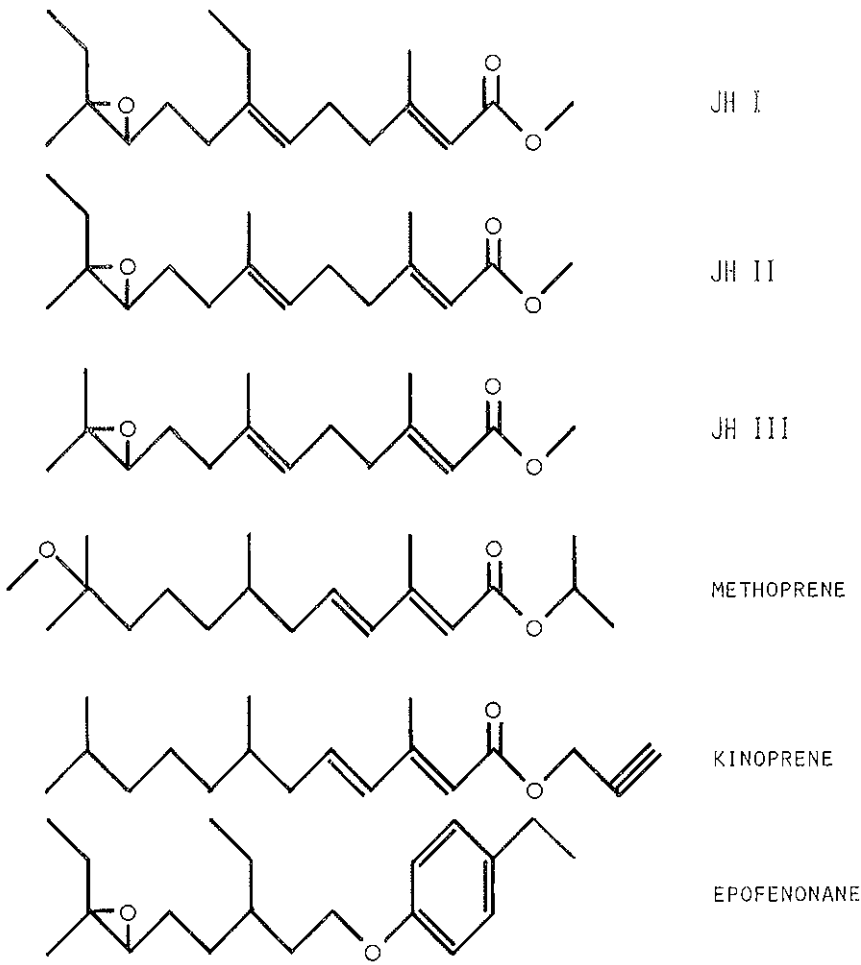


FIG. 1 — Structures of natural juvenile hormones and some of the most active analogs with practical potential.

chemical series ethyl side chains seem to impart high foliar stability to the molecule, e.g. epofenonane (Fig. 1) (HANGARTNER *et al.*, 1976). The general mode(s) of action, the specific selectivity and other aspects of the JH analogs are described elsewhere in greater detail (STAAL, 1975; MENN and PALLOS, 1975; SLAMA *et al.*, 1974; SEHNAL, 1976).

All practical uses have thus far been derived from their irreversible actions on insect metamorphosis. Other possible and often more indirect effects on embryonic development, diapause, phase, caste and morph development, etc., have not yet been exploited to their fullest potential.

It is worthwhile to review the properties that have provided the roadblocks in development of JH type IGRs as well as the favorable factors that have repeatedly tipped the scales towards practical usage.

The disadvantages of JH analogs are:

1) The slow mode of action cannot prevent damage done by immature stages and makes assessment of success possible only after it is too late to take other measures;

2) The sensitive resting stages (pupae, adults in reproductive diapause, etc.) are usually inaccessible;

3) The narrow "windows" of sensitivity combined with less than acceptable stability and persistence of foliar residues.

4) The lack of irreversible effects on adults with most compounds of this type;

5) The relatively high price of manufacturing;

6) The lack of ovicidal effects on eggs deposited on fresh foliar residues, even though direct topical treatment of the eggs is often very effective;

7) The lack of effectiveness of presently known JH analogs on certain insect groups (weevils, grasshoppers, cockroaches, etc.).

The advantages of JH analogs have offset the disadvantages in several specific use patterns in insect control, including a use in the rearing of beneficial insects.

Control of Mosquito Larvae with Methoprene

Methoprene is particularly effective against floodwater mosquitos such as *Aedes aegypti, Aedes nigromaculus*, etc. that develop rather synchronously after flooding of the habitat with the diapausing eggs. Methoprene is, in fact, the most active larvicide for mosquitos ever developed. However even here, persistence had to be increased through micro-encapsulation (plastic walled capsules of 1-10 micron diameter in a water emulsifiable suspension). The effectiveness of this methoprene formulation may be derived in part from ingestion of the micro capsules by the larvae, which feed indiscriminately on particles of this size (Fig. 4). Aedine and anopheline mosquitos are most susceptible. Culicine mosquito species are generally somewhat less sensitive and less synchronized and appear to require a different approach to formulation. Since larvicidal control for mosquitos is not practiced on a worldwide basis, and the adults are not susceptible to methoprene, actual use is restricted. Besides the competitive efficacy of methoprene as a larvicide, another favorable asset is the very selective action on Diptera. This provides a reduced hazard for non target organisms, such as fish, compared with most standard larvicides (TAKAHASHI and MIURA, 1975).

Control of Fly Larvae with Methoprene

It has not been possible to make IGRs penetrate the semisolid substrates of many larval Diptera adequately when applied externally or even hy mixing, without a large percentage of the larvae escaping contact. The solution to fly control in the manure of cattle proved to be oral administration of methoprene with food, water, or mineral supplements (CHAMBERLAIN, 1975; BEADLES *et al.*, 1975). However, due to differential susceptihility of the four most pestiferous fly species in manure, they cannot all be controlled effectively with the presently available mineral block (containing 0,02% methoprene). Only the horn fly (*Haematobia irritans*) is controlled fully and economically. The face fly (*Musca autumnalis*) and stable fly (*Stomoxys calcitrans*) are partly controlled and the housefly (*Musca domestica*) remains largely unaffected. The less susceptible species could be controlled with presently uneconomic doses (Table 1). The surprising aspect of this is that enough methoprene passes the cow's ali-

TABLE 1 — *Fly control in manure by feedthrough of methoprene to cattle.* Data supplied by Moormans Mfg. Co., Quincy, Illinois, USA.

Species [1]	LC_{50}	LC_{90}	Calculated effective food rates based on LC_{90} mg/1000 lbs/day
horn fly *Haematobia irritans*	0.0046	0.0193	7.5[2]
stable fly *Stomoxys calcitrans*	0.1170	0.2110	82.0
face fly *Musca autumnalis*	0.1010	0.2250	87.0
housefly *Musca domestica*	2.2800	7.600	3000.0

[1] All fly species used were moderately OP resistant except for the facefly.
[2] Actual level supplied by mineral blocks.

mentary tract unchanged to exceed the required level in the manure (up to 96-98% of the administered methoprene is broken down during passage and rendered inactive). In 1976 more than 1,000,000 head of cattle in the USA received methoprene with their mineral supplements.

Similar administrations in poultry, although successful in preliminary studies (MORGAN *et al.*, 1975), have failed to yield consistent fly control at economic rates because the target in this case is primarily the less susceptible housefly (*Musca domestica*), and because many larvae are able to migrate from the methoprene containing manure to other pupation sites and thus manage to escape exposure during their most sensitive prepupation period.

In cattle, as well as poultry, the bulk of the methoprene and its metabolites are excreted and do not pass into the animal's internal system to any appreciable degree.

The use of JH analogs against both mosquito and fly larvae was a logical priority since most Diptera do not produce damage as im-

matures and also because of the short development cycles in this type of insect, requiring only a minimal residue stability.

Many other groups of flies of agricultural, nuisance, or medical importance are sensitive in varying degrees to methoprene and other IGRs, and practical applications to these targets continue to be investigated (CHAMBERLAIN, 1975).

Insect Control in Stored Commodities

The principal advantages of methoprene for this type of target are the very low human and vertebrate toxicity, the closed environment (not subject to continuous immigration), and the relatively high activity on several typical storage pest species (beetles as well as moths) (STRONG and DIEKMAN, 1973; WILLIAMS and AMOS, 1974; MCGREGOR and KRAMER, 1975; LOSCHIAVO, 1976). Yet, the development of IGRs with JH activity for stored grain protection has been impeded by the relative insensitivity of the two very important and ubiquitous weevil species, the rice weevel (*Sitophilus oryzae*) and the granary weevil (*Sitophilus granarius*). This insensitivity for IGRs appears to be a rather intrinsic characteristic of weevils in general rather than the result of the sequestering of the larvae of weevils inside the grain kernels (ROWLANDS, 1976; STRONG, personal communication).

While stored food protection applications are not now actively contemplated for methoprene, the compound has recently received an experimental registration for the protection of stored tobacco (for a total treatment of 3.6 million lbs of stored tobacco), in which the cigarette beetle (*Lasioderma serricorne*) is the main pest species.

Control of Greenhouse Whitefly (Trialeurodes vaporariorum) and other Greenhouse Homoptera

Another IGR, kinoprene (Fig. 1), combines a very high and selective JH activity with a direct toxicity on Homoptera at higher doses (STAAL *et al.*, 1973). This compound, therefore, has the characteristics of a highly active IGR, and as well as a direct toxicant of which the precise mode of action (related to the propynyl moiety) is still obscure. As a consequence, all stages of the whitefly, including the eggs (independent of age) and the adults are affected

(no current insecticide affects all stages). Likewise, in aphids, most stages are susceptible or show impaired reproduction through effects on the successive generations of viviparous forms (NASSAR *et al.*, 1973). As yet, kinoprene is only registered for use on ornamental plants in the USA. Applications on food crops might require more expenditures in toxicology than may be justified by the limited market potential. The present formulations of kinoprene appear to have insufficient stability as foliar residues for economic control of Homoptera under field conditions.

Methoprene also works reasonably well against many species of Homoptera (greenhouse and field pests such as aphids, whiteflies, scales, etc.) but the ultimate effectiveness of this compound by itself is fairly marginal compared with other current pesticides for these insects. However, in combination with a more acute toxicant, its long range JH activity contributes a worthwhile element to overall efficacy.

Methoprene appears to affect whiteflies far more than their hymenopteran parasite, *Encarsia formosa*. Recent studies have indicated that a combination of methoprene applications and introduction of *Encarsia* in greenhouses provides far better control than either of these methods separately (DEGHEELE, VAN DE VEIRE and STAAL, in preparation). Laboratory data on the subject of differential activity against target species and beneficial species are not as a rule very relevant, and longer term studies on the dynamic effects on pest/beneficial complexes under actual field conditions are required to provide conclusive data. (The cited study on whitefly/*Encarsia* does reflect actual greenhouse conditions.)

Along the same principle, the use of mermitid nematodes for control of mosquito larvae proved completely compatible with the use of methoprene, integrating the efficiency of both control methods (W. R. NICKLE, personal communication).

The use of Methoprene in Silkworm Culture

An interesting use of methoprene has been developed in Japan, where it was found that a timely administration to nearly mature silkworms can increase cocoon size and silk production by as much as 5 to 15% (depending on the dose and the exact timing), with

only minimal increase in food consumption. (The fact that the pupae themselves are adversely affected by the IGR is not of concern to the silk producers.)

Major Field Crop and Forest Insect Pests

This application constitutes by far the most important use of pest control products and offers more incentive for the development of new pesticides than any other application. It is here that the cost-effectiveness ratio comes to its most severe test with field stability as one of the most crucial parameters.

Most IGRs with JH activity have fallen short of the basic requirement of persistence of foliar residues, primarily because of susceptibility to ultraviolet radiation. Compounds that perform adequately in the protected environments of greenhouses, grain silos, etc. may fail completely in the field, because only the insects exactly in the sensitive stage at the time of application will be affected. Thus, the persistency requirements for an IGR with JH activity are, in fact, more stringent than for a more conventional pesticide that can affect many stages of insect development. Theoretically, the short persistence could be compensated by more frequent treatments, but the economics of this are entirely prohibitive.

A recent breakthrough has been achieved in the JH analog, epofenonane (See Fig. 1), that combines high activity on several insect species with high foliar stability. While the disadvantage of a slow mode of action is still present and the cost/effectiveness ratio is not yet known, the promise of applications for IGRs that were hitherto not available has now been opened. An example of such a use would be the control of scale species in tree crops (Hangartner *et al.*, 1976; Vogel *et al.*, 1976; Jucker *et al.*, 1976). Characteristics of scale insect pests are: their lack of mobility, lack of acute deleterious effects on the host plants, and the lack of sensitivity to many conventional insecticides. These features seem to fit the mode of action of IGRs better than the features of other major types of field pests. JH analogs, in general, do not seem to have difficulties exerting their effects through the heavy "armor" these insects are equipped with.

IGRs may fit the requirements for forest pest control some-

what better than those for agriculture. The high selectivity of IGRs is more of an immediate asset, while the ultraviolet degradation is less severe on leaves (or coniferous needles) other than those directly exposed to sunlight. The efficacy of methoprene on Eastern hemlock looper has been demonstrated (A. RETNAKARAN, personal communication), but the economics are not attractive as yet.

As a more general consideration, forest pests do not rate very high as a primary economic incentive for the development of novel pesticides due to the cyclic nature of their occurrence and the lack of funds for forest pest control in vast forest areas that are not economically productive.

OTHER POSSIBLE TARGETS FOR JH ANALOGS

IGRs of the JH type offer interesting prospects for the control of certain social insects. Several ant species (the fire ant, the Pharaoh ant, the Argentine ant, etc.) and possibly termites, are sensitive to JH analogs (EDWARDS, 1975; TROISI *et al.*, 1974; CUPP and O'NEAL, 1973). Since research on long term efficacy is extremely tedious and this type of target requires years of research into bait formulations, the determination of practical efficacy can only come slowly. It is important to note that generally only pesticides with a slow action, that can be carried as a bait inside the nest in some form, offer prospects for lasting control of ants. With the demise of conventional pesticides such as Mirex, IGRs may offer the next best alternative in this area. The application of IGRs for the control of social insects would constitute one of the most economical uses of IGRs since the treatments can be aimed towards the insects directly through baits rather than towards entire habitats.

IGRs of the JH type that affect social Hymenoptera theoretically pose a hazard to bees. However, as effects on hymenopteran species generally require the ingestion of baits, foliar residues are not usually harmful to worker bees or the colony. Most IGRs are not systemic and thus are not accumulated in pollen and nectar, and therefore, pose little danger. Feeding experiments to bees are perhaps the most severe test but not very indicative for field conditions. It is no surprise that tests of this kind may indicate deleterious long

term effects on broad development rather than workers when fed at very high rates (HRDY and SKROBAL, 1976). Field experiments at more realistic rates would probably not show significant effects.

MOLTING HORMONES

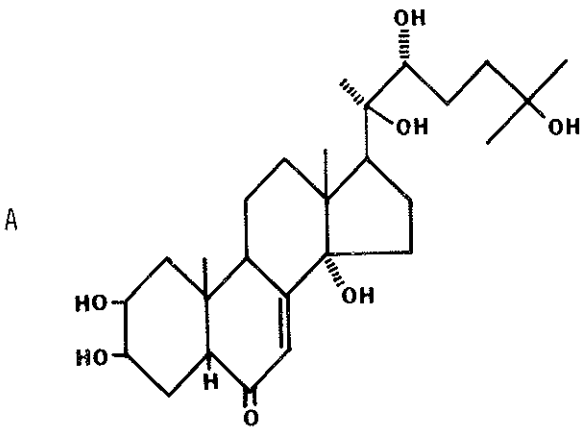
Besides the already mentioned lack of biological efficacy, another element preventing their use as IGRs is the complicated steroid structure. Compounds of this type (Fig. 2a) cannot be economically synthesized and although certain plant sources appear to be rich in phytoecdysones, extraction for insect control purposes could still only be an economical proposition if they would be highly effective.

The use of any chemical resembling steroid hormones, which play very essential roles in man and higher animals would necessitate a more elaborate and expensive testing for possible side effects.

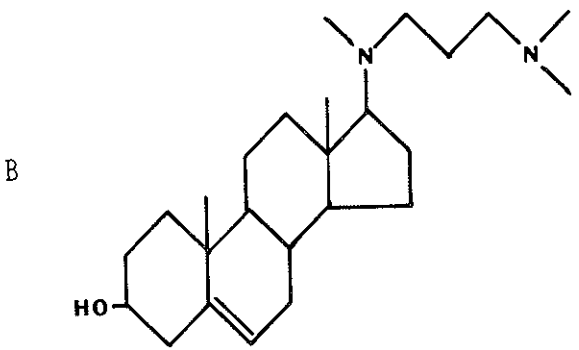
The only practical application of phyto-ecdysones to date is the use in commercial silkworm rearing in Japan. Spray applications induce a synchronization of cocoon spinning that increases rearing efficiency and thus is a benefit. In this case, the application is very direct and thus economical.

ANTI MOLTING HORMONES

Certain azosterols (Fig. 2b) or even simpler nitrogen containing compounds (Fig. 2c, d) appear to be disruptive on the development of phytophagous insects that depend on the conversion of dietary sitosterol to cholesterol (ROBBINS *et al.*, 1975). However, within this group of compounds one would also have to be alert for possible effects on higher animals in which steroid hormones play a very important regulatory role. The development of their use on insects is therefore not an easy proposition. The cost of azosterols would be entirely prohibitive for agricultural use, but the smaller molecules described by ROBBINS *et al.* would offer better promise against phytophagous insects that do not have access to cholesterol in their diet. It may still be possible to find other types of non-steroidal MH inhibitors, affecting essential steps in the synthesis,

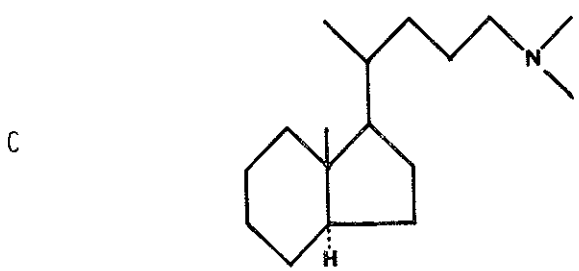


β-ECDYSONE MH



20,25 DIAZOCHOLESTEROL

ANTI MH



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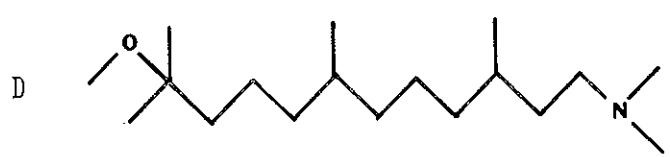


ANTI MH

FIG. 2 — Structure of β-ecdysone and some molting hormone antagonists.

transport, storage, or recognition by receptors of the endogenous hormones.

Molting hormones appear to have a regulatory function in other arthropods than insects (e.g. Crustacea), and can therefore be expected to be less selective in most use patterns. The same caution would most likely apply to possible anti molting hormones.

Anti Juvenile Hormones

Although anti JHs have been only hypothetical until recently, families of compounds with more or less distinct JH antagonistic activity and affecting different target species rather selectively, have now been identified.

I. The precocenes, belonging to the group of ageratochromenes, were isolated by W. S. Bowers from extracts of the bedding plants *Ageratum houstonianum* and are mainly active on the milkweed bug (*Oncopeltus fasciatus*) and cotton stainer bugs (*Dysdercus spp*) (Bowers, 1976). The effect of precocene II (Fig. 3a) is clearly that of producing premature metamorphosis and inhibition of egg development in adults of these species. The mode of action, thought at first to be exclusively antiallatotropic, may be of a higher order, since the prothoracic glands also show signs of degeneration (Masner *et al.*, 1976). This suggests that either several secretive functions are affected simultaneously or that higher regulatory centers such as the neurosecretory cells in the brain are inhibited. The effects of precocene II on precocious metamorphosis and egg development of *Oncopeltus* can be entirely counteracted by simultaneously administered JH analogs, but the acute mortality produced by high doses on *Oncopeltus* as well as other insect species is not influenced by JH and is probably due to an entirely different and less selective property. On any of several holometabolous insects, including economically important pest species, the precocenes have failed to demonstrate effects other than a slowdown of development and toxicity at high doses.

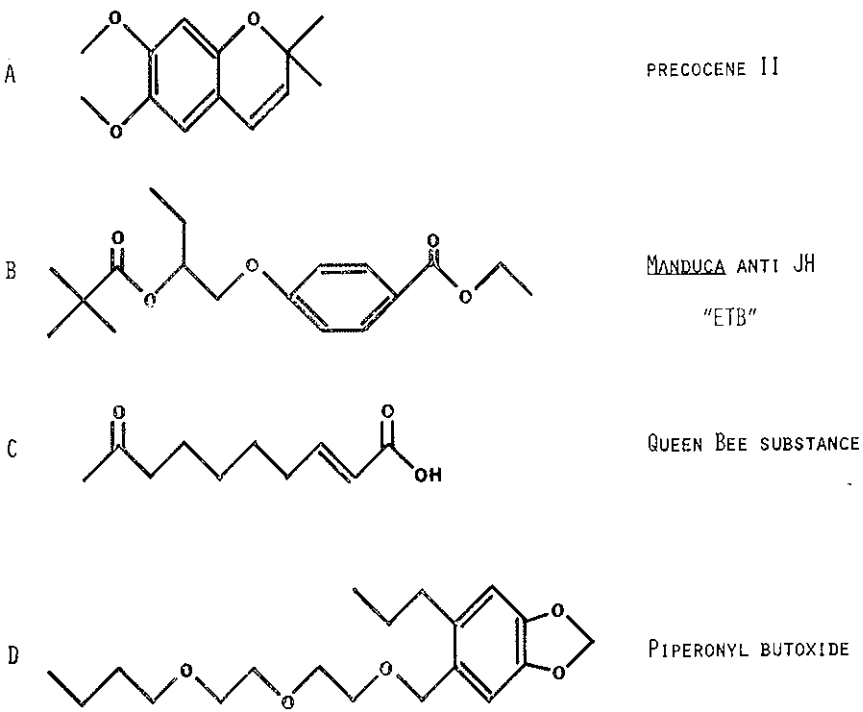


FIG. 3 — Structure of juvenile hormone antagonists.

II. Another group of compounds, represented by ethyl 4-[2-(*tert*-butylcarbonyloxy) butoxybenzoate or ETB (Fig. 3b, see KONDO *et al.*, 1973; OSHIMA *et al.*, 1974) has only shown effects on the tobacco hornworm (*Manduca sexta*). A precise dose on the IIIrd instar produces a black IV instar larva and premature pupal cuticle in the Vth (last) larval instar (Fig. 5) (STAAL *et al.*, in preparation). No modulation of the dose or timing has succeeded in production of pupal cuticle *before* this last larval instar but the black color can be induced as early as in the IInd instar. In *Manduca* larvae the development of an unusual black pigmentation is an indicator of JH deficiency at an intermediate level that is not severe enough to produce premature metamorphosis as follows from surgical ex-

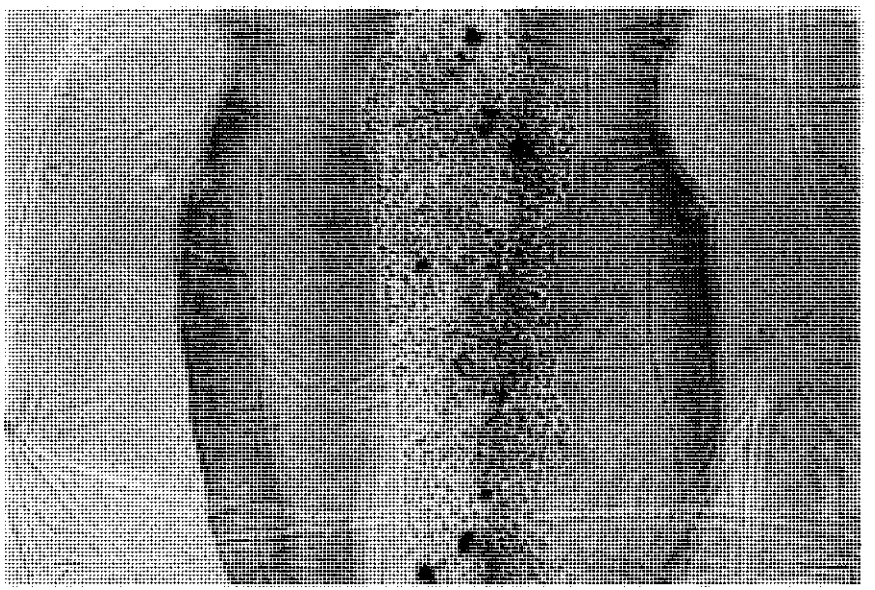
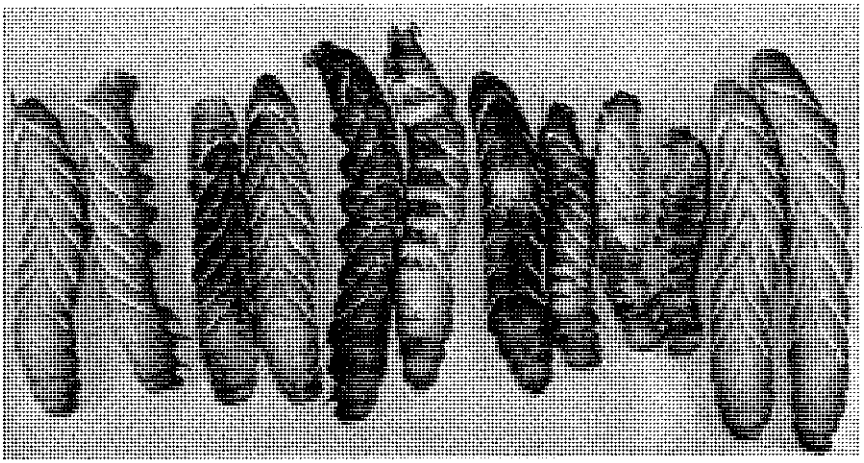


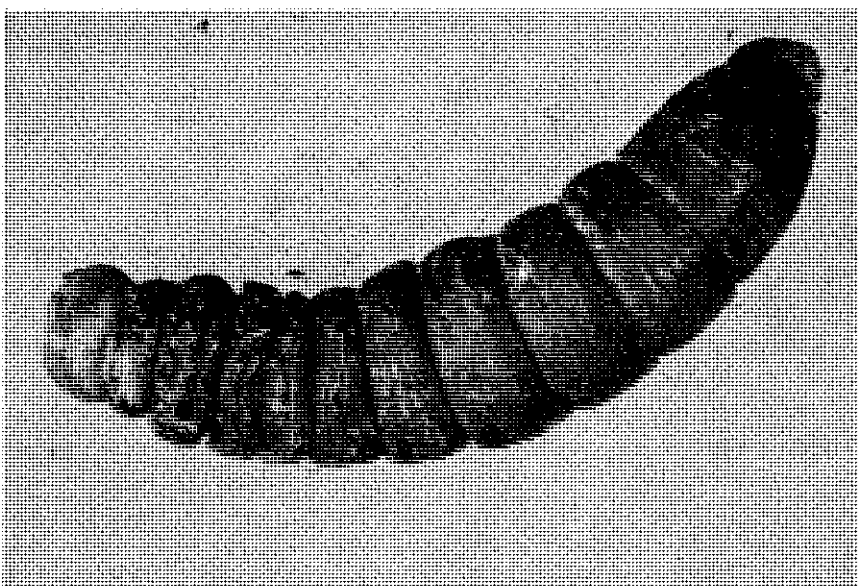
FIG. 4 — Midgut of last instar *Aedes aegypti* larva filled with microcapsules of ALTOSID SR-10 (methoprene) taken up by indiscriminate ingestion.

periments and studies on a spontaneous black mutant of *Manduca sexta* (SAFRANEK and RIDDIFORD, 1975).

Unfortunately, the optimum effect of premature metamorphosis is only produced by a very narrow dose range. The optimum dose is 30 to 100 µg topically on IIIrd instars or 10 ppm in the food when administered prior to the IVth instar. A dose of three to ten times this optimum is no longer effective and the specimens will instead show positive JH effects. A rather high intrinsic JH activity for this type of compound is found in other lepidopteran bioassay systems as well (*Galleria mellonella, Heliothis virescens, Trichoplusia ni*) but the antagonistic activity has only been observed in *Manduca sexta* so far. Since all other JH antagonistic representatives of this family of compounds also possess positive JH activity, it is possible that the two are inseparable. This strongly suggests competition with endogenous JH on peripheral receptors as a possible mechanism of action. As proof of its true antagonistic behavior, the effects of this com-



a



b

FIG. 5, a and b:

a. Group of Vth instar larvae of *Manduca sexta*, treated with ETB (100 µg topically) in the IIIrd instar. To the left in the picture are two untreated larvae, the others show various degrees of precocious pupal cuticle and black pigmentation. The precocious pupae are unviable.

b. Severely affected precocious pupa (Vth instar) after topical treatment with 30 µg of ETB in the IIIrd instar larva.

pound could be counteracted by simultaneous administration of several JH active compounds.

The practical potential of compounds of this type presently appears to be poor due to the high and very narrow dose requirement and the apparent selectivity for certain species.

III. Older work on (E)-9-oxo-2-decenoic acid, the identified component of queen bee substance (Fig. 3c), suggests JH antagonistic effects on egg development of Diptera (NAYAR, 1963; SANNASI, 1969). However, these effects could not be reproduced with this chemical on housefly in our laboratory. The same compound has also failed to produce effects on metamorphosis in a range of different insect species, and its full recognition as a JH antagonist would therefore require more corroboration and proof of its mode of action.

IV. We have also found that piperonyl butoxide (PB) (Fig. 3d) has all the characteristics of a juvenile hormone antagonist in the *Manduca* test system as indicated by the appearance of black pigmentation after topical application or administration in the medium. In a few cases we have also observed prematurely occurring pupal cuticle, but these observations are hampered by the high mortality occurring in specimens treated with doses producing an optimally black integument.

Simultaneous administration of any JH active compound (including compound 3b) prevents the blackness from occurring but not the mortality of higher doses, indicating that PB is a true anti JH. This conclusion is surprising since PB is known as a synergist for pesticides, preventing their degradation in insects by inhibition of the microsomal oxidase enzymes. One would therefore expect a JH protecting effect rather than an anti JH effect. However, the JH protecting aspect is definitely present as well, as follows from applications immediately after allatectomy. It then prevents metamorphosis since a normal but black larval/larval molt is induced instead of a larval/pupal molt. PB by itself does not appear to possess positive JH activity in most test systems, but a low level transitory pseudo juvenilizing effect in the *Galleria* wax test was noted many years ago.

Since the natural regulation of JH function in insects is a com-

plicated sequence from stimulation of the gland by neurosecretory centers, to hiosynthesis, secretion, transport, storage, and finally association with (hypothetical) receptors, other types of JH antagonists with more useful properties may still await discovery.

Conclusions

As could have been expected, IGRs are not a general panacea for insect control. Their use is restricted by the same rules as for other compounds and only a careful weighing of benefits and drawbacks has promoted their use in specific cases. The original speculation that insects may be unable to acquire resistance against compounds resembling their natural hormones has already been deflated by the facts. The route of external application provides a mechanism for resistance, and the natural presence of metabolic enzymes that appear to have a function in the regulation of endogenous hormone titers provides another one. In fact, cross resistance against JH analogs has been found in certain insecticide resistant housefly strains (Cerf and Georghiou, 1972) and the breeding of *Aedes aegypti* mosquitos for several generations under stress of methoprene has yielded increased insensitivity to this IGR (Brown and Brown, 1974). It should be emphasized, however, that although cross resistance and development of resistance under laboratory conditions indicate that JH analogs are not exempt from selective processes leading to resistance, the actual occurrence of resistance in the field cannot be predicted with any precision.

Experience with the useful life of any IGR before sizeable resistance develops in insect populations can therefore only be gained through the years. We may assume, however, that the continuous development of novel types of pest control compounds will be essential in order to stay ahead in the continuous struggle with problem causing insects.

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DISCUSSION

CANONICA

You have reported many activity data of synthetic analogues of natural juvenoids. Their molecules contain asymmetric centers, for example 3 in the case of epofenonane. It means 8 possible stereoisomers, of which only one is probably the true active form. So, the data you have mentioned can be even largely different depending on the content of this form in the mixture checked.

Secondly, you have expressed the general opinion that the extraction of ecdysones from natural sources must be anyway too expensive for practical purposes. I do not think so: for instance, the seeds of an Ipomoea I have mentioned yesterday are cheap and contain about 2% of a mixture of ecdysones. Probably this content can be increased selecting suitable varieties and cultivation conditions.

STAAL

I do agree, of course, that if good herbaceous sources can be found, the price calculations might improve. Unfortunately, even the most active ecdysteroids are not all that efficacious in terms of insect control (the very efficient application in silkworm culture is beside the point here). For agricultural use, compounds have to be inexpensive. For these reasons, I do not see a practical application for ecdysteroids in insect control except for that in the silkworm industry. You brought up a very relevant point about the stereoisomers and the enantiomers. We know that rather pure optical enantiomeric antipodes of JH and JH analogs differ greatly in biological activity. Most likely, although we have no absolute proof in our hands, only one of the enantiomers has all the activity and the other one does not. In the racemic mixtures, then, we would have about half the activity of the pure active enantiomer. The possibility remains that very pure, inactive, enantiomers may possess

JH antagonistic properties. However, at this time absolute enantiomeric purity is difficult to achieve and to measure. As far as geometric isomers of JH analogs are concerned, they also display pronounced differences in activity, but industry has been able to establish fairly stereospecific synthesis procedures that guarantee high purity in this regard.

NAKANISHI

First I want to make an off-the-record remark. Another practical application of ecdysone is — this is limited to your spouse. Professor Takemoto's wife has once in a while a fit of rheumatism and then, whenever she has that, he gives her a 50 mg dosage of ecdysone — inokosterone in this case — and apparently it has a very good effect in curing this, but this is off the record. Now, I would like to ask: there are 3 kinds of juvenile hormones: I, II and III, and there must be some reason for nature to make these things, and when you think that most of the insects in which distribution has been studied, quite a few of them contain more than one juvenile hormone. And also in some cases it has been shown that the titre of these 3 juvenile hormones depends on the larval stage. Now, on this basis I was wondering whether you have tested the effects of different juvenile hormone analogs at different stages of larva to see whether they have different effects, instead of just choosing just one larval stage.

STAAL

I was only talking about practical applications and the only worthwhile target process for this appears to be the inhibition of metamorphosis. We have done extensive studies on the three natural hormones and also on comparable homologs with ethyl or methyl side chains in many analog series. I can state that in our 2,4 dienoate series (which includes methoprene) it really does not make all that much difference whether ethyl or methyl side chains are present, but it does make a tremendous difference in the natural hormones. However, it is interesting to note that in the epofenonane compound the ethyl side chains seem to impart to the molecule a tremendous stability in foliar applications that is not present in the natural juvenile hormones with corresponding ethyl side chains.

NAKANISHI

Excuse me if I am saying nonsense, but what I am asking is, say, the first instar and 5th instar — they may be affected different ways by different juvenoids. That is what I am asking, and have you tested this type of effect because of the different distribution depending on the larval stage of the 3 different juvenile hormones?

STAAL

This is a very interesting, but rather academic point. I would like to investigate this in more detail, but unfortunately it is not practical to remove corpora allata from first instars. I have completed work now on the third, fourth, and fifth instars, and I found that JH I and II are very effective compounds to regulate the larval development, but JH III is not. This leads me to believe that when one analyzes an insect such as *Manduca* for its juvenile hormone titers and one finds approximately equivalent amounts of JH I, II, and III, the JH III is only a rather insignificant artifact that does not contribute much physiologically. There is, however, still a good possibility that JH III plays a predominant role in the reproductive adult, but I have not looked at this aspect in the described experiments. In my opinion, JH III is not active enough to be classified as an essential JH during larval development of *Manduca sexta.*

DORN

I would like to make just a comment on this. We did extensive work with juvenile hormone active IGRs; and we encountered actually all the problems you mentioned in your lecture. However, we found that many IGRs affected the insects not only during metamorphosis but also at earlier stages. Epofenonane, for instance, which performs well against many lepidopterous and homopterous species, disrupted the development of some insects at several stages. After application of epofenonane, the young larvae of the citrus mealy bug, *Planococcus citri*, were unable to complete normal ecdysis and died during the process. When we applied very low dosages so that larvae could survive, we found disrupture of the metamorphosis of the males. You could see the corresponding pictures yesterday in the lecture presented by Sir Vincent Wigg-

lesworth. The females of this species do not undergo a lot of external metamorphosis; however, the ovaries must differentiate and we could limit or even block this process by epofenonane. Consequently, if you treat a mixed population with this compound you get a very good change and complete control since the compound is relatively stable on leaves. Similar results were found with California red scale (*Aonidiella aurantii*). A field trial with summerfruit tortrix moth (*Adoxophyes orana*) performed during this summer in Switzerland provides an additional example of IGR induced disrupture of development at different stages. Very low dosages were applied so that the population was not completely controlled initially. About — I think — 15% of the adults survived. However, the surviving females had significantly lower fecundity than those in the check and the eggs which were laid had a distinctly lower hatching rate. So you see, JH active IGRs are able to disrupt not only the metamorphosis but also completely different stages of the insect life cycle.

STAAL

Thank you very much, Dr. Dorn, for elaborating on my comments. I have not gone into this much detail, but you are right. We did some work on scale insects years ago and obtained very similar results. One can disrupt scale development, which is a very specialized type of insect development, in virtually any immature stage. Scales are undergoing some degree of metamorphosis between every two larval stages and each of these transformations from stage to stage can be disrupted by JH.

I am also particularly interested in your experience with epofenonane in obtaining ovicidal effects on summerfruit tortrix. The eggs of this species are flattened out against the leaf substrate and thus may pick up far more compound from a foliar residue than the usual spherical type of egg. Spherical insect eggs appear not to be affected by foliar residues of JH.

DORN

Well, we did some experiments with such species but we never tried to apply sublethal dosages — we just tried to kill the insects during metamorphosis. I cannot answer your question for this reason.

BOWERS

To refer to the presence of JH III in *Manduca* as a nonsense compound probably just reflects your opinion — not *Manduca*'s. I think this attitude reflects all of our genuine misunderstandings and perhaps ignorance of juvenile hormone and what it actually does at the tissue level in the insect as it is secreted by the insect corpora allata. After all, much of our information about what juvenile hormone is and does has been obtained from these trivial morphogenetic and a few gonadotropic assays. These are just two actions of JH that we can measure easily. I would not be surprised that it has many tissue and metabolic functions for which we are not assaying.

STAAL

My conclusions are based on the substitution assay that I described in detail yesterday, in which the adequacy of substitution compounds on the entire second half of the larval development is evaluated. In order to obtain adequate substitution with JH III applied in the food medium, the doses needed are excessive compared with the doses one would need of JH I or II. However, since titer determinations in the larvae show that these natural hormones are present in quantities of approximately the same order, I cannot conclude that JH III is really an essential hormone. If the animal would make only JH II in the larval stage, this would be completely adequate for its normal development. I agree with you that this substitution assay needs, what appear to be, gigantic overdoses of JH compared with measured endogenous levels to obtain full substitution. It is likely that insects have ways to use and preserve their endogenous hormones in a much more efficient way than exogenously applied hormones.

CURRENT STATE OF THE FIELD USE OF PHEROMONES IN INSECT CONTROL

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Although many insects are able to sense the presence of and respond to other insects of the same species by using the same five senses that are used by man — vision, hearing, touch, taste, and smell — their major method of communicating with each other appears to involve the chemical senses of taste and smell. Those chemicals that are used for communication between individuals of a given species are called pheromones. More specifically, a pheromone is defined as a chemical or a mixture of chemicals that is released to the exterior by an organism and that causes one or more specific reactions in a receiving organism of the same species. Behavioral reactions that may be stimulated in the receiving organism differ greatly according to the species under consideration and its way-of-life. For some species, pheromones cause the receiving individuals to approach and aggregate near the pheromone

(1) Much of the information presented in this paper has been published recently by the author in the following two review articles that relate to the use of pheromones in pest control:

SHOREY H.H., *Concepts and methodology involved in pheromonal control of Lepidoptera by disruption of premating communication. In* N.R. McFarlane (ed.). The Evaluation of Biological Activity. Academic Press, London. In Press.

SHOREY H.H., *Manipulation of insect pests of agricultural crops. In* H.H. Shorey and J. McKelvey (eds.). Chemical Control of Insect Behavior: Theory and Application. Wiley, New York. In Press.

source, whereas in other cases, pheromones might cause the receivers to disperse from the vicinity. In still other cases, pheromones may either stimulate or inhibit various behaviors such as sexual behavior or aggression. Sometimes the same pheromone may stimulate more than one behavior, such as approach of male insects toward a pheromone source followed by copulatory reactions when near the source.

During the past 15 years, entomologists have directed a large amount of effort toward the development of knowledge concerning pheromone communication among various pest insect species. Much of this research effort has been stimulated by the recognition that pest control with conventional insecticides is not always adequate to meet our needs and, in fact, that such control systems often lead to serious problems such as hazards to non-target organisms and the development of insecticide-resistance among the target organisms. As powerful, highly selective, and apparently safe chemical agents, the pheromones, which merely modify the behavior of the target species, may be a valuable adjunct to (and in some cases a replacement for) other pest control techniques.

At this point I should stress that extensive pheromone studies, directed toward an understanding of the pheromone behavior of pest insects and of how the behavior might be manipulated to man's advantage, are being pursued by many investigators working on such diverse insect pests as bark beetles and ambrosia beetles in forests, boll weevils in cotton, and various stored products pests in warehouses and granaries. A description of these various efforts is beyond the scope of this report. Although the remainder of my discussion will be restricted to a consideration of the sex pheromones that are released from female moths and that cause approach and copulatory reactions by males of the same species, many of the principles, goals, and problems are similar to those encountered in research on the other insect types that are not considered here.

Sex pheromone communication among moths

Moths are an excellent group of insects on which principles for manipulation of pheromone communication can be established.

Although not pests themselves, the moths represent the adult stage of many extremely serious caterpillar pests of agricultural, tree and ornamental crops, and grains and other stored food products. Successful mating of many moth species seems to depend strongly, if not totally, on prior pheromone communication between the sexes, and the mating is essential to the production of fertile eggs hy the females.

The cabbage looper moth, *Trichoplusia ni*, can be used to illustrate the role of the female sex pheromone in the communication behavior that leads to mating (SHOREY, 1976). Although the details of premating behavior differ from one species to another, the sequence discussed below probably occurs in a more-or-less similar manner in many other moth species.

Assume that the time is between midnight and dawn and that a female cabbage looper moth is clinging to a plant. Her body is either aligned horizontally, on the lower side of a leaf, or vertically, with her head up. The specialized reproductive segments of her abdomen, segments 8, 9 and 10, are telescoped within her seventh segment when she is at rest. When ready to mate, she protrudes these terminal segments. Her sex pheromone gland, located in the membrane between the eighth and ninth segments, is extended as a turgid hemisphere of glandular tissue. The pheromone molecules evaporate from the gland surface and are carried away on air currents, forming an elongate aerial trail on the downwind side of the female.

When the trail of molecules arrives at the vicinity of a male moth resting on a plant at some distance downwind from the female, he initiates a complex sequence of behaviors that concludes in his copulation with the female. The behaviors are instinctive acts that are preprogrammed in the male's nervous system and are displayed in the correct sequence when he is stimulated by the pheromone. The resting male first hrings his antennae forward, into the airstream. This is probably the invertebrate equivalent of " sniffing " the air. He then extends his wings and vibrates them with an increasing amplitude until his wing muscles are warmed to the temperature level that is necessary for flight. He takes flight and proceeds upwind, following the aerial trail by means that are not well understood. When he arrives near the female,

the high concentration of odor that he now perceives causes him to slow his forward flight speed and to orient toward her over the last few cm by both chemical-sensing and visual means. His visual guidance system, when he is exposed to the high concentration of pheromone, causes him to approach objects that are approximately the size and shape of a female moth. When he arrives next to the female, he hovers below her abdomen and touches her pheromone gland with his antennae and front feet. He then flies to a position beside the female, curves the posterior part of his abdomen toward her, and, if successful union is accomplished, settles down to mate with her.

The essential role of the pheromone as a stimulant of the sequence of male behaviors can be seen if we remove the female moth from her resting spot. In her place can be deposited the amount of pheromone, about 1 μg, which is contained in her pheromone gland. The pheromone evaporates into the air, again forming an aerial trail. When the odor molecules contact a male, he is stimulated to bring his antennae forward, extend and vibrate his wings, fly upwind through the trail, hover below the spot of pheromone and touch it with his antennae and front feet, move to a position beside the spot, and curve his abdomen toward and attempt to copulate with the spot. In large part, the pheromone represents " female " to the male's nervous system; the male can not recognize a female as a potential mating partner if he does not smell the pheromone.

This need for the male to use a sex pheromone stimulus in order to locate and copulate with a female may represent a weak link in the life cycle of the insects. If man can obtain sufficient knowledge concerning the nature of the pheromone chemicals, the exact behaviors that the chemicals stimulate, and the manner in which the behaviors vary according to differing environmental conditions, then he might be able to manipulate the behaviors to his own advantage.

The use of pheromones in pest management systems

Two main strategies are emerging for the use of pheromones in pest management. These strategies are almost direct opposites

of each other. One involves the use of a pheromone for stimulation of the normal approach response of the responding insects, except the response is manipulated in such a way that the insects end up in a location that is disadvantageous for them and advantageous for man. For instance, by following chemical cues and approaching the source of a synthetic sex pheromone, male moths might become ensnared in a trap instead of arriving near a pheromone-releasing female. The other strategy involves the disruption of the normal chemical communication behavior of the insects. In this case, male moths might be rendered incapable of responding to or locating the source of a natural pheromone, and the females would remain unmated. These strategies are considered in more detail in the following sections.

Stimulation of the Approach Response of Pest Insects. Pheromones that stimulate approach responses can be used as bait in traps. Great care must be exercised in the design of an effective trap. It must be appropriate so that the insects, in displaying their normal behavior, will freely enter it and be captured. Variables that should be considered include the size, color, and shape of the trap, the type of orifice through which the insects will enter, the height above the ground, the vegetative habitat in which the trap is deployed, and the concentration of pheromone issuing from the trap (CHARMILLOT *et al.*, 1975; DELLEY *et al.*, 1975; MINKS, 1975; MINKS and DE JONG, 1975; MINKS and VOERMAN, 1973; MINKS *et al.*, 1971; TRAMMEL, 1975).

Height above the ground is a very important variable, and responding insects may be caught in maximal numbers only when the trap is at the appropriate elevation. Among a number of moth species, best responses of males occur when pheromone-baited traps are suspended near the top of the foliage canopy, regardless of whether the vegetation is cabbages or forest trees (ALINIAZEE and STAFFORD, 1972; KAAE and SHOREY, 1973; MILLER and MCDOUGALL, 1973). This generality does not always apply, however, and optimal heights should be determined from behavioral analyses of each species. Also, the optimal height for a given species may differ when certain environmental factors such as

wind velocity vary from low to high (KAAE and SHOREY, 1973).

Some lepidopterous pests are highly restricted to their vegetative habitat, and males of these species are rarely caught in pheromone-baited traps located outside of the type of crop in which they developed as larvae (BRADER-BREUKEL, 1969; SHARMA *et al.*, 1971; TEETES and RANDOLPH, 1970). On the other hand, some insects are relatively independent of vegetative hosts in their pheromone behavior. Males of the cabbage looper moth are attracted equally to pheromone-baited traps placed in cabbage fields or over bare ground (SAARIO *et al.*, 1970).

The concentration of pheromone emitted from a trap may be especially critical. Often, the catches of male moths increase as the concentration of pheromone is increased, up to a certain maximum level. After that, further increases in pheromone concentration are related to corresponding decreases in numbers of males trapped (GASTON *et al.*, 1971; SHARMA *et al.*, 1971). Furthermore, that concentration that gives the highest male captures may vary from one trap to another, depending on the details of trap design (SHARMA *et al.*, 1971). We suspect that the narrow range of the most effective concentrations is related to the behavior of the responding moths. If the concentration near the orifice of the trap is higher than that likely to occur near a normal, pheromone-releasing female, then the males might sense that they are exposed to a concentration indicative that a female is nearby when they are still some distance downwind from the trap. The males might then be stimulated to stop upwind orientation and to initiate short-range, visually oriented searching behavior to locate the nonexistent female.

Pheromone-baited traps have two potential uses in agricultural pest management: 1) survey for the distribution or abundance of the pests, and 2) direct control by removal of the trapped insects from the population.

Pheromone-Baited Traps For Survey of Pest Insects. The only practical usage of pheromones to date in pest management programs has been as a survey tool. Pheromone-baited survey traps have been used either to monitor the spread of a potential pest into previously noninfested areas or to monitor the population

level of an established pest so that conventional pest control techniques can be applied at the most appropriate time.

Pheromone-baited traps are presently used in large areas of the United States to monitor the spread of such introduced insects as the pink bollworm. The trapping technique may be infinitely more efficient than the alternative — visual searching for signs of damage by the insects (MORENO *et al.*, 1973; SHAW *et al.*, 1971). The potential range of insect species that can be surveyed in this way is limited only by the range of species that use pheromone signals for inducing aggregation of other insects of the same species.

In principle, the use of pheromone-baited traps to monitor the build-up of an established pest population so as to predict when insecticidal control methods are necessary should also be highly effective. Often the male and female adults must utilize pheromone communication systems and mate before the damaging larval stage can be produced. Therefore, if male captures in traps can be correlated with subsequent larval populations, spray schedules can be efficiently timed. Such correlations have increased the efficiency of insecticidal control measures directed against such pests as the codling moth, the summerfruit tortrix, the spiny bollworm, and the pink bollworm (BATISTE *et al.*, 1973; KEHAT and BAR, 1975; MADSEN and VAKENTI, 1972, 1973; MINKS, 1975; MINKS and DEJONG, 1975; NEUMARK *et al.*, 1975; RIEDL and CROFT, 1974; ROELOFS *et al.*, 1976; TOSCANO *et al.*, 1974). However, the correlations may often be poor and thus lead to misjudgments. For example, differing weather patterns may cause trapping results to not correlate well with the time of subsequent egg laying by female moths and with the time then needed for hatching of the eggs. Therefore, pheromone trap surveys may have to be supplemented by determination of seasonal oviposition patterns of laboratory-reared females or by the inspection of fruit for larval entries or by a temperature summing technique which gives an estimate of the time lag needed for egg hatch (BATISTE *et al.*, 1973; MADSEN and VAKENTI, 1973; MINKS, 1975; MINKS and DEJONG, 1975).

Pheromone-Baited Traps For Direct Control of Pest Insects. When used for direct control, the trapping method must remove

sufficient insects to cause a reduction in the pest population of the next generation. For many moth species, both males and females are capable of mating many times. If 90 % of the males were captured, the remaining 10 % might be sufficient to inseminate most of the females. Thus, a very large proportion of males probably would have to be captured before any noticeable diminution of the next generation would occur.

The trapping approach, using female sex pheromone-baited traps, is often called the "male annihilation technique". Considerable effort has gone into the evaluation of this technique against a number of moth species. In general, preliminary results have shown that the method may have some usefulness in keeping pest populations from increasing if they are already at a very low level; however, little success has been shown against infestations that are already at a damaging level (CAMERON, 1973; GLASS *et al.*, 1970; ROELOFS and TETTE, 1970; ROELOFS *et al.*, 1970, 1976; TASCHENBERG *et al.*, 1974; TRAMMEL *et al.*, 1974). This restriction to low density populations seems reasonable if we consider that the traps must compete directly with pheromone-releasing females in the field for attracting males. If over 90 % of the males must be removed, and if the concentration of pheromone leaving a trap can not be much higher than the concentration near a pheromone-releasing female, then many more traps than natural females would probably be necessary. Also, the proportion of male moths that a pheromone-baited trap removes from the environment may decrease as the absolute density of the pest population in the field increases (HOWELL, 1974; RIEDL and CROFT, 1974; TASCHENBERG *et al.*, 1974). This factor is, perhaps, directly related to the numbers of competing wild females in the field.

Disruption of Pheromone Communication

Even before the sex pheromones of any pest insects had been identified, a number of writers proposed that if sufficient synthetic pheromone were distributed in the air, normal pheromone-communication systems of certain species would be disrupted and the sexes might be incapable of locating each other for mating. The ap-

proach has a great deal of appeal and has been pursued extensively in recent years, with very promising results.

The behavioral mechanisms that cause communication disruption are poorly known. Some evidence, based on laboratory experiments plus considerable speculation, indicates that three factors are involved: sensory adaptation, central nervous system habituation, and " confusion ".

Sensory adaptation to odors is a common phenomenon in insects. During exposure to a constant level of odorant, the olfactory sensory receptors soon stop reporting to the central nervous system that the odor is present (PAYNE, 1974). The sensory receptors again become responsive to the odor, within a few seconds or minutes after the stimulus is removed.

Habituation is similar in principle to sensory adaptation, except the phenomenon occurs within the central nervous system. If an animal is exposed to a stimulus and if its subsequent responses do not lead to a suitable end result (mating in the case of a male insect stimulated by female sex pheromone), then it tends to be less responsive when it perceives the stimulus again. Habituation of male insects to sex pheromones may persist for many minutes or even hours (BARTELL and LAWRENCE, 1973; TRAYNIER, 1970).

" Confusion " is a direct result of competition between the pheromone released from synthetic sources and the identical pheromone normally released from the insects themselves in the field. If the number of synthetic sources greatly exceeds the number of natural pheromone-releasing insects, or if the sources release much more pheromone than the natural insects, then the responding insects, if they are not adapted or habituated, will be more likely to approach the synthetic sources.

Disruption of communication has been most studied with synthetic pheromones that are identical to the natural pheromones of a particular species. However, disruption might also be accomplished by the use of parapheromones (non-pheromone chemicals that cause behaviors identical to those caused by natural pheromones) or by antipheromones (non-pheromone chemicals that directly block or inhibit responsiveness of insects to their natural pheromones) (KAAE *et al.*, 1973; KLUN *et al.*, 1975; MCLAUGHLIN *et al.*, 1976; MITCHELL *et al.*, 1976; ROELOFS *et al.*, 1976; TUM-

LINSON *et al.*, 1976). Ideally, a blend of chemicals might be found which would disrupt premating communication of a whole complex of pest insects that infest a given crop.

The first demonstration of the feasibility of the disruption approach was accomplished by GASTON *et al.* (1967). They released looplure, the synthetic pheromone of the cabbage looper moth, from an array of 100 evaporators spaced 3 meters apart in 0.1 hectare plots and found that male moths were rendered completely incapable of locating pheromone-releasing females used as bait in traps in the centers of the plot. Since that time, disruption of sex pheromone communication through atmospheric permeation with synthetic pheromones has been demonstrated in a wide variety of moth species.

Especially in the case of the gypsy moth, the plum fruit moth, the oriental fruit moth, and the pink bollworm moth, programs using synthetic pheromones for disruption of premating communication seem to be coming close to practical realization. Generally, for these species, it appears that the release of around 15 grams of pheromone per hectare over the entire pest season may cause most females to remain infertile and thus may cause substantial reduction in the larval pest population of the next generation (ARN *et al.*, 1976; BEROZA, 1976; BEROZA *et al.*, 1974; CAMERON and SCHWALBE, 1974; ROTHSCHILD, 1975; SCHWALBE *et al.*, 1974; SHOREY *et al.*, 1976). The disruption systems for these species appear to be nearing the level at which their use is competitive with standard insecticide-control techniques. Additional fine-tuning of the systems, through further research on the normal communication behavior of the insects and through the engineering of techniques for evaporating the pheromone into the atmosphere at the correct locations and the correct times, should lead to further improvements in the degree of pest control afforded.

Two differing strategies for deploying synthetic pheromones for communication disruption have been studied. One strategy involves widely separated evaporative substrates, spaced as far as 0.1 to 1 km apart, with each substrate releasing relatively massive quantities of pheromone into the air. The other strategy is based on a large number of small substrates placed close together in the field. The small substrates could be dispersed by aircraft or other

conventional insecticide-application techniques, and each substrate would release a relatively small quantity of pheromone.

Working with the cabbage looper moth, SHOREY and GASTON (1974) proposed that neither the separation between substrates nor the release rate of pheromone from each substrate was the critical factor. Rather, the important factor was the absolute quantity of pheromone released into the atmosphere over each unit area of land in a given time. FARKAS *et al.* (1974) obtained over 90 % disruption of premating communication among cabbage looper moths by using synthetic pheromone dispensers placed 400 m apart.

On the other end of the spectrum, and probably the most practical disruption method for many pest species is the use of microcapsules or other microdispersible formulations containing pheromone (BEROZA *et al.*, 1974; CAMERON and SCHWALBE, 1974; SCHWALBE *et al.*, 1974). Such formulations rely for their effect on many pheromone-releasing substrates being distributed over a given area.

CONCLUSIONS

In the end, we must reemphasize studies of the behavior of the pest insects. Insect behavior is often highly stereotyped; this rigidity causes the insects to perform certain behaviors when exposed to appropriate stimuli and forms the basis for behavioral pest control. But insects are also " flexible " in that their behavior is modified when they are in different physiological states or are exposed to various environmental conditions (SHOREY, 1974). As an example, male and female pink bollworm moths aggregate near the tops of cotton plants when the wind velocity is low. However, on windy nights they move down in the foliage to near the bases of the plants before they engage in pheromone communication (KAAE and SHOREY, 1973). If a pheromonal pest management system is to be effective, we must design methods to present the necessary concentrations of synthetic pheromone in the environment at all the times and in all the locations where mating might occur.

An agricultural crop is often threatened by a complex of pest insect species. Pheromonal control of one of the species may be

of little value if normal insecticide application schedules must be followed for control of a number of other species. On the other hand, often one or two key species are the only primary pests of a crop and require rigorous suppression techniques. If insecticide treatments could be minimized and if populations of beneficial insects increased, many secondary pests, which often are abundant only because insecticides upset the normal biotic balance within the agricultural ecosystem, might present little hazard to the crop. The extreme selectivity of pheromonal, behavioral control of a few key pests might cause a number of potential secondary pests to pose less of a problem.

Pheromones should be considered as potentially powerful pest management tools that should be intelligently integrated with other tools to make the environment less suitable for survival or reproduction of agricultural pests.

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DISCUSSION

BOWERS

You have shown that you could control the pink bollworm very nicely through the mass confusion technique with the sex pheromone. However the bollworm is just one of the major pests of cotton and I am curious about whether the cotton crop was marketable. Without the use of any broad spectrum insecticides did other insects or mites do serious damage to the cotton?

SHOREY

That is a very good direct question. I am going to answer it indirectly with something that I meant to say in my talk. Pheromones, although they will enter into certain pest management systems for direct control of pest insects, are also not the panacea that even pheromone researchers thought they would be a decade or so ago. But they will have certain selective or selected uses. One of these uses will be for manipulation or management of key pests of crops. I think this is very important. Often one or two species on a crop will be key pests, and insecticide applications which are necessary for control of those pests often cause other pest problems by disrupting the environment, killing parasites and other beneficial organisms. Such is the case with the pink bollworm in southern California. Before the pink bollworm came into southern California, much of the cotton acreage in that area was under very intelligent management, with insecticide applications in some areas being reduced to zero. And there were fewer pest problems. For instance, the cotton leaf perforator ceases to exist under non-insecticidal situations. It only exists as a pest when insecticides are applied for other pests. The pink bollworm came into California. All of a sudden, the average grower was applying between five and ten insecticide treatments per season and other pest problems established themselves. This indirectly

answers your question. The pink bollworm in that way is a good model insect to use for selective pest control because it is a key pest. In other situations, such as in many orchard situations, there are as many as ten different pest species which attack the crop. Singling out one of them for selective control may not reduce the need for insecticide applications at all. And so a great deal of thought must go into identifying the key pests upon which we should use kindly, selective treatment methods as part of the pest management system.

NAKANISHI

Some pheromones which require cis and trans mixtures, how are they applied? How are those capillaries used?

SHOREY

"Gossyplure" the pink bollworm pheromone — that is one of those pheromones that require a mixture. It is a 1-to-1 ratio of the cis-cis and cis-trans isomers of 7,11 hexadecadienyl acetate.

NAKANISHI

Even if you start with the same mixture there must be different rates of vaporization and things like that. And I was wondering whether you used two capillaries of different diameters.

SHOREY

Again in the case of the pink bollworm it does not make any difference because there are identical vaporization rates of the two isomers and they are used in mixture. However, in some species a very discrete ratio of two ingredients may be needed with greatly different release rates. Then the system would have to be appropriately designed — either with separate capillaries or with appropriate quantities of each ingredient in single capillaries.

BRADER

You have wonderfully evaluated many of the possible approaches

that can be made of pheromones. Personally one point I am missing. I know you did not work on it but I would still like your opinion on it. It is about the pheromone inhibitors.

SHOREY

I have made very small comments in my written manuscript about this. However it is an area that is attracting the attention of many research workers in pheromones and that is related to the complexities of the various chemicals that often make up a particular pheromone blend. Let me talk about biology just for a moment. We often find related species co-existing in an area, each of them having in common certain chemicals in their pheromone blends. In those cases we sometimes find that although the insects have certain chemicals in common, they may have one or more minor components of the pheromone which are not in common. Often the minor component might make the total pheromone more attractive to males of the correct species and at the same time cause males of the related species to be inhibited from approaching that blend. So there are dual messages that are going out, one to males of the correct species saying "come hither", one to members of other species saying "stay away, even though many of the components of this blend are attractive to you". Also in early research with pheromone traps, a number of investigators found that certain chemicals, when put in a trap with living females, would inhibit the males of that species from approaching. Now I speculate that in many of these cases, the chemicals that were placed in the traps are similar to chemicals that are given off from the females of related species. They are inhibitors. When the investigators have then taken those same chemicals that inhibit males from entering traps, and permeated the whole environment with them, the males then freely come in and enter the traps. The males may be adapting and habituating to the inhibitory chemicals which are everywhere in the environment, leaving them free to respond to the females used as bait in the traps. Thus at least in one class of inhibitory compounds, although males are inhibited from entering traps if the inhibitors are put in the traps, the inhibitors do no good if they are dispersed through the area. Again we cannot overgeneralize, because we do not know the biological rationale of most of the chemicals that we are using. We do not know how they are received by the insects in many cases.

So, if we broadly say that chemicals are inhibitory, some of them may be inhibitory because they are tying up the sensor sites as was mentioned in a discussion yesterday. Others may be inhibitory because in the central nervous system they say the wrong species is there, and other chemicals may be inhibitory for still other reasons.

KARLSON

Would you care to speculate what would happen if pheromone application is continued even when the population is very low?

SHOREY

I wish you had said "very high" and then I would have given a different speculation.

KARLSON

I can add this to my question.

SHOREY

I do not know if I am going to answer your question. As a game in Riverside we have started to try to select out what we would call "pheromone resistant strains" of the pink bollworm. Let's just say we are trying to select for alternate pathways of mating communication. In our preliminary research, the results of which are very erratic (none of this is ready for publication yet), we have found that if we saturate the atmosphere in a glass jar with pheromone, an occasional male is capable of finding a female in that jar and mating with her. If we then take their progeny and do the same thing so that we work through F_1, F_2, F_3, F_4 generations, we have been able to get as many as 40 % of the moths to mate in the pheromone saturated atmosphere. But, you see, this is why I wanted to relate your question to high-density populations. In this experiment we were not selecting against distance attraction at all. We were only working with one aspect of pheromone communication, which is the very close range component. Although we have not studied the behaviour of the selected individuals yet, we may

be selecting for those males that are sufficiently active that they step on the female pheromone gland once in a while with their feet and therefore become stimulated to copulate. A different experiment would be to try to select for alternate pathways of distance communication, and now we get back to your low-density situation. What would happen under a continued period of time when the insects are dispersed in the field and if pheromones were kept at a high level all the time? I don't have an answer for that, other than to fully appreciate the fact that we are not going to eradicate the pink bollworm from the face of the earth by the communication disruption technique. Many species release minor components from the pheromone gland. The pink bollworm may have a number of different minor chemicals that are used in a very minor segment of the population and which would then be selected if the air were continuously permeated with the major pheromone components.

WAIN

I would just like to relate a very interesting story that was told to me in New Zealand about twelve months ago relating to the grass grub. The female grass grub beetle lays its eggs on the pasture and the larvae which hatch bite through the roots of the grass killing the plants. This can lead to vast areas of bare soil which, of course, is completely non-productive as far as the animal is concerned. Now DDT can give complete control of this pest when it is applied to the soil. Unfortunately the sheep eating the grass take DDT into their bodies. This accumulates in the body fat. Such carcasses are unsaleable abroad so DDT has had to be prohibited for use in grass grub control. As a result, this pest has now become a very very big problem again. Research workers in New Zealand therefore began to try to isolate the sex attractant for the male beetle. Many virgin females were extracted in the normal way and the extract was fractionated. These fractions were tested in the field for their capacity to attract the flying male beetles. None of them were effective but the research workers found that the extracted bodies of a few female beetles stuck on to a card were very effective in attracting male beetles. It was subsequently found that the attractive material was something present in the glue which was used to stick the female to the card. In such ways are new discoveries sometimes made!

SIDDAL

Because the release of pheromones by females and the perception of pheromones by males is temperature-sensitive, is there any research being devoted to formulation for release of pheromone with a negative temperature co-efficient so that the unnecessary loss of pheromone in the hot day-time could be avoided?

SHOREY

I know of no practical research going on in that area. Apparently there are some matrices that do have temperature coefficients of release that would be favourable in this regard. They would tend to close down at the inappropriate time of day and open up and increase their release rate at the appropriate time. However I did not mention any of the types of research that have been done with such pheromone release substances. We spent two seasons working with a large number of micro-encapsulated formulations of pheromone, with great frustration. Although many investigators in the pheromone field have reported promising results with micro-encapsulated formulations, to my knowledge they have never developed a truly adequate release substrate. For the best pheromone release substrate, we should have a constant release over time, whereas essentially every formulation that is available has a release rate which rapidly decreases over time.

ABO-KHATWA

In extension to Dr. Siddal's question I am just wondering as to the potency of these pheromones if applied to field crops situated nearby an air pollution source. Is it possible that pollutant particles can mask or interfere in any way with the pheromonal perception by target insects?

SHOREY

I know of no such masking effect. I suppose it is possible. Some of the early naturalists that worked with pheromones remarked that the normal chemical masking agents did not interfere with pheromone responses. Fabré commented that the odor of naphtha in a room contain-

ing female moths had no influence in preventing the male moths from entering in response to female pheromones.

BOWERS

I am interested in the males which you selected that seem to be able to copulate (I mean these 40 %) in spite of an atmosphere permeated with pheromone. Have you looked at their electroantennograms and seen if they respond to the pheromone?

SHOREY

No, in fact that is why I hesitated to even mention that experiment. We lost the colony and we are starting all over again.

JACOBSON

I have a comment that I would like to make in connection with Dr. Wain's story about the grassgrub beetle. There is in fact a sex attractant in the grassgrub beetle which has been identified as phenol and which does apparently seem to work under certain field conditions. I do not think it stands a very good chance of being used in a practical way but also I would like to point out that with many of the lepidopterous insects, once the female has been mated, she is no longer attractive to males. In connection with the hair pencils Dr. Shorey spoke about, and which we find in I think probably all of the noctuids, we are coming around to believe more and more that the pheromone which is produced by the hair pencils of these males may play a part in causing the female to cease release of her sex pheromone. This has been shown definitely in the case of the tobacco budworm, *Heliothis virescens*, and my group is now working on this problem. I think that if we can identify this material and if the structure is simple enough so that it can be synthesized, such a compound which causes females to stop releasing the sex pheromone to attract the male could play quite a large part in either suppression or control of these insects.

NAKANISHI

Since the capillaries are left in the fields for a long time — I am not

familiar with the photosensivity but wouldn't it be better to use the black capillary rather than the white one?

SHOREY

Conrel Co has determined that the pheromone within the capillary remains stable.

In our own experiments we added U.O.P. 688 which is an anti-oxidant to the pheromone. With this addition the pheromone remains fully attractive after several months in the capillaries in the field.

IMPACT OF NATURAL PLANT PROTECTANTS ON THE ENVIRONMENT

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Introduction

Even the most selective and specific pesticides will affect the ecosystem in the target area. If only the target pests are decimated, neighboring organisms in the system will also be affected such as, for instance, organisms that depend upon the target species for food or shelter. Effects on nontarget species often extend beyond target areas, because pesticides and their metabolites and/or degradation products move away from the site of deposit by drift, volatilization, leaching, and/or surface transport in water or on sediment. The degree to which pesticides move throughout the environment by these processes is related primarily to their chemical stabilities, their solubility characteristics, and their absorption on soil particles.

Pesticides may also affect lower terrestrial or aquatic organisms, including organisms that are important to vital natural biological waste degradation and/or oxygen production mechanisms. However, harmful effects to such organisms will probably not be as obvious or dramatic as, for instance, kills of fish, birds or mammals, and may therefore go unnoticed unless and until they result in more apparent consequences.

Persistent pesticides or their metabolites and degradation pro-

ducts may accumulate in the environment, especially in or near treated areas. Accumulation will occur in local soils or waters when the rate of pesticide input into the area exceeds the rate of degradation and transport out of the area.

Natural pesticides may be applied to the soil, to plant foliage, as a space treatment against flying insects, as a residual treatment to stored products, to water, or released into the air from traps or mechanical means (attractants, repellents, communication disruptants). Although attractants are generally not insecticidal, materials used for insect control in the United States are defined as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended by the Federal Environmental Pesticide Control Act of 1972 (FEPCA), and toxicological data are needed for registration of compounds for which pesticidal use is contemplated. Mammalian toxicity categories of pesticides set by the EPA are shown in Table 1.

Use and development of chemical pesticides is increasingly constrained in the United States by laws and regulations adopted in the interest of environmental protection and occupational health and safety. Furthermore, development of new pesticides is made more difficult and expensive by the new regulatory regime.

Relatively little information is available on the toxicity and hazards of pesticides or potential pesticides such as pheromones, plant insecticides (such as pyrethrins and pyrethroids), microorganisms, and hormones, and their residues, to nontarget organisms under field conditions. Information is especially sparse concerning the effects, if any, of residues of these materials on lower aquatic and terrestrial organisms. Although there is a literature available on the effects of individual compounds on isolated organisms or systems in the laboratory or greenhouse over short periods of time, such studies are usually far removed from field conditions, and their results do not answer the question of their significance in regard to field conditions.

From the foregoing, it is obvious that the decisive factors which will determine those natural products that may be used as plant protectants are 1) persistence and degradation, 2) effect on beneficial (nontarget) organisms, and 3) phytotoxicity and toxico-

TABLE 1 — *Mammalian toxicity categories of pesticides*.*

Toxicity Parameter/Category	Highly Toxic	Moderately Toxic	Slightly Toxic	Relatively Nontoxic
Acute oral LD_{50}, mg/kg	Less than 50	Over 50-500	Over 500-5,000	Over 5,000
Acute dermal LD_{50}, mg/kg	Less than 200	Over 200-2,000	Over 2,000-20,000	Over 20,000
Acute inhalation LD_{50}, µg/liter	Less than 2,000	Over 2,000-20,000		
Dose probably lethal to a 150 lb man	A few drops to 1 teaspoonful	1 teaspoonful to 1 oz	Over 1 oz to 1 pt or 1 lb	Over 1 pt or 1 lb
Signal word required on label	Danger-Poison Skull and Crossbones	Warning	Caution	

* From "Production, Distribution, Use, and Environmental Impact Potential of Selected Pesticides", EPA, 1974.

logical effects on warm-blooded animals. No attempt has been made to make this presentation an exhaustive review, but merely to cite major representative examples in each of these categories. Finally, comments are made on the outlook for practical use of the natural products.

PHEROMONES

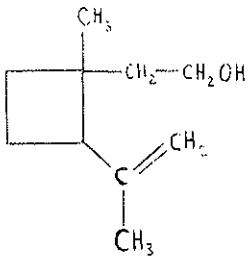
Persistence and Degradation

BEROZA *et al.* [1] described a two-stage laboratory apparatus for measuring relative emission rates of pheromones (and other behavior-controlling chemicals), particularly from slow-release formulations. One stage ages formulations for long periods of time by passing air at a fixed temperature over planchets containing microencapsulated pheromone. Another stage measures relative emission rates from samples after suitable intervals of aging while air at 100 ml./min. passes over them. These procedures, used in combination with field testing, may expedite the acquisition of data needed to develop suitable formulations and to determine total release of pheromone into the air.

Spraying disparlure, alone or with a diluent, is not satisfactory for use as a confusant because the nonpolar lure will dissolve in the leaf waxes upon which it settles, resulting in severe curtailment of volatility and loss of effectiveness. It is more apt to persist on a solid support with a hydrophobic surface (hydrophobic paper, plastic, cork) because disparlure will wet and adhere to such a surface and will also resist leaching by rain [2].

Grandlure, the natural sex pheromone of the highly destructive pest of cotton known as "boll weevil" (*Anthonomus grandis*), consists of two alcohols and two aldehydes, as shown in Figure 1. Soil and water persistence studies with grandlure were carried out by HENSON *et al.* [3]; oxidative decomposition products from the aldehydes were isolated and identified as the two acids, two esters, and one aldehyde shown in Figure 2. The grandlure alcohols were stable.

For the soil studies, individual grandlure components (10 mg each) were applied to moist soil surface in beakers and held in



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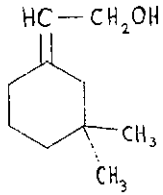

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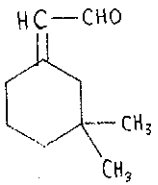


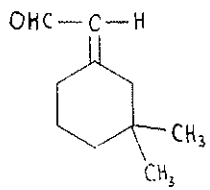


FIG. 1 — Boll Weevil Sex Pheromone (Grandlure).

constant temperature chambers at 32° or 21° for 0, 4, 8, 12, 24, 32, and 48 hours, then the soil was analyzed. For water studies, individual components (10 mg) were placed in 100 ml of water in beakers, then held at same temperature for the same periods of time. It was found that 98% of all components were lost from soil within 24 hours at 32° and no grandlure remained in soil after 32 hours at either temperature. Losses of grandlure from water were less rapid than from soil, all components being dissipated from water within hours at 32°; the aldehydes were lost within 24 hours. Alcohol II was depleted in 32 hours and alcohol I in 48 hours. When two types of grandlure dispensers (bare filters and cardboard physical barrier filters) were washed with water, they showed a

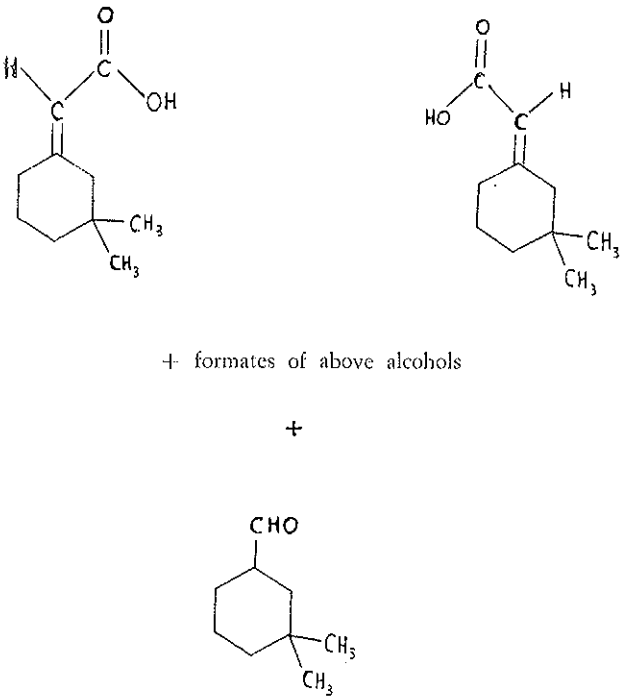


FIG. 2 — Oxidative degradation products of grandlure.

marked difference in loss of the compounds. Loss from bare filters was nearly quantitative; those enclosed in cardboard cylinders lost only 8-12%. The question of whether grandlure could escape from the filters into surrounding soil was investigated by imbedding physical barrier filters containing 10 mg grandlure in 100 ml of moist soil for 24 hours at 21°, then analyzing. Neither grandlure nor the oxidation products were detected in the soil around the Leggett trap; the volatility of the compounds apparently forced them into the atmosphere rather than into the soil. The same was true of soil containing imbedded grandlure dispensers. The physical barrier grandlure formulations currently in use showed no tendencies toward excessive soil and water contamination even under extreme conditions.

Effect on Nontarget Organisms

The sex pheromones are usually very highly specific for their own insect species or several closely related species. No information is available on the effects of pheromone oxidation products or metabolites on beneficial insects.

Toxicology

In 1975, BEROZA *et al.* [4] reported on the acute toxicity of 3 insect sex attractants (disparlure, looplure, and muscalure), a synthetic compound active as a pink bollworm sex attractant (hexalure), and an inhibitor for looplure. Disparlure is the natural pheromone of the gypsy moth (*Lymantria dispar*), looplure is the natural pheromone of the cabbage looper moth (*Trichoplusia ni*), and muscalure is the natural pheromone of the house fly (*Musca domestica*). These 5 compounds were tested for acute oral and aerosol inhalation toxicity in rats, acute dermal toxicity, eye irritation, and primary skin irritation on rabbits, and for toxicity to rainbow trout and bluegill sunfish. The data for rats and rabbits are shown in Table 2.

With regard to acute oral toxicity, under the FIFRA classification system a material with an acute oral LD_{50} equal to or less than 50 mg/kg is highly toxic, a LD_{50} of 50-500 mg/kg is toxic, a LD_{50} of 500-5000 mg/kg is nontoxic. Therefore the four attractants under discussion are nontoxic when administered orally. Disparlure, hexalure, and muscalure caused no deaths at the dose levels tested, although they did evoke reactions such as reduced activity, ruffed fur, diuresis, and slight muscular weakness lasting up to 2 days.

With regard to acute dermal toxicity, LD_{50}'s equal to or less than 200 mg/kg are highly toxic, 200-2000 mg/kg are toxic, and 2000-20,000 mg/kg are slightly toxic. The looplure inhibitor is therefore classed as slightly toxic, with a LD_{50} of 3700 mg/kg. The other materials were not tested at concentrations greater than 2025 mg/kg but since they caused no deaths they are not more than slightly toxic. In the animals treated with looplure inhibitor, ataxia, muscular weakness, and reduced temperature were noted. All test chemicals caused local skin reactions characterized at the end of the 24-hr.

TABLE 2 — *Toxicity of insect pheromones and an inhibitor to rats and rabbits*.*

Chemical	Acute oral LD_{50} (mg/kg) (rats)[a]	Acute dermal LD_{50} (mg/kg) (rabbits)	Eye irritation (rabbits) in			Primary skin irritation score[c] (rabbit)
			1 hr.	*24 hrs.*	*48 hrs.*	
Disparlure	>34,600	>2025	12.0	1.7	0	2.8
Looplure	≥13,430	>2025	6.0	0	0	2.8
Muscalure	>23,070	>2025	6.0	0	0	1.7
Hexalure	>34,600	>2025	8.0	1.3	0	1.9
Looplure inhibitor	≥11,730	3700	6.0	10.1	7.5	8.0

[a] Undiluted material; four animals/dose.

[b] Dose 0.1 ml/ 0 = no irritation, maximum score of 110 is weighted summation of grades for the 3 ocular tissues (cornea, iris, conjunctiva); 6 rabbits/test.

[c] Dose 0.5 ml; 6 rabbits/test; max. score of 8.0 for an average of 24- and 72 hr. ratings.

* Modified from BEROZA *et al.*, Toxicol. Appl. Pharmacol. *31*, 421 (1975).

contact period by erythema and edema. With looplure inhihitor, focal 2nd degree burns were noted at 24 hr; 7-14 days following removal of compound, desquamation and pustulation were noted on all animals. As for skin irritation, only the looplure inhibitor was a skin irritant.

None of the materials was an eye irritant.

Toxicity data for fish are shown in Table 3. Looplure was the most toxic substance, with a LC_{50} (median lethal concentration) of 3.7 ppm for rainbow trout and about 3 ppm for bluegill sunfish. No deaths or adverse reactions were observed with disparlure and looplure. With the higher doses of looplure inhibitor, both species of fish became quiescent and flaccid, swimming or lying on their sides with slow respiration; trout also showed dark discoloration of the integument.

TABLE 3 — *Toxicity of insect pheromones and an inhibitor to fish*.*

Chemical	Concn. (ppm)	Lc 50 (ppm)[a] Rainbow trout (hrs.) 24	48	96	Bluegill sunfish (hrs.) 24	48	96
Disparlure	0.1-100	>100	>100	>100	>100	>100	>100
Looplure	0.1-100	>100	>100	>100	>100	>100	>100
Looplure inhibitor	1.8-4.4	3.7	3.7	3.7	3.1	2.9	2.8

[a] Ten fish used at each concentration; disparlure and looplure tested at 4 concn. and looplure inhibitor at 5 concns.

* Modified from BEROZA *et al.*, Toxicol. Appl. Pharmacol. *31*, 421 (1975).

Outlook for Practical Use

In general, those pheromones tested toxicologically have had a low order of toxicity. In the small amounts that will normally be required in field work (as little as 10 μg for use in traps and approximately 0.4-8 g/acre or 1-20 g/ha. for use by the air-permeation

method), the use of these chemicals should present no environmental problems from a toxicological standpoint.

In April 1976, the EPA issued to Conred, Norwood, Massachusetts, an experimental use permit (No. 36638-EUP-1) allowing the use of 90 lb. active ingredient of the pink bollworm sex pheromone [(*Z*,*Z*)- and (*Z*,*E*)-7,11-hexadecadienyl acetates] on cotton as a confusion agent in the control of the pink bollworm. A total of 6,600 acres is involved, and the program is authorized only in the States of Arizona and California. The permit is effective Apr. 6, 1976 to Apr. 6, 1977. All cottonseed treated under this permit must be used for seed purposes only, or exported, or destroyed [5].

Pyrethroids

Persistence and Degradation

According to a pesticides Data Sheet on pyrethrins issued by the World Health Organization in 1975, pyrethrins for agricultural, horticultural, and forestry uses are neither persistent nor phytotoxic, but contamination of watercourses should be avoided [6]. Despite surprisingly high intravenous toxicity to rats, as described below, the relatively short-lived effects in survivors of an intravenous dose bordering on the lethal suggests a rapid detoxication of the pyrethrins. By their speed of action after intravenous injection, it seems that pyrethrins act *per se* and no metabolic conversion to a more toxic molecule is required for their action [7].

Effect on Nontarget Organisms

Schuyler and Massing [8] found that ground ultra low volume (ULV) applications of synergized pyrethrins at 2.62-2.8 g/ha. gave satisfactory control of mosquitoes and no mortality of nontarget insects such as honey bees, damselflies, dragonflies, ladybird beetles, and monarch butterflies.

Toxicology

The Data Sheet issued on pyrethrins by the World Health Organization (WHO) in 1975 lists the common formulations used in agriculture, horticulture, and forestry as 0.2-0.4% dusts, 0.5-1.0% ULV sprays, 0.1-0.5% pressure packs, and 0.003-0.015% emulsifiable concentrates to be "applied just before harvest on growing bush and vine fruits, deciduous fruits and nuts, forage crops and vegetables (and) ornamentals. Spray or dust formulations are used on freshly picked fruits and vegetables in the field, in storage and in processing plants." Susceptible pests are given as "sawfly larvae, lepidopterous caterpillars, leafhoppers, aphids, beetles, and thrips."

VERSCHOYLE and BARNES [7] administered natural pyrethrins and several synthetic analogs to rats both intravenously and orally. The results (Table 4) showed that all compounds were much more toxic by the intravenous route. The related synthetic compound, bioresmethrin, which is even more toxic to insects is less toxic to mammals, both orally and intravenously. Still other related synthetics are even more active insecticidally, while retaining low oral toxicity to rats. After oral dosing with bioallethrin, tremors were noted about 1 hour later. Final stages of poisoning consisted of convulsive

TABLE 4 — *Comparative oral and intravenuous toxicities of pyrethrins and analogs to rats.**

Compound	Oral toxicity (mg/kg) (LD_{50})	Intravenous toxicity (mg/kg) (lethal)
Pyrethrin I	260-420 (♂)	5.0 (+)
Pyrethrin II	>600 (♂)	1.0 (+)
Natural pyrethrins	1400 (+)	5.0 (+)
Bioallethrin	1030 (+)	4.0 (+)
Resmethrin	>3000 (+)	160-170 (+)
Bioresmethrin	>8000 (+)	340 (+)

* Modified from VERSCHOYLE and BARNES, Pesticide Biochem. Physiol. 2, 308 (1972).

twitching, prostration, coma and death (3-24 hours after dosing). After intravenous injection, the pyrethrins were the most rapid in action, animals collapsing with convulsive tremors immediately after injection; most deaths occurred within 10 min. Pyrethrin II was the most toxic compound tested intravenously [9].

The occurrence of pyrethrum dermatitis among users of unrefined pyrethrum extracts has been known for many years. Such extracts produced moderate reactions in guinea pigs sensitized to dried, ground pyrethrum flowers, and considerably stronger reactions were produced by non-dialyzable fractions of 0.9% saline and 25% sodium thiocyanate extracts. Further tests on sensitized guinea pigs have indicated that crude pyrethrum oleoresin contains two types of allergens: *a*) glycoproteins or glycopeptides with molecular weights of 60,000-200,000, and *b*) sesquiterpene lactones, mainly pyrethrosin which is present in very low concentration. No allergenic activity was obtained from pyrethrin II [10].

The pyrethrins are absorbed by the respiratory route in mammals, poorly from the gastrointestinal tract, and insignificantly through the skin. Allergic reactions may result from dermal exposure, but the material does not accumulate in mammalian tissues. Rats fed a maximum dietary level of 5000 ppm pyrethrins for 2 years showed no reduction in growth and survival; liver damage was seen at 1000 to 5000 ppm but not at 200 ppm. According to the WHO [6], allergic manifestations, particularly contact dermatitis, have been encountered in persons occupationally exposed to pyrethrum flowers or crude pyrethrum extracts, but "the general population will not be expected to be affected by pyrethrins under normal conditions of use." Of 200 human subjects (177 females and 23 males) patch-tested with 1% aqueous pyrethrins, none showed primary irritation or sensitization. Pyrethrins are nontoxic to birds and bees, but toxic to fish and cold-blooded animals generally.

Outlook for Practical Use

The pyrethrins are already in use against a wide range of agricultural, horticultural, and forest pests. They do not leave persistent residues and have a long record of safe use. Despite their high toxicity

to warm-blooded animals by the intravenous route, they have very low oral toxicity to mammals. In addition, the much more insecticidally active synthetic pyrethrin analogs are significantly less toxic to mammals and are not expected to cause dermatitis on handling.

Microorganismic Products

Persistence and Degradation

Conidia of the fungus *Nomuraea rileyi*, isolated from the green cloverworm, *Plathypena scabra*, appear to be useful in controlling cabbage looper larvae on soybean leaves. The average half-life of the conidia is 2 days. Of numerous fungicides, herbicides and insecticides registered for use on soybeans, almost all fungicides inhibited the growth of *N. rileyi* [11, 12].

The addition of carbon and molasses to Thurcide (a *Bacillus thuringiensis* formulation) extended its insecticidal activity and spore residual when applied to Eastern red cedar foliage. The estimated projected half-life of the formulation (in the absence of rain) was 7 days [13].

The persistence of spores of *B. thuringiensis* (commercial formulations) on oak leaves in the field was determined in connection with control of the wintermoth, *Operophthera brumata*, and the green oak tortrix, *Tortrix viridana*, in Czechoslovakia [14]. Within the first week after aerial application, the viable spore count on leaves was reduced 2-3-fold, but the efficacy of the preparations was dependent on the environmental temperature during the first week.

The half-life of spores and endocrystals of *B. thuringiensis* on soybean leaves was less than 24 hours, although some insecticidal activity was detected at 7 days post application [15]. The viability of *B. thuringiensis* spores applied to stored wheat decreased rapidly immediately after application and at a lower rate throughout the storage period; the decreases were directly proportional to storage temperature [16].

The nucleopolyhedrosis virus (NPV) of *Heliothis* spp., *Baculovirus heliothis*, showed a half-life of a little more than 2 days when applied to soybean leaves as an aqueous suspension, and no viral

activity could be detected after 14 days exposure [15]. However, wettable powder formulations of the NPV were more active and 2-5 times more persistent under both simulated and natural sunlight [17]. The use of a commercial adjuvant provides protection against inactivation of the virus by sunlight, retards evaporation of water-virus mixtures, and stimulates feeding by the larvae [18].

In a comparison of NPV and entomopoxvirus of the spruce budworm, *Choristoneura fumiferana*, in Canadian forests, CUNNINGHAM *et al.* [19] found that the entomopoxvirus applied by air did not persist well, whereas excellent persistence was obtained with the NPV.

WILSON [20] demonstrated that 5-6 hours of direct sunlight will inactivate spores of *Nosema fumiferanae*. Sandoz adjuvant V was found to be a good ultraviolet protectant for the spores when mixed in the spore formulations prior to spraying on trees for spruce hudworm control [21].

Effect on Nontarget Organisms

Bacillus thuringiensis infects a variety of Lepidoptera and it is therefore possible that it may be accused of endangering some butterfly species desirable for its intrinsic beauty. *B. popilliae*, which was extensively used in the eastern United States more than 30 years ago to cause milky disease epidemics in Japanese bettle, does not infect silkworms or bees [22].

Toxicology

A variety of acute and chronic exposures of mice, rats, guinea pigs, swine, fish, and human volunteers to *B. thuringiensis* failed to reveal any pathogenicity or toxicity. Use of the bacterial product as a feed additive did not affect Japanese quail, chickens, or cattle [23].

Outlook for Practical Use

Not one of over 40 entomopathogenic viruses tested *in vivo* was toxic or pathogenic to vertebrates, and extensive tests in the

United States have demonstrated *B. thuringiensis* and *B. heliothis* are not toxic or pathogenic to man, other animals, and plants [23]. The entomopathogenic fungi currently under consideration, such as *Beauveria, Entomophthora, Coelomomyces,* and *Hirsutella,* have been shown to be nontoxic or noninfectious to vertebrates, but they might be allergens.

About 15 years ago the U. S. Food and Drug Administration granted full exemption from residue tolerances for the commercial product, Thuricide.

In 1973, IGNOFFO [24] published an excellent review of the effects of entomopathogens on vertebrates, and in 1975 he published a review including the environmental impact of these materials [17].

In January 1976 the Environmental Protection Agency registered, for the first time ever, a pesticide made from a naturally occurring insect polyhedral virus; this product by Sandoz, bearing the trade name "Elcar", has been approved for use against two highly destructive cotton pests, the cotton bollworm (*Heliothis zea*) and the tobacco budworm (*H. virescens*). Other viruses are now being tested for the control of two timber-defoliating insects, the gypsy moth (*Lymantria dispar*) and the tussock moth (*Orgyia leucostigma*). Also a fungus that is the natural enemy of a weed common to rice fields is being tested in Arkansas, and a protozoan (*Nosema locustae*) is being tested in Montana and Wyoming for control of grasshoppers.

Several commercial products based on *Bacillus thuringiensis* are currently available on the U. S. market. These products are exempted by EPA from the requirement of a tolerance and are registered for the control of lepidopterous insects on a considerable number of crops.

INSECT GROWTH REGULATORS

Persistence and Degradation

ROWLANDS [25] tested the uptake and metabolism in stored wheat grain of Cecropia JH-1, Bowers' 2b (a methylenedioxyphenyl compound), and Altosid (Methoprene) (Figure 3) and found the residual half-lives in freshly harvested wheat (19% moisture) to be 1-2 weeks, 5-6 weeks, and 2-3 weeks, respectively. Metabolic changes

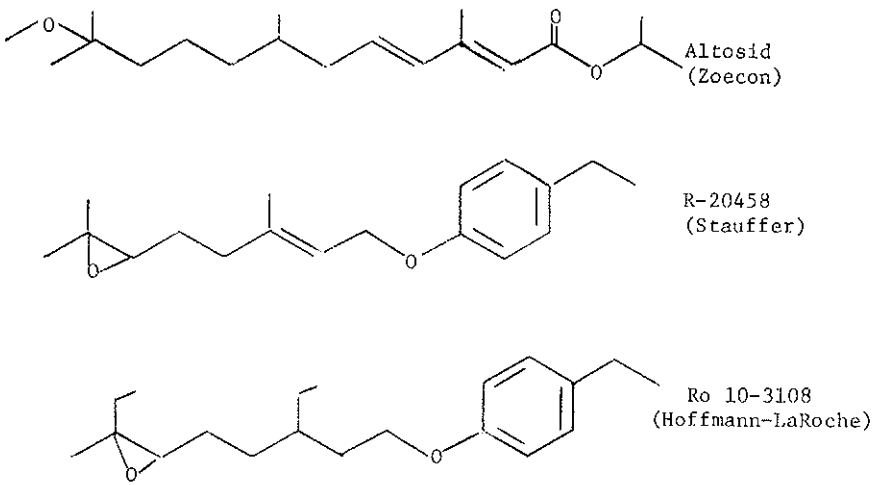


FIG. 3 — Insect Growth Regulators.

observed included opening and hydroxylation of epoxide groups and ester cleavage.

Altosid sprayed on potted balsam fir trees as 200 µl of an emulsion (equivalent to 3.07 liters/ha.) remained active against spruce budworm (*Choristoneura fumigerana*) on the foliage for at least 15 days; it was not broken down by ultraviolet light or leached by water, but it lost its activity within 12 hours when suspended in tap water [26].

Deuterated Altosid in pond water containing unknown microorganisms showed a half-life of 30 hours at 0.001 ppm and 40 hours at 0.01 ppm. Incubation for 3 days at 0.42 ppm gave 3 primary metabolites, but incubation of ^{14}C-labeled Altosid in another sample of pond water gave a completely different metabolic profile with the principal metabolite being 7-methoxycitronellic acid [27].

According to QUISTAD *et al.* [28], the most abundant photodecomposition product of Altosid was 7-methoxycitronellal; decomposition by sunlight was quite rapid in aqueous form but much slower in methanol. Altosid showed an initial half-life of 10 days at 1 kg/ha. on sandy loam soil, but decomposition was much slower on auto-

claved soil. Small amounts of nonpolar metabolites were isolated, with more than 50% being converted to CO_2. The data obtained indicated rapid and extensive breakdown in soils [29].

The reported biochemical behavior of several candidate compounds in rodent, bovine, plant and microbial species indicates ready degradation, in some instances, to fragments which were readily reincorporated into natural constituents from the metabolic pool [30].

Metabolism studies with the Stauffer compound R-20458 (Figure 3) in the rat, mouse, and a steer demonstrated extensive biotransformation of this compound *in vivo* and *in vitro*, and the ready elimination of residues of this chemical and its metabolites in urine and feces. These findings indicate that tissue residues are unlikely to occur from feedlot treatments with this compound [31]. Oral administration of R-20458 to steers showed that it was completely metabolized and the metabolites were quantitatively excreted in the urine and feces. After application to the hair and skin of steers, 30% of the dose was absorbed and subsequently excreted through the urine and feces during the first 7 days after treatment; 40% of the dose remained at the application site after 7 days, of which more than 90% was unchanged R-20458 [32]. The epoxide moiety of R-20458 in the rumen is reduced to an olefin [33].

HANGARTNER *et al.* [30] have recently reported on the field evaluation of a new compound designated Ro 10-3108 (Figure 3), an efficient agent for plant protection. Stability studies in the laboratory and persistency studies outdoors show that this compound, in contrast to most known insect growth regulators, is sufficiently stable to ultraviolet light and hydrolysis for practical purposes to give good control of natural populations of summerfruit tortrix moth (*Adoxophyes orana*) and scale insects when used as a 0.1% spray on apple trees.

Effect on Nontarget Organisms

Short term acute toxicity studies with Altosid in the laboratory and field on 35 nontarget aquatic species including Protozoa, Platyhelminthes, Rotatoria, Annelida, Arthropoda, Mollusca, Chordata, and

Thallophyta showed no visible effects when exposed to field rate concentrations. However, larvae of several aquatic Diptera showed some sensitivity to Altosid [34].

Compound Ro 10-3108 compares favorably with standard insecticides as far as the effect on nontarget organisms such as freshwater eels, mosquito fish, shrimps, skimmers, diving beetles, and water fleas is concerned. In addition, it did not harm bees, parasitic wasps, and predators of San Jose scale when it was used at 1 kg/ha. to spray a rape field four times during blossom [30].

Toxicology

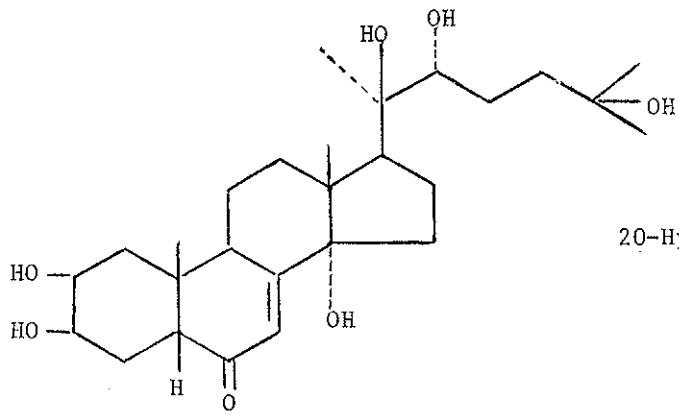
In 1971, SIDDALL and SLADE [35] found that one of the Cecropia juvenile hormones produced no signs of toxicity when given to mice in a single oral dose of 5,000 mg/kg. When the animals were observed for 21 days following treatment and then subjected to comprehensive blood and tissue analyses, no adverse effects were noted.

Published reports on the acute and subacute toxicity of several candidate juvenile hormone mimics to mammalian, bird, and fish species showed that they are only slightly toxic to these test species by oral, dermal, eye, and inhalation routes [31].

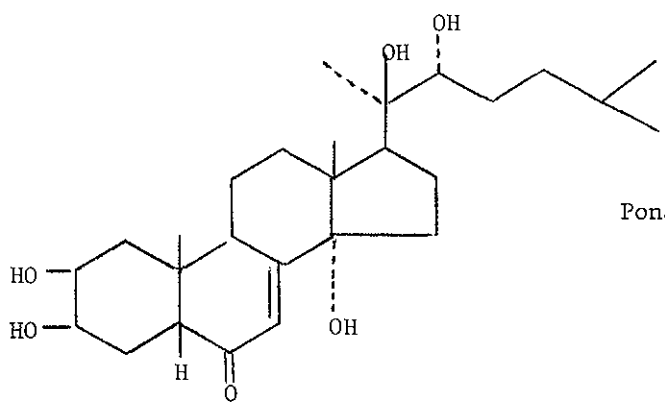
Altosid showed an oral LD_{50} greater than 50,000 mg/kg in rats and a LC_{50} in bluegill fish and trout in excess of 80 ppm and 4.4 ppm, respectively. It had no apparent effect on birds and showed only minimal eye and skin irritation in rabbits [36].

Compound Ro 10-3108 showed acute oral LD_{50} values for mice and rats in excess of 8000 mg/kg. Skin and eye irritation tests showed favorable results, and guppies and rainbow trout survived a 96-hour exposure to a 5000-ppm suspension of this compound [30].

The insect ecdysone 20-hydroxyecdysone (Figure 4) and the phytoecdysone cyasterone were nontoxic to mice when administered orally at 100 μg/mouse/day for 90 days. Ponasterone A (Figure 4) was nontoxic to rats when administered orally at doses as high as 50 mg/animal/day for 5 days; no abnormalities were detected in the treated rats [37].



20-Hydroxyecdysone



Ponasterone A

FIG. 4 — Molting Hormones.

Outlook for the Future

In 1975, WOLFF [38], writing in the Rockefeller Foundation Illustrated, said "Altosid is required to pass the same environmental test as any other chemical insecticide before it can be released commercially. So far the product has been registered for tests against mosquitoes in agricultural fields and pastures in California, Florida, and 21 other states. In another, ingenious, application Zoecon proposes to deliver Altosid via salt-licks for cattle. Enough of the hor-

mone purportedly remains unmetabolized, even after passage through the bovine's four-part stomach, to block the metamorphosis of houseflies and hornflies, which breed in manure piles. Even if Altosid survives the EPA gantlet, however, it shares with all other JH materials certain liabilities as a practical insecticide. For one, JH and its mimics are short-lived in the environment, while the insect's susceptibility to the hormone is also fleeting; thus there is a "gate" of only a few hours, or a few days at best, when insect and insecticide must be introduced to each other."

In April 1976, the EPA issued to Zoecon Corporation an experimental use permit (No. 20954-EUP-5) allowing the use of 375 lbs. of Altosid in small bodies of water to evaluate the control of mosquitoes over a total of 123 acres in 20 States. The permit is effective from Apr. 7, 1976 to Apr. 7, 1977 [5].

The outlook for the use of insect growth regulators against agricultural insects is uncertain. Only a few areas in plant protection appear to be suited for ready control by these morphogenetic agents. This is primarily because of the problems of critical timing in application, short persistence in the environment, lack of immediate control, and reinvading populations. In addition, further work is needed to assess the safety of these compounds in terms of their carcinogenic, mutagenic, and teratogenic potential, and their long-term environmental behavior.

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DISCUSSION

MARINI-BETTÒLO

I thank you very much Dr. Jacobson for this important contribution, which covers the point of view of the requirements for a new product to be used in insect control.

I also thank Dr. Jacobson for having distributed a document containing the guidelines proposed in the United States for the registration of an attractant.

This document gives many interesting details and may stimulate some further discussion on a point which has been raised already on many occasions during this study week.

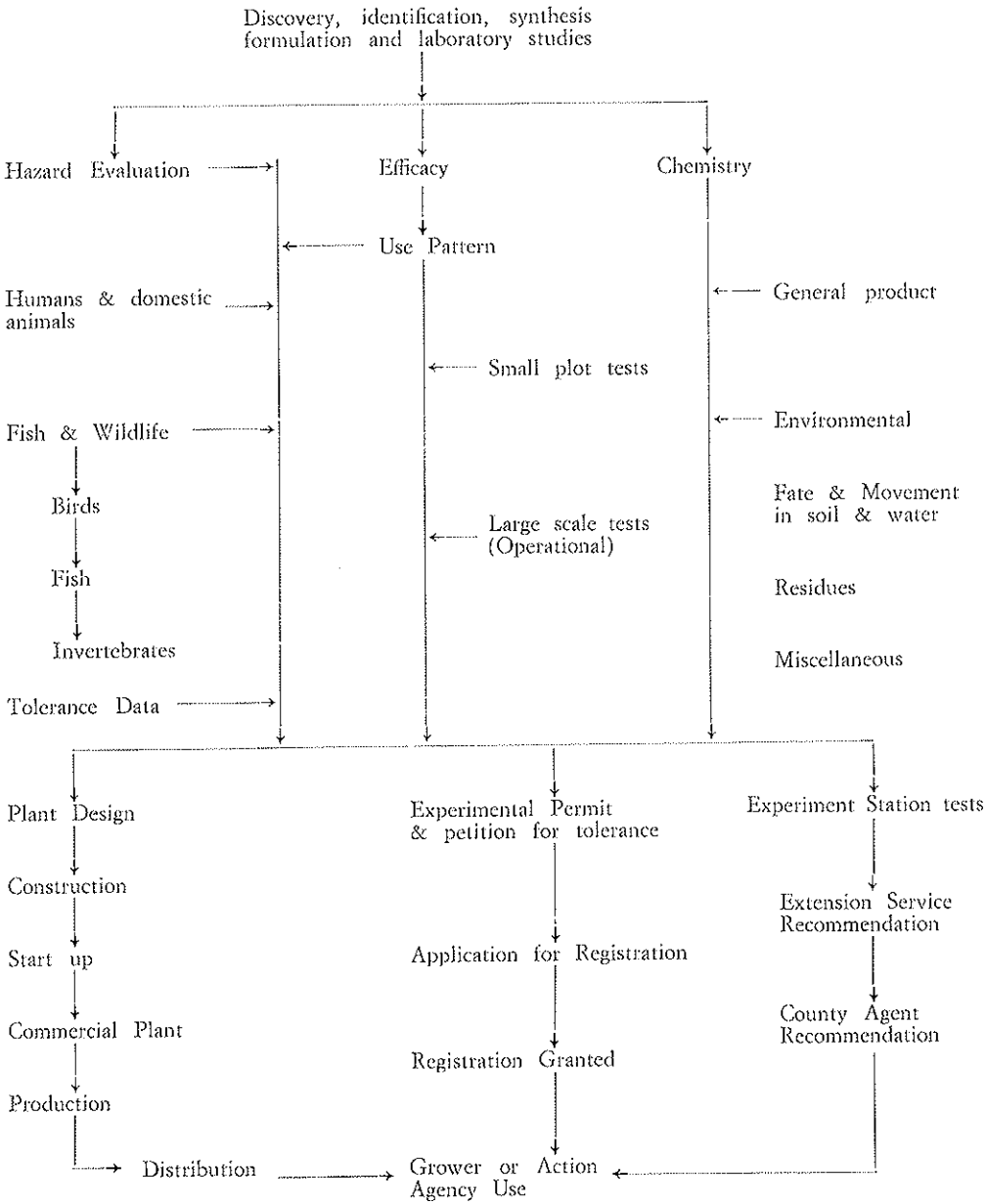
MODEL FOR THE DEVELOPMENT OF DATA TOWARD REGISTRATION OF AN ATTRACTANT (PHEROMONE)

HAZARD EVALUATION

HUMANS & DOMESTIC ANIMALS

A. *Basic Tests* (Required for both technical and formulated product) ($ 1,200 est. 1972 dollars for technical product).

1. Acute oral LD_{50}. - Use the rat to provide complete description of effect observed including full details of behavior changes and gross pathological findings at post-mortem. If the material is used in domestic situations, a test must also be performed on the product as diluted for use. LD_{50} values beyond 5,000 mg/kg in the acute oral or dermal test are of no practical value and should not be administered.
2. Acute dermal LD_{50}. - Use rabbit (guinea pig and rat also acceptable). Results should determine the LD_{50} and systemic effects as well as local dermatological effects.
3. Acute primary dermal irritation. - Use rabbit (guinea pig and rat also acceptable).
4. Acute primary eye irritation. - Use rabbit.



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B. *Conditional Tests*

1. Acute inhalation LC_{50}. - Required where respirable dust, mist, gas or vapor occurs through the physical and chemical nature of the product (formulated or technical). Other routes of administration may be required. ($ 500, est. 1972 dollars).

2. Subacute tests:

a. Subacute dermal. - Required if conditions of use may result in repeated skin contact, dusting or bathing pets, impregnation of fabrics or leather, etc. Guinea pig is the animal of choice for skin sensitization and the rabbit is the animal of choice for subacute dermal data. Tests will be performed both on the technical grade and formulated product.

b. Subacute inhalation. - Required if conditions of use result in repeated inhalation exposure.

* *c.* Subacute oral. - Required if use results in residues on food or feed or if significant oral exposure is expected. Two mammalian species must be used, one a nonrodent.

* *d.* Teratology studies are required on the technical grade product if its use may reasonably be expected to result in exposure to female humans or if the use may result in residues in food or feed. ($6,000-$8,000, 1975 dollars).

e. Neuroxicity. - Required if changes in behavioral patterns or other central nervous system effects are observed, or if a test compound is an organophosphate or carbamate pesticide or otherwide results in cholinesterase inhibition.

* *f.* Metabolism studies. - Required if pesticide use results in residues on food or feed. Studied in one or two species of animals in relation to use pattern (i.e. if residues are in the feed of dairy cattle, metabolism of the pesticide in the cow should be studied).

C. *Chronic tests*

* 1. Oncogenicity. - Required if the pesticide use results in a residue on food or feed. Lifetime feeding studies are required in at least two mammalian species. The rat, mouse, and hamster are the animals of choice. One species must be the rat.

* 2. Feeding studies. - Required if the pesticide use results in residues on food or feed. Two mammalian species must be used in lifetime feeding evaluations. One species must be the rat. The other species will be determined by registration division. In most instances lifetime feeding studies also will provide data on oncogenicity that will be acceptable. (Feeding & oncogenicity $75,000-$125,000, 1975 dollars).

* 3. Reproduction studies. - Required if the pesticide use results in a residue on food or feed. Use at least one mammalian species. Should be the same rodent species used in the feeding study. ($22,000-$25,000, 1975 dollars).

4. Other chronic tests may also be required.

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* Required if residues result in food or feed or if exemption or tolerance is required.

D. *Special Studies*

* 1. Mutagenicity studies. - Required if a tolerance or an exemption from the requirement of a tolerance is required. ($15,000-$25,000, 1975 dollars).

2. Potentiation. - Required if the toxic effects of the pesticide could be potentiated by another chemical in the formulation or by another pesticide product with which it may be used.

3. Reentry studies. - Required for cholinesterase inhibiting pesticides.

4. Other studies may be required as appropriate.

FISH AND WILDLIFE

A. *Basic Tests* (required for outdoor use)

1. Avian acute oral LD_{50}. - Required for one species of wild waterfowl (mallard or upland game bird).

2. Avian subacute dietary LC_{50}. - Required for one species of wild waterfowl and one species of upland game bird.

3. Fish acute toxicity. - Required for a cold water and a warm water fish using technical grade.

4. Invertebrate acute toxicity. - Required for a sensitive aquatic invertebrate, such as *Daphnia* sp.

B. *Conditional Tests*

1. Shrimp, oyster, and crab acute toxicity. - Required if pesticide used in or may contaminate estuarine or marine environments.

C. *Chronic Tests*

1. Reproduction studies on Bobwhite and Mallard normally will be required if pesticide is persistent, stored or accumulates in plant or animal tissue or is used where wild birds may be subjected to repeated or continued exposure.

2. Subacute or chronic fish and or invertebrate reproduction studies normally will be required if the pesticide is used in, or is likely to contaminate aquatic environments.

D. *Special Studies*

1. Requirements depend on use pattern.

EFFICACY

Data are required to substantiate efficacy claims made for the pesticide. Evidence of product efficacy will be demonstrated through laboratory and/or field testing procedures which simulate actual use conditions. Information should include the following:

A. Data to support the minimum effective dosage and effective dosage range.

B. Description of application techniques including equipment used in application, method, timing, and site of application.

C. Evaluation of the action of the product in destroying, repelling or mitigating the pest.

D. Measurement of toxic effects to plants or animals that are host to the pest as appropriate.

Specific guidelines are currently being drawn up by EPA for attractants and pheromones and this information will be included as soon as it is received.

Chemistry

General Product Chemistry (required)

A. *Basic manufacturing process*:

Information is required on the basic manufacturing process and on the purity of starting and intermediate materials.

B. *Information required for active and inert ingredients*:

Structural formula, melting point, boiling point, vapor pressure, density, or specific gravity, hydrolysis rate, solubility, and various solvents, dissociation constants, stability, physical state, color, odor, composition of technical product giving names and percentages of impurities.

C. *Assay and impurity assessment for technical products*:

Analysis and methods employed are required for the principal component and for the significant technical impurities in each pesticide chemical.

D. *Information required for formulations*:

Miscibility in various solvents; if applicable the pH, boiling point, flash point, specific gravity, viscosity and vapor pressure; explosive characteristics if any; corrosion hazards; capability of acting as an oxidizing or a reducing agent; and for liquids, the weight of the active ingredients per gallon.

E. *Storage stability*:

Data, usually chemical analysis, covering a period of at least a year should be submitted to show storage stability. This should be done as commercially packaged since EPA requires commercially packaged materials to be stable for as long as it would be expected to remain in channels of trade.

Environmental Chemistry (required for outdoor use)

A. *Pesticide fate and movement in soil*

1. Edaphic and climatic descriptions:

The common textural classification and the following physical and chemical properties of the surface soil should be determined. Organic carbon, cation exchange capacity at pH 7, pH, bulk density and water retentivity at 1/3 bar.

Field studies should be supported by descriptive data to locate the field plots within a county or parish, amount of rainfall and/or irrigation water accumulative to each sampling and soil and/or air temperature data from the State climatic division within which the field studies were performed.

2. Soil metabolism studies:

   These studies should be designed to provide the basic information on the rate, type and degree of degradation of the parent pesticide and its transformation products including bound residues. Studies with radiolabeled pesticides will be required. Sufficient data should be collected for periodic analysis to allow graphic plotting of the amount of parent compound until 10% or less remains. Clearly defined and validated analytical procedures for the separation and identification of degradation products in polar and nonpolar metabolites should be available. Specific activity of the radiolabel should be capable of tracing metabolites down to 10 ppb in the soil. Basic soil metabolism studies should be performed under aerobic conditions. For standardization and comparison, a soil with sandy loam, loam, or silt loam surface texture is preferred.

3. Soil persistence studies:

   Data derived under actual use conditions are required under the main geographic and/or climatological areas for which the registration is proposed. Use four sampling sites and statistically valid sampling schemes over sufficient timeframe to allow a definitive assessment of the degradation profile. Each site utilized should represent a major soil series in a particular geographic region. If a pesticide or its degradation products are highly persistent special long-term studies to determine buildup of these substances may be required.

4. Leaching studies:

   Laboratory scale leaching studies on the parent compound and its soil degradation products will be required.

B. *Pesticide fate and movement in water*:

Typical studies which may be required for a new pesticide product are as follows:

1. Dissipation rate in distilled water.
2. Degradation in water containing suspended solids.
3. Degradation studies in bottom sediments.
4. Translocation.
5. Livestock and poultry drinking water.
6. Tests involving moving waters.

C. *Pesticide residue studies*

1. Fish and Wildlife studies:
   Fish residue studies must be conducted to determine the amount of uptake of the pesticide and its major degradation products by fish until a plateau has been reached and the rate of dissipation of any residue taken up by fish when placed in pesticide-free water.

2. Crop uptake studies:

   Required where use pattern is proposed for croplands.

D. *Photodegradation studies are required on surfaces in water and in air depending on the nature of the compound and its use pattern.*

E. *Volatilization studies may be required depending upon use pattern.*

F. *Microbiological studies*

1. Effect of pesticides on microorganisms:
   Tests on soil microorganisms or relevant enzyme systems of natural soil populations will be required to assess the overall action of a pesticide.

2. Effect of microorganisms on pesticides:

   Because biodegradation relies extensively on the action of microorganisms, a qualitative and quantitative assessment of their role in the degradation of all pesticides likely to enter aquatic environments in significant quantities will be required such as aquatic herbicides, household pesticides, and industrial microbicides.

KNÜSLI

Well, the Chairman allowed that we could eventually come back to the whole registration matter after Dr. Jacobson's paper, so I intend to make use of this. I think that this audience here was shown and realized what an effort it means to bring forward a compound, be it natural or unnatural, to the level of practical use, and that they recognize how many premature gray hairs such efforts can cause. Now I must say as an individual I am pleased that there exists a registration procedure because as Dr. Staal said, I feel too I am a consumer and I would like to be safe, and I would like that my kids and eventually my grandkids survive, and therefore I appreciate such a procedure as I appreciate also that I am examined for weapons when I enter an airplane for example. But I think that it became evident that the whole system of the requirements may have reached or already passed what is justifiable, and I wonder whether we can eventually deal with this aspect a little bit more or not. I told it during lunch to my colleagues in the case the table salt or the mustard you put on the hamburger would be a pesticide agent you would hardly be able to bring these two products through registration — and this shows you the limits which we have reached. But I am doubtful whether a recommendation to the registration authorities from this audience — although this audience has a substantial

weight — could help. Eventually we could try to draft proposals for a differential registration procedure, for example depending on the toxicological characteristics of a compound or on the persistency or on the picture of the degradation or on the size of the application of a product; and I wonder whether you have additional ideas along such lines.

MARINI-BETTÒLO

Thank you Dr. Knüsli I think we must consider that Dr. Jacobson works at the U.S. Department of Agriculture and has also to deal with the Food and Drug Administration for the regulations and for the registrations. In the document which has been distributed there are a great number of requirements to be fulfilled for registration. This is due to the fact that we cannot foresee what can happen in the future with the use of these substances. In effect, in the past, several inconveniences have happened. This is the reason why we have so many regulations and so great a number of trials to be fulfilled before registering a new product. Some of them are probably unnecessary for certain substances. Anyhow we need a new approach in establishing these regulations otherwise we would prevent research and thus the finding of new compounds which are necessary for protecting the crops all over the world.

JACOBSON

I must tell you that I myself have not yet had very much to do with Food and Drug Administration or with EPA in connection with my work — not directly. I can give you my opinion of the situation. Now, you understand that this is just my opinion and I am not representing the U.S. Department of Agriculture when I give you this. I feel, and I've felt for quite a long time, that EPA has gotten what we call in the United States "too big for its britches", and they have put so many obstacles in the path of licensing or registering these materials that I think they have to be taken down a peg now. Just before we came in here to reassemble, Dr. Shorey was telling me that there seems to be a glimmer of light in possibly getting them to reconsider some of the stringent regulations that they put on these things. In one respect I can understand the way EPA and FDA are thinking on this thing. As I was telling Dr. Bowers during lunch, I agree with most of what

he said when he was talking about the necessity of putting public pressure on in order to try to get around these overly stringent regulations that EPA has put on registration, but I think in order to be somewhat realistic — I think especially in the United States one must realize that the majority of the public wants strict regulation because they want to be able to feel safe in using certain materials, in knowing what they are eating and that they are not subjecting themselves to hazards. So if he feels that the public can put on a certain amount of pressure — sufficient pressure — to overcome these stringent regulations, I think that this is a mistake, especially in the United States where we have so many environmentalists — but I'm somewhat encouraged by what Dr. Shorey has said and all I can say is that we will have to see what happens. I do know that very recently, within the past 2 or 3 weeks, the United States Congress has passed a new Toxic Chemicals bill or act, and I don't know whether this puts more power in the hands of the Administrator of the Environmental Protection Agency as to which chemicals he wants to turn down and accept. There's no way of knowing right now whether the regulations are going to become more strict or more lax.

CHAPMAN

Of course I agree with everyone that this program of registration is extremely important but I think I also agree with Dr. Knüsli that perhaps we are not in a position to do very much about it. Now this is not a reason for not talking about it and discussing it and perhaps making a recommendation, but what I rather fear is that we might get bogged down in the problem of registration and miss out on things that we could actually achieve — because it seems to me sitting here during the last 3 days we've heard a lot of very interesting information — I think some of the chemistry has been quite outstanding.

Now, if I were standing here with a million pounds in my pocket to give away to someone who wanted to register a chemical, I haven't heard anything that would convince me, and the reason that I haven't heard anything to convince me is, not that people haven't been doing good work, but that it's all so fragmentary and disorganized that it is not answering any of the questions that I would want answered if I was going to put up a million pounds.

I think if one takes the example where most work has been done

— and that seems to be azadirachtin — the only advance really in the last 30 years — one might almost say 300 years — as far as I know, is Professor Nakanishi's identification of the chemical structure. But 30 years ago people knew that neem was a very potent insect repellent. I suspect that the Indians knew it even 300 years ago. And a lot of people are working on this now and they're doing good work but we're going round and round in circles. If I ask people: can you tell me if neem or azadirachtin is effective against the insects where I might want to use it, like *Heliothis,* or the Colorado beetle or other insects of that sort, in 9 cases out of 10 they couldn't tell me, because the insects they've worked on are the ones that happen to be convenient in their laboratory. If I ask them how many persist, mostly they can't tell me. If I ask them if it's got a systemic action, they cannot tell me. If I ask them what the yield of a neem tree is and how long it is sustained, they cannot tell me. Now if I am going to put up a million pounds, I want to know these things. These are very simple problems, and what it needs is for all these people who have been working on neem or whatever it happens to be, to get together in order to provide answers to these problems. And I think that's something that this meeting really could provide. It could provide the basis for people getting together and thrashing out these problems of what is needed. And instead of people going out and working on the long-haired caterpillar that feeds on goodness knows what, to see if neem works on it, he might actually do it on an insect where we wanted to know the answer; and we could then pool that information. Then if you come to me I might provide my million pounds. So, really what I am saying is please do not let us get too bogged down in registration.

WAIN

I would just like to add a word to what Dr. Elliott was saying if I may about the British NRDC. The NRDC I always regard as an institution which provides patents without tears for academics. In the past one or two quite important discoveries have been made in universities and government departments, which have not been covered by patents. The classical case of course is the discovery of penicillin in England which was not patented. All the developmental work on penicillin, however,

was patented in the United States. So Britain had to pay royalties to America for the exploitation of its own discovery.

This is the main reason why the NRDC was set up and I and many others have found the service most valuable. As far as the inventor is concerned the problems involved in obtaining patent protection are minimal. Not only this but the NRDC also deals with the licensing and commercial exploitation of the patents.

NAKANISHI

What I am going to say is probably fragmentary as usual but first a very brief comment on NRDC and the difference between Research Corporation and NRDC. As you say, NRDC does provide us with funds, with strings attached to a certain extent, and with these funds you can probably hire a technician and then go on to the developmental aspects of a finding. On the other hand, Research Corporation provides no funds; instead you have to solicit exclusive licenses — and as far as we are concerned, we would rather prefer the latter one, because it saves us trouble of even thinking of what type of decisions we should make, and these should be handled by professionals rather than people like ourselves. Another main objection about NRDC is: If I were a British subject and if this Insect Institute in Nairobi were another British organization, probably I would go through NRDC. However the ICIPE is international.

We have to go through some neutral organization like Research Corporation, that is as far as university people are concerned, provided some company is interested in it. I am mentioning this because I think that Dr. Elliott may have slightly misunderstood what I had meant. It is a pity once you have isolated a crude product — you usually go to a lot of trouble to collect it — and if you are just looking for one of its bioactivities, there are many possibilities that it may have other activities besides, for example against insects. What I had in mind was to go through these commercial assay organisations and then they would run for you, say, 30 or 40 ..... screenings and just give you the results. Probably after this meeting I think my way to approach the African compounds is to send these to these commercial places and get a lead on 2 or 3 bio-activities and then if it is worthwhile we will go through the licensing through the Research Corporation and then

several commercial companies will deal with these. As far as I know, in the USA if a natural product is new you can patent it.

And then whatever new activity is found will go through that patent. If it's a known compound, then you can patent it for that particular purpose. But my feeling is that as exemplified by penicillins, steroids and pyrethroids, the natural products rather than giving you the final product, just give you a lead — and I think it is really the starting point to what Dr. Knüsli calls unnatural products. But this sort of thing has to be, for obvious reasons, done by the experts with much more expertise in the various fields. As I mentioned yesterday that I was getting disenchanted with the so called static but I am optimistic again and I am interested in seeing what type of cooperative scheme results from this meeting.

SOMERVILLE

I would just like to make a few comments on registration.

I consider this is basically a problem of people — a Risk-Benefit analysis in which we are balancing the benefits to agriculture against possible detrimental effects can range from instances such as Flixborough and Seveso to the other end of the scale, where there can be minor dermatitis or very limited environmental effects. I think it's very important that we take each instance in its own light, that we don't try and generalise and, I would like to suggest that there are some things that we can do as a group of scientists. One of them I think is to encourage that there is always a dialogue with the bureaucrats, as we tend to call them. In my experience the bureaucrats tend to be fairly reasonable people and if you do talk to them they will listen and accept reasonable argument. The other thing I think we can do is to encourage the development of the predictive tests which are beginning to come into use, such as the mutagenic test which bears some relationship to carcinogenicity which has been developed in Ames' laboratory at Berkeley. If we can develop tests such as this and tests with human cell lines I think that we may in the long term be able to reduce the cost of these registration requirements as well as minimize the risk to people.

MARINI-BETTÒLO

I think that we shall stop talking about registration now because it goes a little bit beyond our work aim.

BALLIO

You briefly mentioned in your lecture the problem of grain storage. I would like to have your comments on research recently carried out in some countries (Australia, United States and Italy) directed towards the possibility of storing grains under anaerobic atmospheres, like nitrogen, CO_2, etc. As both nitrogen and CO_2 are natural products, I feel that this point is pertinent to the main topics of the Study Week. One major interest of this approach resides in the fact that the treatment does not involve toxicity problems and might afford pest control under non-polluting conditions.

JACOBSON

I am sorry I can't answer that question — I just do not know enough about the subject.

KNÜSLI

I have another point where I think this audience expects an answer by me. It was said — and this was quoted although I think already by other speakers outside this audience — that the so-called big companies who have the money and who could afford studies around natural matrices would not do it, while small outfits who would be willing to do such studies would not have the money, they would be broke. Now I think what is common to the small and to the big outfits is that they live by trade, by selling a service to a client who is willing to pay something for the service because the service has a value for him. So I think we have to consider how the means available to the outfits are attributed to the various projects. I can give you some figures as to the distribution of the R and D budget of my company in the field of agricultural chemicals. Ten percent, roughly, go to chemical syntheses. Forty percent go to biological evaluation, including worldwide biological field evaluation. Twenty percent go to technical development — when I

say technical development, that means formulation, process development, and so on — but not investments for production; and about 20%, this comes close to the figure quoted by Dr. Brader, goes to registration; that is, all the studies for residue analysis, for metabolism, for toxicology and other registration matters. You have a remainder of 10 %, which goes to patents and I do not know what. So these are roughly the figures. Now of course I said R and D, I would estimate that of the money spent about two-thirds to two-fifths may go to the development phase, and one third to two-fifths go to the research phase. So the development phase circles around the promising offspring of research. Now whether you deal in research with natural or natural-related or unnatural compounds, or any other approach, each of these approaches has in principle the chance to reach the development phase and to be involved in all the efforts in the development phase. As a research director I have the choice how to distribute the funds. I could consider to put all eggs in one basket and for example propose: let's invest all the research funds in projects dealing with natural structures. I know that my colleagues of Zoecon to whom I feel very related followed such an approach, and if I'm right that caused them some gray hairs. In my case I would never do that because I would fear that, following exclusively this strategy, the probability of going broke in 10 or 15 years would be high. I prefer to attribute, for example, a fifth or a fourth of the funds for research along leads of natural origin, and that we do in fact. In other words, in contrast to what has been said a so called big company as ours doesn't exclude the natural or natural-linked approach. It devotes a substantial quantity of research money to it but invests at the same time in other strategies which are at least as promising as the strategies based on natural products.

JACOBSON

Mr. Chairman, before we go on to the next presentation, I would like to say that when I finished my presentation this morning Dr. Siddal told me that he felt that some of my data with regard to Altosid were erroneous and I think that if this is actually so, the accurate information should be brought out.

SIDDAL

I simply referred to the time at which the compound became commercially available, which is already in 1975, and there was already issued a permit for experimental use in 1973, and I felt that this was an important point only because it illustrated that the total elapsed time from the beginning of what Dr. Knüsli has referred to as research in 1969 until the first commercial registration in 1975 is about 6 years and that this is what one may expect as a sort of absolute minimum time for taking a natural product through the necessary steps to bring it into practical use. I think in the manuscript of Dr. Jacobson it would be very simple to change the data to reflect the actual situation.

DORN

I have also a question concerning toxicology. You presented the toxicological data for the pyrethrins. As you mentioned for practical applications they are most often combined with piperonyl butoxide. In no textbook could I find any toxicological data about such combinations. I would now like to ask you how the addition of the synergists does alter the mammalian toxicity of the active substances.

JACOBSON

I think that probably Dr. Elliott would be much more informed to be able to answer that question.

ELLIOTT

The Wellcome Foundation published a review of the toxicology of piperonyl butoxide by N. C. Brown (*) which dealt with some of the aspects of these compounds discussed at the Study Week.

(*) Report A 28/52, November 1970.

NATURAL PRODUCTS FROM THE TROPICAL TERMITE *MACROTERMES SUBHYALINUS*: CHEMICAL COMPOSITION AND FUNCTION OF « FUNGUS-GARDENS »

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Overview

At the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, scientists of the termite research programme are conducting physiological and ecological research on the tropical termite *Macrotermes subhyalinus*; one of the major grass-feeding termites in semi-arid regions. As is the case with other ICIPE research programmes, it is felt that a thorough knowledge of the physiology and ecology of this insect may lead to the design of control methods that may disrupt these processes selectively, thus providing environmentally safe control mechanisms.

Natural products from the termite *M. subhyalinus* were recently isolated, identified and some of their physiological roles were investigated. Among these products, the juvenile hormone (JH) (methyl(2E, 6E)-10, 11-epoxy 3, 7, 11-trimethyldodeca-2, 6-dienoate or JH III) was identified (Meyer *et al.*, 1976) as a major JH in the hemolymph of the physogastric queen. Lüscher, in a series of publications, has shown that in the case of lower termites, JH plays an important role as a pheromone in the termite colony regulating the production of various castes. Whether JH plays a similar role in higher termites, such as *Macrotermes sp,* is still under investigation. Lüscher (1976), has also suggested that JH may act as a gonadotropic hormone in termites, thus regulating the synthesis of the large quantity of protein needed for the enormous egg production of the

termite queen. Since the increase in protein biosynthesis requires an increase in energy demand which is often accompanied by increase in oxidative metabolism, we investigated (ABO-KHATWA and LÜSCHER, 1977) the influence of JH III and other JH's on the oxygen uptake of fat body and ovarian homogenates and mitochondria *in vitro*. Unlike cockroach studies, JH had no significant influence on basal or oxidative metabolism on fat body and ovarian tissues of the termite queen. But of interest was the fact that addition of β-ecdysone (10μM) increased significantly the basal and oxidative metabolism of the ovarian tissue of the termite queen. We, therefore, investigated the possibility of the presence of ecdysones in the ovaries of the queen. Preliminary results have shown the presence of an ecdysone-like compound of a polarity higher than that of β-ecdysone. The chemical identification of this compound is currently under investigation.

Another natural metabolite that was capable of eliciting the construction of an emergency "royal cell" structure by termite workers, has been recently isolated from the fat body of the termite queen (PRESTWICH and BRUINSMA - personal communication). The chemical identity of this pheromone is still in progress.

INTRODUCTION

In this paper, we would like to report on other natural products found in the "fungus-gardens" of *Macrotermes subhyalinus* in relation to termite nutrition. Termites belonging to the sub-family Macrotermitinae are well known as builders of large mounds commonly seen in tropical Africa. These termites are mainly grass feeders, able to cultivate a basidiomycete fungus belonging to the genus *Termitomyces*. The true nature of "fungus gardens" and their symbiotic relationship with termites has been a subject of controversy since KÖNIG discovered the "fungus gardens" in the eighteenth century. There is no agreement in literature as to whether "fungus gardens" are constructed from fecal pellets or from masticated plant material. Furthermore, it is not clear whether "fungus gardens" provide termites with nutritional material and vitamins (GRASSÉ, 1937) or are primarily concerned with humidity control (GHIDINI, 1938) combined with heat production inside the nest

(LÜSCHER, 1951). SANDS (1969) has reviewed the literature showing several conflicting opinions on this subject.

Fig. 1 shows a photograph of a fungus comb; a special structure constructed by termites, covered with white nodules or conidia of the fungus belonging to the genus *Termitomyces*.

We studied the fungus-termite relationship by: first, conducting a detailed chemical analysis of fungus comb to assess its nutritional value to termites. Second, carrying out some metabolic studies on fungus as well as on termites to determine their capabilities to oxidize some of the metabolites that were discovered in fungus combs. Third, analysing quantitatively and qualitatively, the lipid composition of "fungus-gardens" and those of termites in order to examine their relationships.

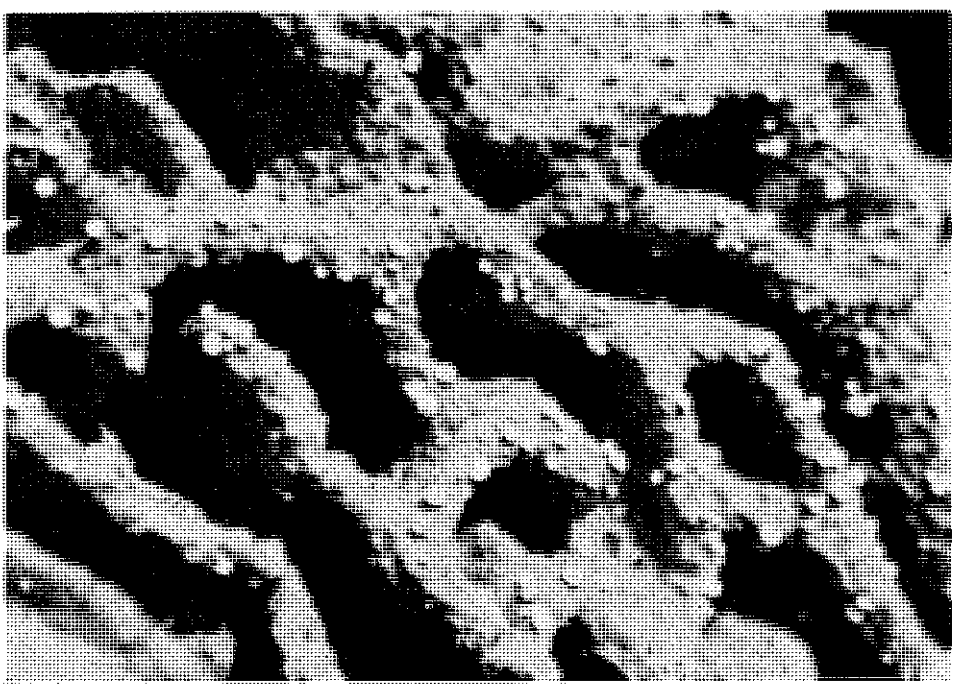


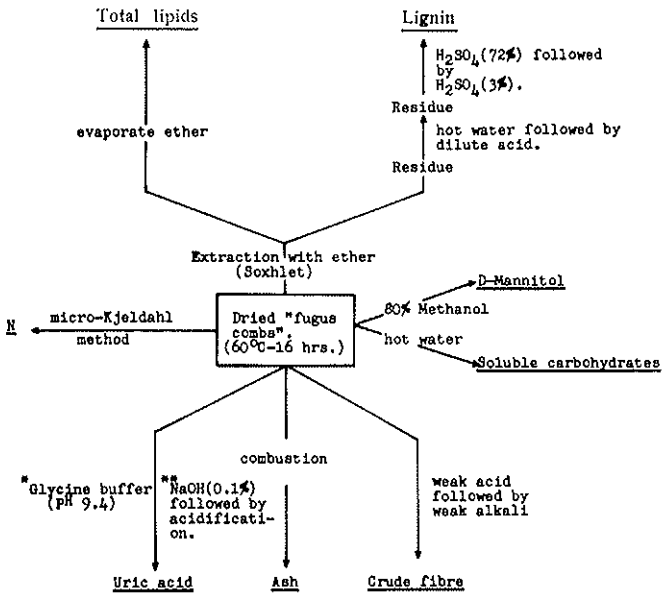
FIG. 1 — A piece of fungus comb showing the white nodules or conidia fungus of the genus *Termitomyces*.

MATERIALS and METHODS

a) *Collection of Samples and Chemical analysis*

Intact fungus combs, *Termitomyces* conidia, and various castes of the termite *Macrotermes subhyalinus* were collected from mounds located in Kajiado district, 100 Km from Nairobi, during April 1975 - September 1976. For extraction and chemical analysis, all samples were dried in an oven at 60° C for 16 hours. Moisture, pH, lignin and crude fibre content of dried fungus combs were determined by standard methods (ALLEN *et al.*, 1974). Nitrogen, cellulose and inorganic analyses were conducted by the National Agricultural Laboratories, Nairobi, Kenya. Total lipid content was determined by either a continuous extraction method (Soxhlet) using anhydrous diethyl ether or by extraction with chloroform: methanol mixture (2:1 v/v). The latter method produced a significantly higher yield of lipid than the former. The separation of lipid classes was conducted by using a florisil column (12 gm; deactivated with 7 % water) according to the metbod used by CARROLL (1961). Reducing sugars were determined by the anthrone method after homogenization in hot water, acidification by 1N H_2SO_4, and hydrolysis in a boiling water bath for 2 hours. The intensity of the colour produced was determined spectrophotometrically, against a standard glucose solution, at 625nm according to ASHWELL (1957). Uric acid was determined chemically by using the sodium tungstate method (DAWSON *et al.*, 1972), and enzymatically by using Uricase enzyme (BDH - standard kit) method.

D-Mannitol was extracted from fungus combs, by 80% aqueous methanol. This sugar was identified by using infrared spectroscopy, n.m.r., and by gas-liquid chromatography (5% SE-30, 3mm by 75mm column, 190° C, N_2 carrier gas at 25 ml/min) as a hexa-actate derivative (synthesized by acetylation of the sugar alcohol using acetic anhydride/pyridine mixture). Melting points of crystallized D-mannitol and that of a standard material were identical (165-167° C). Fig. 2 outlines a diagram showing the various stages of organic analysis of fungus combs.



An cutline diagram showing the various stages in the proximate analysis of « fungus combs ».

FIG. 2

b) *Biological Work*

To determine the ability of fungus and termite extracts to oxidize D-mannitol and other psysiological substrates, freshly collected conidia of *Termitomyces* were homogenized in saline (0.9% KCl), centrifuged at 800g and the supernatent was used as the source of enzyme. Minor workers of *M. subhyalinus* were homogenized in 0.25M sucrose and 2mM EDTA, pH 7.4. The homogenate was filtered through four layers of cheesecloth; the filtrate was centrifuged at 800g and the clear supernatent was used. The reaction mixture (3ml) for both extracts of conidia and termite workers consisted of 0.25M sucrose, 30mM KH_2PO_4, 15mM KCl, 2mM EDTA, 5mM MgCl, 50mM Tris-HCl, pH 7.4 and a physiological substrate in a given concentration as indicated in Table 1. The oxidation rate was

TABLE 1 — *Chemical Composition of Fungus combs of the Termite* Macrotermes subhyalinus.

Content	*% Content (based on the dry weight)*
Water	8.3 - 10.0 (46.7 - 47.4)[a]
Ash	12.1 - 12.5 (85.5 - 87.0)[b]
Crude fibre	23.5 - 25.0
Lignin	14.8 - 18.0
Cellulose	7.0 - 8.7
Reducing sugars	1.0 - 2.4
D-Mannitol	5.5 - 6.9
Uric acid	0.05 - 0.1
Chlorophyll	<0.005
Total nitrogen	1.20 (0.08 - 0.1)[b]
Total lipids	1.80 - 2.10
P (ppm)	236 (22 - 27)[b]
Ca (m. e%)	11.6 (4.0 - 4.8)[b]
Mg (m. e%)	7.0 (3.8 - 4.4)[b]
Na (m. e%)	0.7 (0.02 - 0.18)[b]
pH	4.2 - 4.7 (6.1 - 6.5)[b]

[a] % of water in fresh wet weight.
[b] Values correspond to % contents of a soil material collected from the same mound.

followed polarographically, at 30°C, using a Clark O_2 electrode coupled to a recording system.

To culture the fungus under laboratory conditions, *Termitomyces* conidia were inoculated, under sterilized conditions, on a synthetic agar medium consisting of (per 100 ml); 2 gm bacto-agar, 3 gm sugar (mannitol, sorbitol, glucose, sucrose or mannose), 0.2 gm $NaNO_3$, 50mg each of $MgSO_4$ and KCl, and 1 mg each of Fe SO_4 and $ZnSO_4$. Inoculated agar media were incubated at 30° for several weeks.

RESULTS

Table 1 shows the chemical composition and some physical properties of a fungus comb sample.. The given figures represent the average composition from various samples from the same mound without allowing for the possible variations that might exist in different parts of the fungus combs (see Table 2).

The analytical figures presented in Table 1 show that water content of fungus combs was about half of its fresh weight. The ash content and crude fibre (lignin and cellulose) accounted for one-third of the sample dry weight. The amount of reducing sugars was relatively low (1-2.4 %) while the amount of the sugar alcohol, D-mannitol was surprisingly high (5.5-6.9 %). Uric acid, a main end product of nitrogen metabolism in several insect species, was relatively low (0.05-0.1 %). Other organic components such as

TABLE 2 — *Average Chemical Composition and pH of Various parts of the Fungus combs of* Macrotermes subhyalinus.

Component	*Parts of fungus combs* *			
	Green	*White*	*Eaten*	*General*
Reducing sugars	5.7	17.1	24.3	9.9
Total lipids	20.5	22.7	26.2	21.6
Total nitrogen	12.2	11.1	11.8	11.6
Uric acid	0.66	0.78	0.30	0.56
Total Chlorophyll	0.56	0.02	0.06	0.07
Vitamin C **	0.08	0.10	0.09	0.11
Vitamin A	0.014	0.004	0.003	0.009
pH	4.74	4.25	4.20	4.24

* A fungus comb sample was collected from one termite mound during June, 1976. The sample was separated into various parts depending on its degree of "maturity". Thus, *Green* stands for those parts that were newly deposited on the upper surface of the comb; *White* for those parts that were distinctly white; *Eaten* for parts of fungus comb that were showing markings of mouth parts of termites; and *General* for the middle parts of fungus combs that were probably half "mature". Values are averages of 2-3 determinations, expressed as mg/gm dry weight of fungus combs.

** Vitamins C and A were determined by standard methods conducted by DR. L. O. Abe (Department of Botany, University of Nairobi).

lipids, chlorophyll, and polyphenolic compounds (possibly humic and fulvic acids) were recovered from fungus combs. Polyphenolic compounds (chocolate brown dust-like powder) were extracted with 0.1 % NaOH and their presence could account for the relative acidity of fungus combs (pH 4.20-4.74). The average total nitrogen content (representing the protein and non-protein nitrogen) was relatively low (1.2 %).

Among the inorganic cations found in fungus combs, at relatively high concentrations, were phosphorus (10 times more than the amount found in the surrounding soil) and calcium. Other minerals such as magnesium and sodium were 2-4 times more in fungus combs than in the surrounding soil.

D-Mannitol was identified by using infrared spectroscopy (Fig. 3) and n.m.r., (Fig. 4). Identical spectra were obtained when compared with a standard material. The sugar alcohol, D-mannitol, was further identified as a hexa-acetate derivative as shown in Fig. 5.

It is generally believed (KALSHOVEN, 1936) that when fungus combs reach a certain stage of "maturity", termites eat specific parts

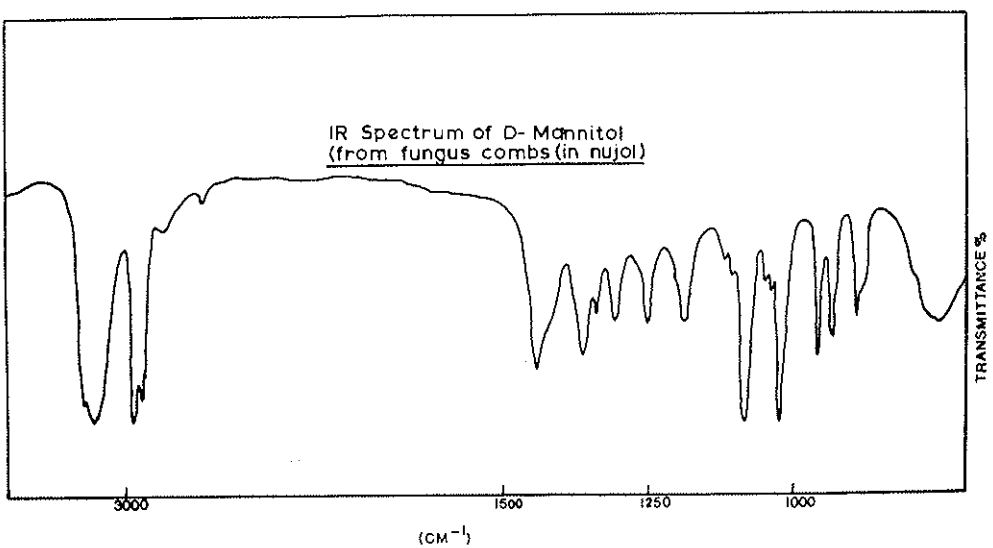


FIG. 3

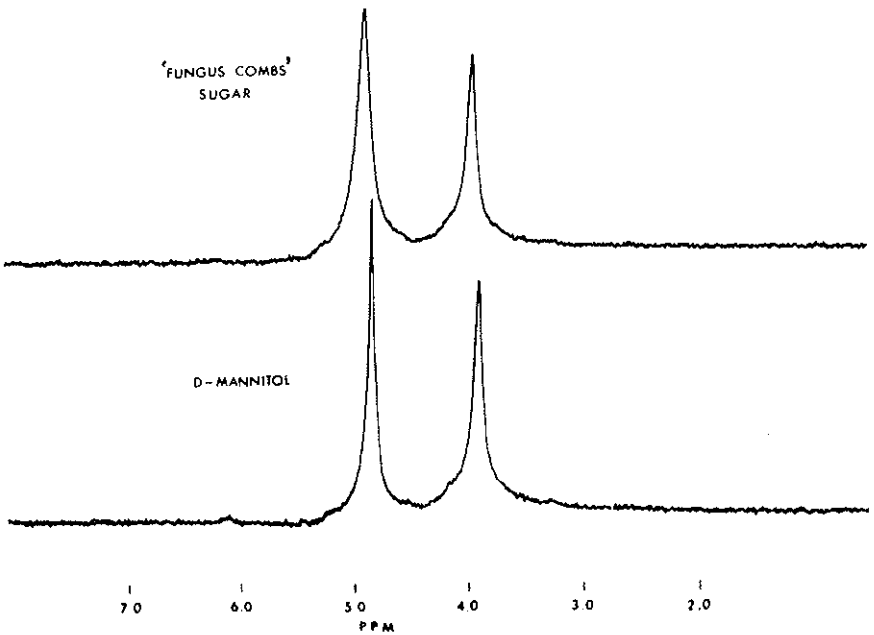


FIG. 4 — n.m.r., spectra of fungus combs sugar and that of a standard D-mannitol.

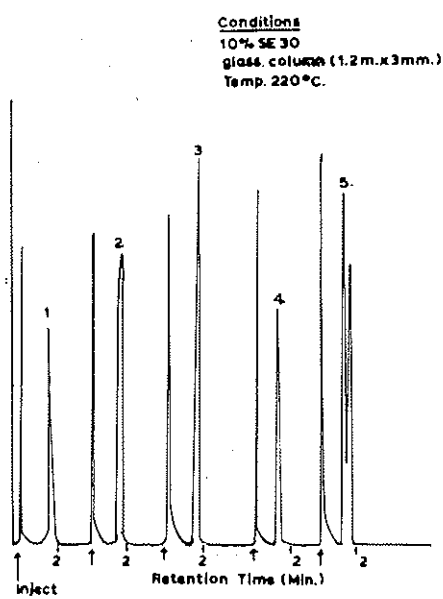


FIG. 5 — Identification of D-mannitol (as a hexa-acetate derivative) by glc. 1: acetate derivative of fungus sugar; 2: D-mannitol acetate; 3: both 1 and 2; 4: D-glucose acetate; 5: both 1 and 4.

of the comb away from below while they reconstruct it by depositing new material on the upper surface. Table 2 shows significant variations in some of the fungus combs constituents, when these combs were analysed after being separated into various parts depending on their stages of "maturity".

It is interesting to note that those parts of the fungus combs showing signs of feeding by termites (Eaten) contained significantly higher quantities of reducing sugars and lipids and lower quantities of uric acid as compared to other parts of the comb. Other constituents such as nitrogen, chlorophyll and vitamins were intermediate. On the other hand, the "White" parts of combs contained the highest amount of uric acid deposits (0.7 mg/gm). The white colour of these parts of the comb could be due to its high content of uric acid salts.

Some of the valuable nutritional ingredients present in fungus combs were reducing sugars and the sugar alcohol D-mannitol. Table 3 shows the oxidative ability of homogenates of *Termitomyces* conidia and of minor workers toward various physiological substrates including a number of reducing sugars and the sugar alcohols D-mannitol and sorbitol.

As can be seen from Table 3, both the sugars mannose and galactose were oxidized readily by extracts from fungus conidia and termite workers. In contrast, D-mannitol was oxidized by fungus conidia but not by termites, although in the former case the oxidation rate was far less (3.1 natoms O/min/mg protein) than the rate for the corresponding sugar D-mannose (11.6). But in the case of the stereoisomer sorbitol, it was the reverse. Thus termites were able to oxidize it while fungus conidia were not.

Table 3 also shows that *Termitomyces* conidia extracts were able to oxidize efficiently low molecular weight alcohols such as methanol, ethanol, and propanol. Termite extracts, on the other hand, were not able to oxidize these alcohols to any extent. We have also noted (not shown in the table) that the addition of KCN (10^{-4}M) during the course of respiration inhibited completely the oxidation of all the physiological substrates tested except those of alcohols that were oxidized by conidia extracts.

Fig. 6 shows the results of an experiment in which *Termitomyces* conidia were allowed to grow on synthetic agar media (fungus

TABLE 3 — *Oxidation Rate of Various Substrates by* Termitomyces *Fungus (conidia) and by minor workers of* Macrotermes subhyalinus.

Substrate [a]	*Oxidation Rate* [b]	
	Fungus	*Termite*
Sugars:		
D-Glucose	—	3.0
D-Mannose	11.6	6.0
D-Galactose	6.6	4.4
D-Fructose	—	—
Sugars alcohols:		
D-Mannitol	3.1	—
Sorbitol	—	8.9
Alcohols:		
Methanol	30.1	—
Ethanol	30.1	—
n-Propanol	5.0	—
iso-Propanol	—	—
Intermediates:		
Acetate	5.1	6.0
Lactate	—	10.4
DL-α-glycerophosphate	23.2	4.0
Succinate	3.7	11.8
Glutamate	5.2	4.4

[a] Final substrate concentration was 20 mM except alcohols which were added at final concentrations of 1 %.

[b] Oxidation rate is expressed as natoms 0 per min. per mg protein.

inoculations were done *in locu* under sterilized conditions using a flame and ethyl alcohol) in the presence of various sugars serving as carbon sources. Only agar media containing D-mannitol (3 %) were able to support the growth of monocultures of *Termitomyces*. The inclusion of other sugars in the agar media resulted in the growth of a variety of other foreign fungi some of which were tentatively identified as *Aspergillus* and *Fusarium* sp. The source

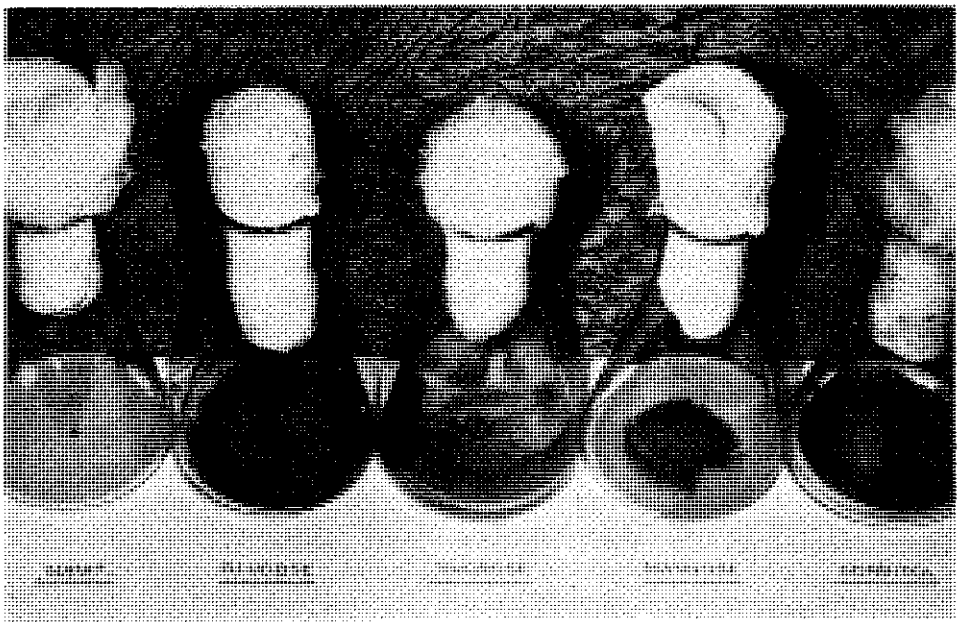


FIG. 6 — Influence of D-mannitol and other sugars on the growth of *Termitomyces* conidia in synthetic agar media.

of these fungi is probably due to their association witb *Termitomyces* conidia which although unable to proliferate into the "garden" substrate, were able to grow when favourable conditions existed.

Fungus combs of *M. subhyalinus* contain relatively smaller amounts of lipids and nitrogen compared to various termite castes as shown in Table 4. The amount of total lipids and nitrogen in fungus combs were inferior compared to those present in soldiers or workers. On the other hand, *Termitomyces* conidia contained the highest amount of nitrogen, similar to that of major soldiers. Termite larvae (three larval stages were combined) contained the highest amount of total lipids, followed by major soldiers.

The lipid composition of fungus combs, conidia, and of various castes of termites were further studied both quantitatively and qualitatively. The results are shown in Fig. 7 and can be divided into 3 groups as follows:

TABLE 4 — *Total lipids and Nitrogen Content of Fungus combs, Conidia, and Various castes of* Macrotermes subhyalinus.[a]

	% *Total Lipids*	% *Total Nitrogen* [b]
Fungus combs	1.8	1.2
Conidia	2.1	7.9
Larvae	11.9	5.7
Major soldiers	9.8	7.9
Major workers	4.3	6.1

[a] Values are averages of 2-3 independent determinations based on dry weight.
[b] From: G. BÜHLMANN (unpublished results).

a) *Lipids of the neuter castes*

Considerable differences in the lipid composition were noted among larvae, major soldiers, and major workers. The amount of total lipids decreased in the same order. The hydrocarbon (HC) content of larvae (29.7 % of total lipid fraction) was found to increase considerably in major soldiers (56.4 %), while it decreased in major workers (5 %). In contrast, phospholipids (PL) and free fatty acids (FA) remained practically unchanged. Major workers contained the highest amount of neutral lipid (NL) (33.4 %) followed, in decreasing order, by larvae (17 %) and major soldiers (8.5 %).

b) *Lipids of the queen fat body and hemolymph*

Both (NL) and (PL) fractions were the predominant lipids of the fat body (39 % and 46.2 % of the total lipids, respectively). On the other hand, (HC) and (FA) accounted for the major lipids in the hemolymph (33.3 % and 17 %, respectively).

c) *Lipids of fungus combs and conidia*

The lipid composition of fungus combs differed considerably from those of the conidia. In the former case lipids were predominantly mono — and triglycerides (both fractions accounted for 74.2 % of the total lipids), while conidia lipids contained, in addition, high amounts of (PL) (25 %) and (FA) (20 %).

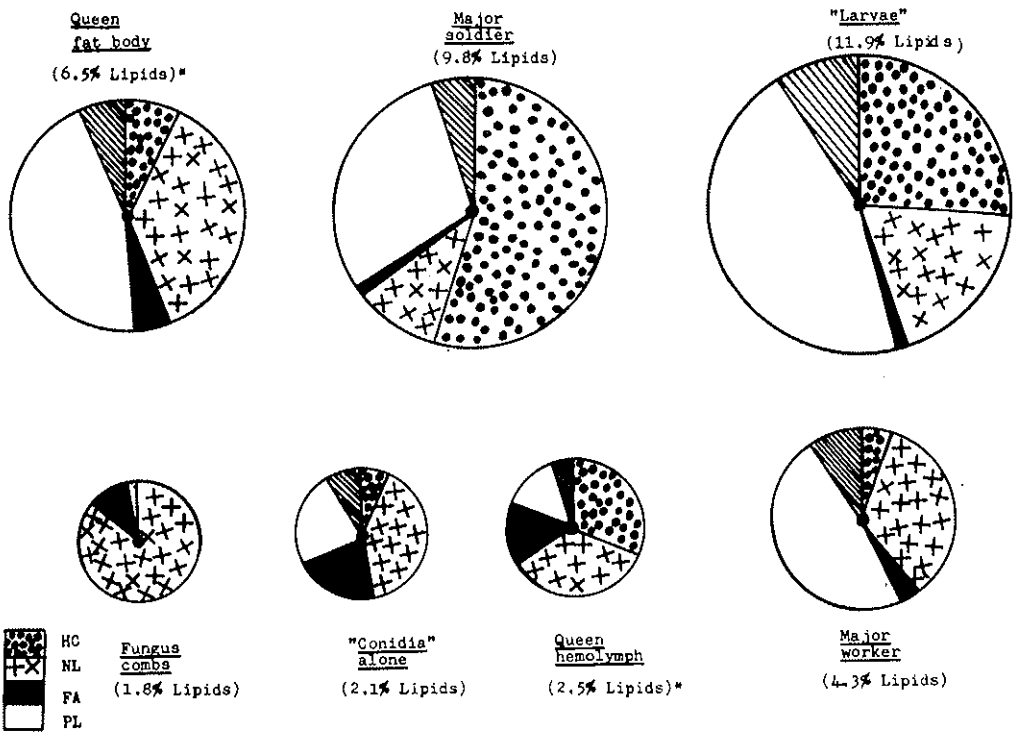


FIG. 7 — Lipid composition of fungus combs, conidia of *Termitomyces* and of various castes of the termite *M. subhyalinus*. HC (hydrocarbons), NL (neutral lipids; a sum of sterol esters, free sterols, tri-; di-; and monoglycerides fractions), FA (free fatty acids), PL (phospholipids).

By cross examining the preceeding results one striking similarity of lipid composition was noted between major workers and the queen fat body.

DISCUSSION

Very little is known about the chemical composition of "fungus gardens" of the sub-family Macrotermitinae. Most researchers in this field agree that they consist mainly of lignin and cellulose but detailed study on its composition is lacking. Therefore, we carried out a detailed chemical analysis of the organic constituents present in the

"fungus-gardens" aiming at the understanding of the very complex problem of termite nutrition.

The water content of fungus combs is relatively high (47 %) compared to that of the surrounding soil. Usually an average size of a *Macrotermes subhyalinus* mound contains about 10 Kg fungus combs which corresponds to 5 Kg of water per mound. This amount of water is significantly less than the amount lost every day. According to WEIR (1973), a small mound of *M. subhyalinus,* having two or three openings, loses as much as 8.5 Kg water/day i.e. 2000 Kg/year. To compensate for such great water loss, these mounds must have access to the water table in addition to the water produced from metabolic activity of termites. WATSON (1972) has estimated that a colony of about one million termites of *M. natalensis* produces about 4 Kg of water per week. To keep a constant humidity level inside the mound, it is possible that fungus combs (provided with its large surface area and its high water-holding capacity) regulate humidity by taking up excess moisture or by releasing it when the atmosphere inside the mound becomes dry (HESSE, 1957).

The ash content of fungus combs of *M. subhyalinus* (12.3 %) is consistent with those reported for *M. natalensis, M. goliath,* and *O. redemanni* as shown in Table 5. It differs, however, from those reported for *M. falciger* and *O. badius.* It is possible to assume that the high ash content reported for the latter group is due to the presence of a certain amount of soil used by termites in constructing their fungus combs.

The lignin: cellulose ratio present in combs of *M. subhyalinus* is found to be considerably higher (2) than that of *O. redemanni* (0.49) and *O. obesus* (0.55) as shown in Table 5. Normally, undigested woody material contains a lignin: cellulose ratio ranging from 0.33 - 0.75 (SANDS, 1969). According to BECKER and SEIFERT (1962), these ratios are roughly reversed in the feces of many termites and the same probably applied to the newly deposited combs. Thus, the high lignin: cellulose ratio in combs of *M. subhyalinus* suggests that digested plant material must have been used for the construction of these combs. This hypothesis is also supported by the fact that these combs contain appreciable levels of uric acid

TABLE 5 — *The Ash, Lignin and Cellulose Composition of Fungus Combs of various Species of Macrotermitinae.*

Species	% *Ash*	% *Lignin*	*Lignin/ Cellulose Ratio*	*References*
O. redemanni	10.7	—	0.49	BECKER and SEIFERT (1962)
O. badius	27.7	23.8	—	CMELIK and DOUGLAS (1970)
O. obesus	—	—	0.55	BECKER and SEIFERT (1962)
M. natalensis	13.0	15.0	—	BECKER and SEIFERT (1962)
M. falciger	23.9	—	—	HESSE (1957)
M. goliath	10.1	43.3	—	CMELIH and DOUGLAS (1970)
M. subhyalinus	12.3	16.3	2.0	Present Study

which was previously shown (HUNGATE, 1941) to be present in termite feces.

One of the major constituents of "fungus-gardens" was the sugar alcohol D-mannitol. This sugar was also identified in "fungus-gardens" of *M. goliath* and *O. badius* (CMELIK and DOUGLAS, 1970). Whether this sugar alcohol was formed by *Termitomyces* as a by-product of fungal metabolism, or originated from plant material is not certain. However, we recently recovered large amounts of mannitol from various grasses and from the bark of *Warburgia ugandensis* (Canellaceae) trees (ABO-KHATWA and KUBO, unpublished observations). Mannitol is also known to be a common constituent of many fungus species (ALTMAN and DITTMER, 1968).

The nutritional value of mannitol to termites is not certain although some insects such as cockroaches, honeybees and silk-worms are able to utilize it in their diets (ALTMAN and DITTMER, 1968). We tried to establish a bioassay method in order to study the influence of feeding mannitol solutions on the longevity of various castes of termites. Preliminary results on termites kept under laboratory conditions for extended periods showed no significant difference between the rate of survival of termites fed on mannitol solutions and those fed on plain water (BÜHLMANN, personal communication). The fact that extracts from termite workers are not capable to oxidize D-mannitol to any extent (Table 3) has further supported our hypothesis about the incapability

of termites to utilize mannitol. Extracts of fungal conidia, on the other hand, oxidize readily D-mannitol. The presence of mannitol dehydrogenase in fungal conidia extracts has been recently established (ABO-KHATWA, unpublished results).

Our results show that the presence of D-mannitol in fungus combs is essential to allow the growth of monocultures of *Termitomyces*, which is often the case in their natural habitat associating with the macrotermitines. Sugars other than mannitol allow spore germination and proliferation of foreign fungi that are probably associated with *Termitomyces*.

An additional function of mannitol may be derived from the fact that dissolving mannitol in aqueous solutions results in heat production or absorption, depending on its final concentration. Table 6 shows the change in heat content ($\Delta\tilde{H}$) or enthalpy when mannitol or sorbitol are dissolved in water at 25°C.

Mannitol at lower concentrations (0.1 - 0.2 mole/Kg H_2O) produces positive (endothermic) enthalpies (i.e. absorption of heat) while at higher concentrations (0.3 - 1.0 mole/Kg H_2O) it produces (negative) exothermic enthalpies (i.e. production of heat). By simple calculations of the amount of mannitol and water in fungus combs of *M. subhyalinus* one reaches a value of about 0.7 mole/Kg water which corresponds to exothermic reactions being carried out in these combs. An additional advantage of the presence of mannitol in the fungus combs is that unlike sorbitol, mannitol possesses a dual effect on either heat production or absorption, depending

TABLE 6 — *Effect of Mannitol and Sorbitol on the Enthalpies (Heat Content) of Water at 25° C.**

Alcohol Concen. mol/Kg H_2O	$\Delta\tilde{H}_2$ *(Cal/mol)*	
	Mannitol	*Sorbitol*
0.1	170	- 80
0.2	140	−220
0.3	− 10	−260
0.7	−180	—
1.0	−230	−310

* From: J.H. STERN and M.E. OCONNOR, « J. Phys. Chem. », *76*, 30772 (1972).

on its final concentration in the combs. This reversible effect could be an important factor in regulating the temperature of fungus combs thereby regulating the temperature inside the mound throughout the year.

Another nutritionally important group of substances present in fungus combs are the nitrogenous constituents. Our results show no significant difference in the nitrogen content of various parts of fungus combs of varying stages of maturity. These findings suggest that fungus combs nitrogen is derived essentially from plant material while fungus tissues contribute very little. The fact that the nitrogen content of fungus combs is significantly much lower (1-2 %) than that of the fungal conidia (7.9 %) indicates that termite requirements for nitrogen are probably obtained from the white nodules (conidia) and not from the fungus combs substrate. An additional source for nitrogen, however, could result from its recycling in termite colonies through the eating of dead and injured members.

Part of the nitrogen compounds found in fungus combs was uric acid (*). The amount of uric acid present in the combs depends upon its stage of maturity; being at high concentration in the newly deposited material (0.66 mg/g dry weight) and at low concentration (0.33 mg/g) in the older combs. It is assumed that the reduction of uric acid concentration during comb maturation is a result of fungal growth. Furthermore, the presence of uric acid in the combs of *M. subhyalinus* suggests that the origin of the building material for these combs is in fact fecal material which has been shown to contain uric acid (HUNGATE, 1941).

The importance of lipids in terms of metabolic and structural function in insects is well documented. Moreover, the diet on which insects feed has a strong influence not only on the total lipid content but also on the relative proportion of lipid classes. Since termite workers are responsible for feeding other castes (larvae, soldiers and reproductives) the quantity and quality of lipids present in these castes ware examined together with those of fungus combs and

(*) CMELIK and DOUGLAS (1970), were not able to detect the presence of uric acid in fungus combs of *M. goliath* and *O. badius*. The enzymatic method used in this study is more sensitive, in determining uric acid, than their murexid reaction method.

conidia. Considerable differences of lipid composition are found between the various termite castes. For example, lipids from major soldiers are mainly hydrocarbons which must have been associated with their heavily sclerotized heads and mandibles. The frontal glands of these soldiers are known to produce enormous amounts of hydrocarbons (mainly paraffins and olefins of C_{23} - C_{31}) (PRESTWICH, personal communication). The presence of large quantities of hydrocarbons in soldiers of a number of Australian termites (MOORE, 1964 and 1969) and of *M. goliath* (CMELIK, 1969a) have also been reported. Major workers, on the other hand, contain small amounts of hydrocarbons; instead, neutral lipids and phospholipids are the predominant lipids.

Lipid composition of queen fat body and that of hemolymph show considerable differences. The fat body is characterized by its high contents of triglycerides (11.4 % of the total lipids) and phospholipids (46.2 %) as compared to the hemolymph (1.6 and 10.7 % respectively). The latter however, contains higher amounts of hydrocarbons (33.3 %) and free fatty acids (16.8 %) than the fat body (6.5 and 3.2 % respectively). Similar results were obtained from studies on queens of *M. goliath* and *M. natalensis* (CMELIK, 1969b).

A striking feature of the lipid composition of the queen fat body is its similarity to that of workers. This finding suggests that lipids present in the saliva of workers when they are received by the queen will be channeled directly to the fat body without major metabolic alterations.

Limited similarities between the composition of lipids from fungus combs or conidia and that from various termite castes do exist. A similar finding was also reported (CMELIK, 1972) concerning the similarity of fatty acids and sterol composition from "fungus-gardens" and that from workers of *M. falciger*. These findings indicate that most of the termite castes, if not all, are capable of modifying the lipid composition of their diets according to their needs.

Based on our findings, we conclude therefore, that "fungus-gardens" seem to contribute little to the nutrition of termites and that they probably provide them only with essential ingredients such as vitamins and nitrogen. The latter requirement should be

obtained mainly from the white nodules (conidia) which are rich in nitrogen content. The main function of "fungus-gardens" associated with macrotermitines however, seems to be concerned with heat and water regulation within the mound and annual fluctuations of mannitol content could be a factor in temperature control. Currently, I am investigating the role of fungus in the degradation of cellulose thereby making it more readily digested by termites. Preliminary results have shown that fungal conidia contain high cellulase activity which remains practically unchanged after storing dried conidia for 6 months (ABO-KHATWA, 1977).

SUMMARY

Chemical analysis was performed on "fungus-gardens" and on various castes of the termite *M. subhyalinus* with the ultimate objective of studying the importance of "fungus-gardens" to the nutrition of termites. A sugar alcohol was isolated from "fungus-gardens" and was identified as D-mannitol by means of spectroscopic and chromatographic methods. Metabolic and feeding studies showed that termites do not utilize mannitol but the latter was found to be essential in supporting the growth of monocultures of *Termitomyces*. An additional function, of mannitol, seems to be concerned with temperature regulation inside the mound throughout the year. The nutritional value of "fungus-gardens" to termites is questionable but might provide them with essential ingredients such as vitamins and nitrogen. The latter requirements should be obtained mainly from the white nodules (conidia) which are rich in nitrogen content. Considerable variations existed between the lipid composition of "fungus-gardens" and those of termites. This finding suggests that in case the termites receive certain lipids from "fungus-gardens" they are capable to modify it according to their needs.

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DISCUSSION

CANONICA

Mannitol seems to be widespread as a constituent of fungi. Many years ago I investigated the chemical constituents of *Sarcodon imbricatum*, a higher fungus frequent on our mountains, and I have found it contains about 20% of mannitol. This fungus is not toxic for tbe mammals, but I could never observe it is attacked by insects.

BELL

There's one point which you made that I should like to ask you about: you said that in these lipids were hydrocarbons ranging in chain length from C-18 to C-25. That is to say a mixture of even-numbered hydrocarbons and odd-numbered hydrocarbons and I would have supposed that the hydrocarbons arose by the decarboxylation of fatty acids and would therefore have been odd-numbered.

Would you like to comment on this?

ABO-KHATWA

I have to make some corrections here. Recently, we have discovered that the frontal glands of soldiers of *Macrotermes subhyalinus* produce high amounts of paraffins ond olefins of chain lengths ranging from $C_{23} - C_{21}$. The biosynthetic pathways of these odd-numbered hydrocarbons in termites have not been worked out.

WAIN

I too was interested in the mannitol question and wanted just to recall that about in 1944, when we were working on the fungicidal action of copper, we had reason to look at the exudate from fungal spores.

The cupric ion is very toxic to plant life and it will kill fungi, but if the copper is precipitated, as in Bordeaux mixture you can control the fungus without killing the plant. We were able to demonstrate that one reason for this is that the fungal sporae exudate can bring copper into solution from the Bordeaux deposit by complex formation. This finding led us to make a comprehensive examination of the exudate produced by spores of *Neurospora sitophila*. To our surprise some 25 per cent of the dry weight of the extract was found to be mannitol.

I would just like to ask Dr. Abo-Khatwa whether his fungus produces any antibiotics? Does it repress the fungal growth of other species that are deliberately introduced?

ABO-KHATWA

We have tried to offer various extracts of this fungus in the drinking water of termite workers that were kept under laboratory conditions for extended periods. This treatment was done in order to suppress the growth of alien microorganisms which often infect termite colonies causing high rates of mortality. We found that these extracts did not inhibit the growth of microorganisms; so it is unlikely that the fungus itself produces antibiotics. On the other hand, if termites are removed from their fungus gardens alien fungi will start growing. Apparently, termites themselves either secrete or excrete antibiotic substances that prevent the growth of foreign fungi. The problem of how termites control fungus growth is indeed a very intriguing one.

SCHILDKNECHT

Five years ago we found in the fungus garden of the leaf cutting ants some substances controlling the fungus in the ants' special glands. Have you ever looked at these glands in the termites too? And in this connection, if you are putting out the termites from the garden, is the garden any longer formed with one kind of fungus?

ABO-KHATWA

The mechanism of constructing fungus gardens by the leaf-cutting ants is different from that of termites. Rather than eating and digesting

the plant material, the ant cuts it into small pieces and adds them to a fungus garden in the nest. When alates start nest founding, the female carries a small pellet of the fungus in a pouch below her mouth parts so that she can use it to inoculate her excreta. In fungus-growing termites, it is generally believed that fungal conidia and/or the fruiting bodies, i.e. basidiospores are carried inside the gut of the swarming alate.

KARLSON

For a biochemist it seems rather unbelievable that these lipids should be transferred from one termite to the other without being digested and the products of hydrolysis absorbed and possibly recombined. So I would rather suspect that it is a coincidence that you find the same lipid content in major workers and in the queen, and that this is different from the other termite casts. I wouldn't believe this until I get very hard evidence that lipids of this size — especially phospholipids — can pass to the fat body through the various membranes without being digested.

ABO-KHATWA

Well as I mentioned early, in a termite colony there are various nutritionally dependent castes such as soldiers, the royal pair... etc. which are not able to feed themselves and must be nourished by workers. In this case some means of trophallaxis such as saliva exchange takes place. We do not know anything about the biochemical composition of this saliva, but indeed it is regarded as a readily digested material. Accordingly, one tends to think that probably the absorption process of the saliva components (lipids, proteins, etc.) by the digestive system of the receiver termite may in fact surpass the secretion of the enzymes that are capable to alter and modify it. This has been shown to be the case in the physogastric queen (*). I fully agree with you that a direct proof is needed to substantiate my claim. Probably radioactive tracers can be used in this case.

(*) NOIROT-TIMOTHEE, C. and CH. NOIROT, « Ann. Sci. Nat. Zool. Biol. Animale » (12) 7, 185, 1965.

KARLSON

This is well known in many species, not only in insects, also in mammals and even in man, that certain types of dietary fatty acids, e.g. unsaturated or branched fatty acids, are stored in the body fat. But the fats and lipids are first digested. Moreover, these termites are certainly able to synthesize fatty acids from carbohydrates; thus, they have their own source of fatty acids to make fat or phospholipids.

BALLIO

I refer to Dr. Bell's question about the odd-numbered hydrocarbons. There is one biosynthetic path which involves the condensation of two fatty acids to give branched β-ketoacids with decarboxilation to give ketone which can be reduced and in that case you lose one carbon atom and you have an odd number of hydrocarbon.

STAAL

I'm just curious whether your fungus ever forms the fruiting bodies of the perfect stage which would enable identification of the species.

ABO-KHATWA

Yes it does. All fungi species belonging to the genus *Termitomyces* fructify early in the rainy season. However, the fruiting bodies or the basidiospores are not allowed to grow from fungus gardens since they are being actively tended by termites.

STAAL

In that case the determination of the identity of the fungus species should not present much difficulty.

ABO-KHATWA

This is a mycological problem which should be looked at.

MARINI-BETTÒLO

I think that we should in a certain way study more thoroughly these fungi, trying to obtain submerged cultures in order, to have a greater production of the ir metabolites. And surely there may be some active or promoting substances which may give the reason of many facts that now doesn't come so easily out because of the rough presentation of the analytical data, only based on the content of glucides and lipids.

KNÜSLI

Are these fungus gardens an absolute necessity for the survival of termites? In other words, has it been possible to create fungus-free termite colonies?

ABO-KHATWA

Some species of higher termites are not associated with fungus such as the grass feeding termite *Trinervitermes* but others such as members of the subfamily Macrotermitinae do have a truly symbiotic nature with fungi. We have succeeded to keep incipient colonies of *M. subhyalinus* without fungus garden i.e. fungus-free for a period of nearly two years; after which the colony was not able to survive.

KNÜSLI

The background behind my question is: can you administer fungicides which kill the fungi so that as a second reaction you have also a depression of the termite population?

ABO-KHATWA

Yes, it is an idea but here again we are trying to avoid as much as possible the use of pesticides. Another possibility however, is spraying the inner part of the mound, where the fungus gardens are located, with sugar solutions. This treatment in theory will allow the growth of a variety of foreign microorganisms that might interfere with the symbiotic relationship between termites and their specific fungi.

GILBERT

Just a brief comment on the hydrocarbons. We looked at the fungus of three different nests of Attasexdens in three different areas and hydrocarbons were found — even and odd ones — the odd hydrocarbons in the region C_{25} to C_{31} — the dominant ones; and we found that in one place where the ants were cutting only one species of plant these four odd hydrocarbons also occurred in leaves of the species on which the ants were growing the fungus. But the whole set of even hydrocarbons from about C_{16} — if I remember rightly — through the C_{36} also occurs. In the higher region, above C_{30}, the even hydrocarbons tend to be about as abundant as the odd ones, whereas in that middle region — C_{25}, C_{30} — the odd ones are almost exclusively present. There are branched ones also in the higher region, whereas below C_{30} there are linear ones by the Mass spectrum. It's quite a complicated story and it is not the same story from one nest to another nest. I feel that you really have to work on natural fungus in an analysis of this type. I would prefer to do so rather than on cultured fungus because the ants in the case — probably the termites too — contribute quite a lot to what is going on, I am sure.

BELL

One comment — I may have missed this, but it is a propos of the last remark too — and that is that the termites apparently require fungus, but do you find the fungus growing in the wild without the termites?

ABO-KHATWA

I am not aware of such cases.

CHAPMAN

Just for information on this point of the fungicide, Dr. Wood in Nigeria is in fact looking at this and one of the key problems of course in administering anything like this, is finding a bait that the insect will take back with the pesticide on it — this is one of the things they are looking at specifically, at the moment. We have, incidentally, just appointed a research fellow to look at the biology of termitomyces and she will be based in Nigeria, but will no doubt come to Kenya too.

NAKANISHI

It is not a direct comment of your studies, but what was the other name of the Dutch Research Scientist working with Glenn?

ABO-KHATWA

Mr. O. Bruinsma.

NAKANISHI

When Glenn Presswhich who is mainly working with Jerry Meinwald did this beautiful work with Bruinsma, they identified the queen building pheromone. They have a beautiful picture of the termites building galleries. The pheromone turned out to be palmitoleic acid plus other regular fatty acids.

They sent it to Nature but for some reason it was turned down by the referees. I thought it was an absolutely fascinating work. Although more has to be done regarding vapor pressure, etc.; as Abo-Khatwa mentioned they have to keep a distance from this drop of palmitoleic acid. They build the galleries in a circle with a 2 centimeter distance from the droplet of fatty acids, a fascinating story.

SOMERVILLE

I have a general question, on nitrogen economy. You suggested that these fungi might be involved in supplying nitrogen, but can you in fact do a nitrogen balance within the community? — is it possible that the mannitol is supporting nitrogen-fixing microorganisms?

ABO-KHATWA

There is no evidence in literature that *Termitomyces* fungi or temites belonging to Macrotermitinae are able to fix nitrogen. Only few numbers of subterranean lower termites possess nitrogen fixing ability. The sugar alcohol D-mannitol is known to be assimilated by a variety of microorganisms particularly fungi species belonging to basidiomyces.

SOMERVILLE

I do not know enough about the situation to be able to comment in an informed way. At first sight it would appear that you might be short of nitrogen going into the community and that it might be a possible use for the sugar as an energy source for nitrogen-fixing microorganisms. Has anybody tried acetylene reduction tests?

ABO-KHATWA

A nitrogen balance sheet for a termite colony should give the answer, but to obtain such a sheet one has to know also how much nitrogen is recycled through the eating of dead and injured members. The acetylene reduction method as an indicator of nitrogen fixation has been tried before and was found to be a reliable method.

STAAL

I have a question about the philosophy of the control of termite species that build these big mounds. Is it not true that the main damage by these termites is the mechanical obstruction presented by these mounds for agriculture? If one is going to control the termites by bringing the bait into their nest or immediate surroundings, one is still faced with that big mound which is as hard as a brick and would need mechanical destruction. However, if one destroys it mechanically with the termites in it, the termites are probably gone as well. Therefore, if one would have to do that anyway, why try to kill the termites first? Continued tillage would not allow rebuilding of mounds anyway. Besides, these mounds are mainly built out of very small soil particles that appear to be a considerable source of fertility for the land if they are disintegrated and spread out.

ABO-KHATWA

The main damage caused by mound-building termites in semi-arid regions of Africa and Asia is that they feed on herbaceous plants and grasses thus competing with herbivotous mammals including man. In addition, they are known to compete with man for all of his timber-work. Aside from this, the physical presence of these mounds presents a serious

obstacle to cultivation as they impede the mechanical preparation of the land. The destruction of termite mounds by mechanical means and removing the queens has been practiced for a long time. Although this method can give a significant reduction in termite numbers it is not the most effective since in many cases these colonies can manage to replace the queen and the egg-laying machine continues. Much has been said on the suitability and the degree of fertility of termite mounds for crop growing. Obviously the growth of crops on these mounds, or on areas where they have been levelled and mixed with soil depends upon the type of crop and on the physical and chemical properties of the mounds and soils. At ICIPE we have recently appointed a soil chemist to look into this problem.

STAAL

I just thought that the destruction of mounds would destroy that termite nest pretty effectively, and I may be wrong, but I have seen a lot of agriculture in Africa on places where these mounds formerly were and they seem to be practically eradicated in these fields, without any other means than mechanical.

CHAPMAN

If I could come back at Dr. Staal here, I think one of the key points here is actually that one may not be concerned in fact with killing off the micro-termite that you are talking about, but the same principles can be applied to species which are entirely subterranean. The thing is that when you develop agriculture you obviously totally owe it to the termite fallout and you eliminate a lot of spaces, as you say, by digging up the mounds. But in fact there are species of microtermites which then increase a hundred-thousandfold. And most of the species of microtermes are non-moundbuilders, so you don't know they're there until they come and eat your crops, so that you really need to get at them in some way...

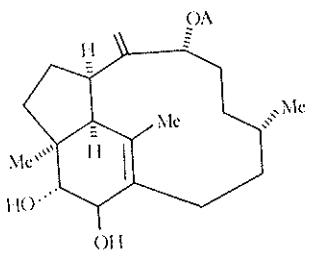
NAKANISHI

Yes but one more comment on Glenn's work and then I would like to ask you a question. He is now scrutinizig all sorts of termites in

Africa, and one set of compounds he has found in the secretion is the trinervitenes mentioned earlier. The colonies from several species have been sent to Cornell and now Tom Eisner will be studying them in more detail.

When the soldiers are attacked by ants, for example, they shoot out their defense secretion which is filled up in the heads.

In the particular case of *Trinervitenes gratiotus,* the soldiers shoot out a defense secretion, for a distance of 5 cm, aimed at their predators, the ant which is much longer in size. Glenn Prestwich at ICIPE has analyzed this. Tom Eisner at Cornell is very interested in the things and they are going to do a systematic study of these secretions. The sticky substance in the defense secretion is mostly monoterpenes. But also diterpernes. The secretion strangles the ants, and they become immobile and are killed. I will first write the structure, just for chemists maybe,



of one of them; it is a very complicated structure. The secretion contains a series of these compounds, about 6 or 7, which constitute a new class of compounds which we have named trinervitenes. You may be interested to know that from the few milligrams that were available, we were able to clarify all the structural moieties, except that we could not exactly place three methylene groups and the angular methyl group. Carl Djerassi has a computer program called CONGEN which gives one all possible combinations of structural moieties. When the information on this diterpene was given to the computer, the outcome was over 1,000 structures!

But finally a miniscule amount fortunately could be obtained as cristals and hence the structure was done by X-rays by John Clardy. These substances are produced only by the soldiers and not by the workers. It may represent a new defense mechanism but the major constituent is according to Tom Eisner of Cornell not toxic at all.

Well coming back to another question, I wanted to ask you something about the enthalpie of fungus combs. I do not want to start difficult physico-chemical problems: you said that you have a negative enthalpie when you dissolve that amount of mannitol in water but I don't see how you can explain the function it plays in regulating the temperature. Have you thought about this?

ABO-KHATWA

The dynamic nature of fungus gardens through the continuous removal of older parts and depositing new ones by termites must have some influence on its final composition of water and mannitol. This continuous process could cause significant changes in the enthalpies of fungus combs. Any changes in the temperature of fungus combs themselves should have some influence on the temperature inside the mound. We must however, not overlook other heat sources such as those from the metaholic activities of fungi and termites.

MARINI-BETTÒLO

Well, I think that we have gone far enough with the discussion and we must think it over with this new problem posed by termites as a model.

IV

PRODUCTS INVOLVED IN PLANT PARASITE INTERACTION

Natural products and plant disease resistance
Phytotoxins

CHEMICAL ASPECTS OF PLANT DISEASE RESISTANCE

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England

There have been notable advances in recent years in the control of plant diseases which, however, still cause extensive losses of human and animal food. Although bacteria and viruses contribute towards these losses, it is the fungi which cause the greatest damage. These are the pathogens that cause the blights, wilts, scab and mildew diseases of fruits and vegetables, the rust and smut diseases of cereals and root rots that affect many crops.

Plant diseases have taken their toll right through the ages. The "blasting" in the time of Moses, recorded in the Bible, was the same rust disease described by the Romans, which still causes enormous losses today.

One of the most widely known disasters due to plant disease was that arising from the failure of the potato crop in Ireland in 1845 and later years. This resulted from the destruction of the potato haulm and tubers by the fungus *Phytopthora infestans* — potato blight. The consequences of potato blight in Ireland during the "hungry forties" were serious indeed — no less than a million people died of starvation and one and a half million emigrated. Many other disasters could be quoted. A prosperous coffee industry in Ceylon was wiped out by rust diseases, and banana production in several countries has been seriously affected, if not eliminated by the fungus causing Panama disease (*Fusarium oxysporum*, var. *cubense*).

But disasters such as these are by no means the whole story, for attack by plant diseases is going on all the time, not necessarily destroying most of the crop, but seriously affecting yield and quality. In unsprayed orchards, attack by apple scab (*Venturia inaequalis*) leads to blemished fruit of low market value. Blighted potato haulm depresses the yield of tubers and these may then rot in the clamp.

Scientific research has provided chemicals with which to attack many plant diseases and thereby increase the yield of crops. The importance of this and other developments in the field of crop protection cannot be over emphasized, for in this troubled world of political unrest and economic hardship the most deep-rooted and serious problem is undoubtedly that of world food shortage. Indeed, if it were possible to divide equally the total available food in the world, everyone would go hungry. Furthermore, the problem gets more acute each year, for not only is the population of the world increasing rapidly, but advances in medical and chemical science give each person a greater expectation of life. With this world situation it is clearly of paramount importance that crop losses due to diseases and pests should be reduced to the very minimum.

Apart from cultural operations, proper crop rotations, use of seed and planting material free from disease and so on, there are two important methods which can be used to achieve this objective.

To produce a variety of a crop plant which is resistant or immune to a particular disease is a most effective and inexpensive method of crop protection from the farmer's viewpoint, particularly if the yield and quality of the new variety are satisfactory. Much attention is being given to this important aspect of crop protection. It has been established that resistance towards a number of plant pathogens, including certain fungi and bacteria and viruses, are heritable characters in that they can be transferred from parent to offspring in accordance with the principles governing the inheritance of morphological characteristics. With many diseases, however, suitable parental material possessing resistance has not yet been found. Another difficulty in breeding for resistance to fungal diseases is that, in many cases, resistance to not just one but a large number of physiologic races of the fungus, each with its own specific parasitic properties, must be incorporated in the resistant variety. Furthermore the fungus, by mutation and sexual reproduction, has

the capacity to produce new races, many of which possess a high degree of virulence. In this way a crop variety which, when first introduced by the plant breeder, shows great promise may all too quickly become susceptible to the disease. This variability in host-parasite relationships ensures that both host and parasite have the opportunity to survive without either becoming dominant; however, it complicates the problem man has to face in seeking his food supplies. The research worker in this sphere can never relax; he must fully understand the biology of the pathogen and keep a close watch on its changing behaviour.

For many years now, chemicals have been available to control plant diseases. Although some of these materials are applied to the seed or to the soil, most of them are used on the plant itself. Some are able to eradicate fungal infections such as the powdery mildews, which are restricted to the surface of the leaves. Most of the fungi that attack plants, however, penetrate the leaf cuticle and ramify through the tissues. It is extremely difficult to eradicate these infections once they have become established, and against this type of fungal attack chemicals with a protective action are usually employed. They must be applied to the leaves before the fungal spores are likely to reach the plant; once the spore has germinated and penetrated the host tissues, the fungicide is powerless. All such chemicals applied to plants should injure the cells of the fungus without damaging those of the host plant, that is they should not be phytotoxic. The copper preparation Bordeaux mixture fulfills these requirements and, over the years, it has proved to be a protective fungicide of first rate importance.

A protective fungicide is subject to removal from the plant by weathering. Not only this but only those parts actually covered by the deposit are protected against fungal pathogens and all new growth is liable to be attacked. These disadvantages, however, can be overcome by using a systemic fungicide, that is, a compound which, being taken up by the plant, acts directly or indirectly on the pathogen within the tissues.

As is well known, spectacular successes have been achieved by chemotherapy in the control of certain human and animal diseases using such materials as sulphonamides and antibiotics. The plant, however, differs markedly from the animal in that it lacks a central

circulatory system with blood and lymph to transport the defensive materials. The distribution of any applied chemical throughout the tissues of a plant following application to roots, leaves or stem is a slower process depending largely upon diffusion from cell to cell. In spite of this limitation, the control of certain plant diseases with chemicals acting systematically has been successfully accomplished and a number of materials are now used commercially for this purpose.

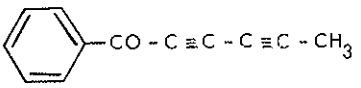
A pyrimidine derivative, dimethirimol, discovered at Jealott's Hill Research Station in England, was one of the first effective synthetic compounds. It was found to give a spectacular control of powdery mildew on the leaves and stems of cucumber, a single application to the soil providing control for ahout six weeks. A related compound ethirimol gave good control of powdery mildew of barley when applied as a seed dressing. Two other compounds, carboxin and oxycarboxin, were discovered in Canada. Carboxin is effective as a seed dressing against loose smut of barley and wheat, neither of which is controlled by organomercurial dressings, and oxycarboxin shows promise against wheat rust. Benomyl ("Benlate") from the Du Pont organization in the USA is highly active against various powdery mildews. However, it is a good fungicide as well as a systemic fungicide and it has been found to perform well against many other fungal diseases of vegetables, flowers and fruit. Amongst other synthetic systemic fungicides, triforine and tridemorph are worthy of mention; both are effective against powdery mildews as is the formamide systemic fungicide (N-(2,2,2-trichloro-1-methoxyethyl)-formamide) discovered in the writer's laboratory [10].

At the present time the main uses of systemic fungicides seem to be the control of powdery mildews, loose smuts and certain rust diseases. They are able to provide protection, to eradicate established infections and, in some cases, can control deep seated pathogens which have previously not been accessible to chemicals. Most of the evidence, however, indicates that whereas the compounds move upwards in the transpiration stream, downward movement does not readily occur. Their use on trees and other woody plants has been disappointing. With their extensive root systems these are not only more difficult to treat through the soil but translocation does not seem to occur so readily with woody as with annual plants.

There is now considerable evidence that fungi which were originally susceptible to commercial systemic fungicides are developing resistance towards them. This is now creating serious problems; indeed in some cases as, for example, when the chemical is used repeatedly in a confined area such as a glasshouse, control may become negligible. Such a situation has in fact already arisen in cucumber mildew control with a number of the commercial compounds which originally were highly effective.

In our laboratory we are following another approach to plant disease control which is based on the fact that in nature, most plants are completely resistant to most of the fungal pathogens to which they are continuously exposed during their growth. Vines, for example, though susceptible to downy mildew disease, are immune from potato blight, cereal rust, apple scab and so on. Thus, throughout the plant kingdom, resistance is the rule rather than the exception. Although such resistance can sometimes be related to morphological characteristics such as a waxy leaf epidermis, there is evidence that natural resistance to infection may be associated with the presence of protective chemicals within the plant cells.

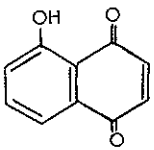
Naturally occurring fungicides include a wide range of chemicals of diverse structure. Micro-organisms produce many antifungal compounds. One of these, griseofulvin — an antifungal metabolic product of *Penicillium griseofulvum P.*, is absorbed and translocated in plants and is active as a systemic fungicide [33]. Many antifungal compounds occur in plants and it is of interest to note that leaves of certain species were used for treating fungal infections of man long before the presence of any fungicidal component was suspected. *Artemisia capillaris* plants, for example, used for many years by paddy field workers to control fungal infections of the feet [34], have been shown to contain the potent fungicide capillin:



Capillin

Numerous phenolic compounds are found within the tissues of plants and since many of these possess fungicidal properties they

may operate in the complex of factors associated with disease resistance. Such compounds have been intensively studied in recent years, [16, 22, 25]. The group is a wide one embracing phenols, phenolic acids, their glycosides and sugar esters and many other classes of compounds possessing one or more phenolic groupings in the molecule. Among such compounds possessing high fungicidal activity is the quinone 5-hydroxy-1,4-naphthoquinone (juglone) isolated from the leaves and pericarp of walnut [20] in which it occurs as a glycoside of the corresponding hydroquinone.

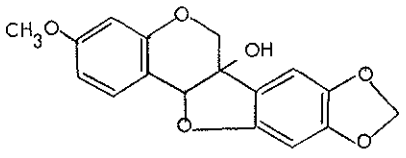


Juglone

Research on phenolic substances in relation to plant disease resistance has centred not only on the possible protective effects of preformed phenolic compounds but also on whether these substances are mobilised or their synthesis is promoted at the site of infection. Another aspect which has received much attention is that the phenolic compound might be liberated from its glycoside or sugar ester at the infection site in response to infection [21, 27]. Chemical changes in infected tissue due to the activity of polyphenoloxidases and peroxidases leading to the production of quinones and other fungicidal compounds have also been investigated [22].

A new group of naturally occurring fungicides has been discovered in recent years from research on the so called phytoalexins. These chemicals, which will also be discussed at this meeting by Dr. CRUICKSHANK, are fungitoxic substances which are produced in plants in response to fungal infection and they arise from effects on specific metabolic systems of the host. The phytoalexins therefore fit in with the concept that defence against invasion is a dynamic process which becomes initiated when the pathogen has made contact with the tissues of the host plant. The existence of phytoalexins was predicted by MÜLLER and BÖRGER [26] in 1940 and since that

time a number of these compounds have been isolated and their structure determined. Of these, the chromano-coumaran, pisatin, has received much attention.

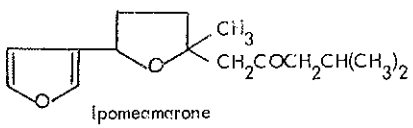


Pisatin

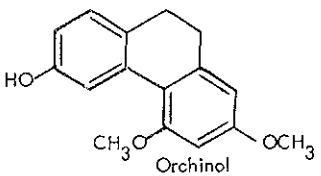
Healthy tissues of pea (*Pisum sativum*) contain little or no pisatin. The compound was obtained by CRUICKSHANK and PERRIN [11] from the diffusate obtained by inoculating the pod cavities with a spore suspension of *Monilinia fructicola* and incubating under high humidity at 20°C for 40 hours. The structure of pisatin was determined by PERRIN and BOTTOMLEY [29]. It has a wide antifungal spectrum and its formation is stimulated in pea pods by a range of fungal species whether these be pathogens or not [11]. It has been suggested that pisatin plays a central role in the immunity of pea plants to fungi which are not pathogenic towards this host. Thus, CRUICKSHANK and PERRIN [11] found that while non-pathogens of pea are very sensitive to pisatin, pathogenic fungi are not; they suggested that resistance arises when infection stimulates phytoalexin production in the tissues at a concentration above the level at which fungal growth is inhibited. Susceptibility, on the other hand, was considered to reflect either an inability of the fungus to stimulate enough phytoalexin or a capacity of the fungus to tolerate the chemical [11]. Studies made by DE WITT ELSHOVE [15], however, indicate that pathogens of pea such as *Fusarium oxysporum* f. sp. *pisi* and *Mycosphaerella pinodes* are pathogens because they are able to metabolize pisatin whereas other fungi, not pathogenic to peas, possess no such detoxicating mechanism and their growth is therefore inhibited by the pisatin present in the host tissues.

Other phytoalexins include ipomeamarone, a sesquiterpenoid compound isolated from sweet potato roots infected with black rot (*Ceratocystis fimbriata*) and orchinol and tririncol isolated from two

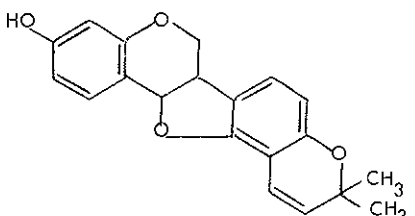
genera of *Orchidaceae*. A phytoalexin produced in French bean and also in pea, is phaseollin which, like pisatin, has a chromano-coumaran structure [28]. Carrot produces a phytoalexin with an isocoumarin-type structure and rishitin, which is a phytoalexin with a terpenoid structure, has been isolated from potato tuber tissue infected with fungal pathogens.



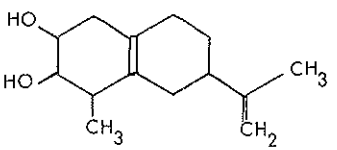
Ipomeamarone



Orchinol



Phaseollin



Rishitin

Although phytoalexins are synthesized and accumulated by plant tissues in response to the presence of pathogenic and non-pathogenic fungi, phytoalexin formation can be stimulated by other means, in absence of a fungus. Thus, for example, pisatin formation is induced by droplets of water in which spores have previously been suspended. Chemical, mechanical and heat treatment, and anaerobic storage of host plant tissues have also been shown to promote the synthesis of the phytoalexin. All these "stress" factors then, both biological and non biological, can initiate the change in host plant metabolism upon which phytoalexin formation depends. This suggests that the production of phytoalexin might arise from a shift in an already existing biosynthetic mechanism; indeed, the initiation of a completely new synthetic pathway involving specific enzymes is not possible as it would require the operation of new genes.

It would be logical, therefore, to expect traces of the phytoalexin to occur in the normal uninfected plant, as well as substances which can yield the phytoalexin in response to the activation or suppression of simple enzyme reactions which are already established

within the host. In the case of pisatin PERRIN and CRUICKSHANK [30] showed that many inorganic and organic chemicals, including metabolic inhibitors, can initiate the formation of the phytoalexin and they concluded that pisatin production results from a metabolic derangement which may be due to the inactivation of a sulphydryl-containing enzyme.

The detection and isolation of phytoalexins has recently been greatly simplified by the use of a new technique whereby plant tissue extracts are subjected to thin layer chromatography. The developed chromatogram is then sprayed with spores of an appropriate fungus suspended in a nutrient solution after which the chromatogram is held at high humidity in an incubator for several days. During this period the darkly pigmented fungus grows over the plate except in those areas where the antifungal compounds are situated. The results of a typical assay are shown in Plate 1 which were obtained using extracts of healthy and infected cowpea stems. It can be seen that the phytoalexins are produced in response to infection and are not normally present in the healthy tissues. Such properties suggest that plants, like animals, may resist disease by producing defensive chemicals which can restrict the growth of the invading pathogen. A number of studies have been made to determine whether the levels of phytoalexins which can build up within the plant's tissues are high enough to confer fungicidal protection [1, 13, 23, 37].

The results of these studies all indicate that phytoalexin production represents an important means by which disease resistance operates. This conclusion is further supported by an investigation carried out in the writer's laboratory, in which further attention was given to phytoalexins present in bean. In addition to phaseollin first reported by PERRIN in 1964, three more phytoalexins — phaseollidin, phaseollidinisoflavan and kievitone — were isolated. These antifungal compounds were found to be produced only in infected tissues and are not present in the surrounding healthy cells [4]. Furthermore, it was found that the cells of cultivars which showed natural resistance to infection contained high levels of the phytoalexins whereas those of cultivars in which the pathogen (*Colletotrichum lindemuthianum*) was spreading, contained extremely low levels. It was concluded that the accumulation of phytoalexins was

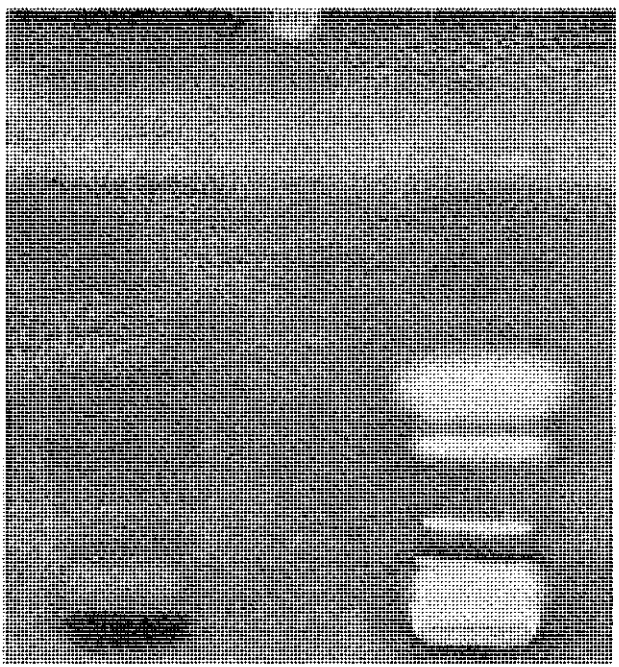


PLATE 1 — Thin layer chromatograms of extracts of (*on left*) healthy and (*on right*) diseased cowpea tissues after spraying with a suspension of fungal spores and incubating the plate. The presence of antifungal compounds is shown by the white areas where the fungal growth has been inhibited.

more than sufficient to account for the inhibition of fungal growth within resistant tissues.

Another development in this field is the demonstration in our laboratory that infecting a plant with a virus disease can also lead to the formation of phytoalexins. Examples of this are capsidiol and glutinosone, both of which have been extracted from virus-infected tobacco leaves [2, 8]. The benzofuran phytoalexin vignafuran [32] and 2'-O-methyl-phaseollidinisoflavan [31] are both found in cowpea after infection with tobacco necrosis virus; kievitone and phaseollinisoflavan are other phytoalexins which are produced in beans, together with phaseollidin, after infection with this same virus [7] (Fig. 1). It is clear therefore that phytoalexins can operate

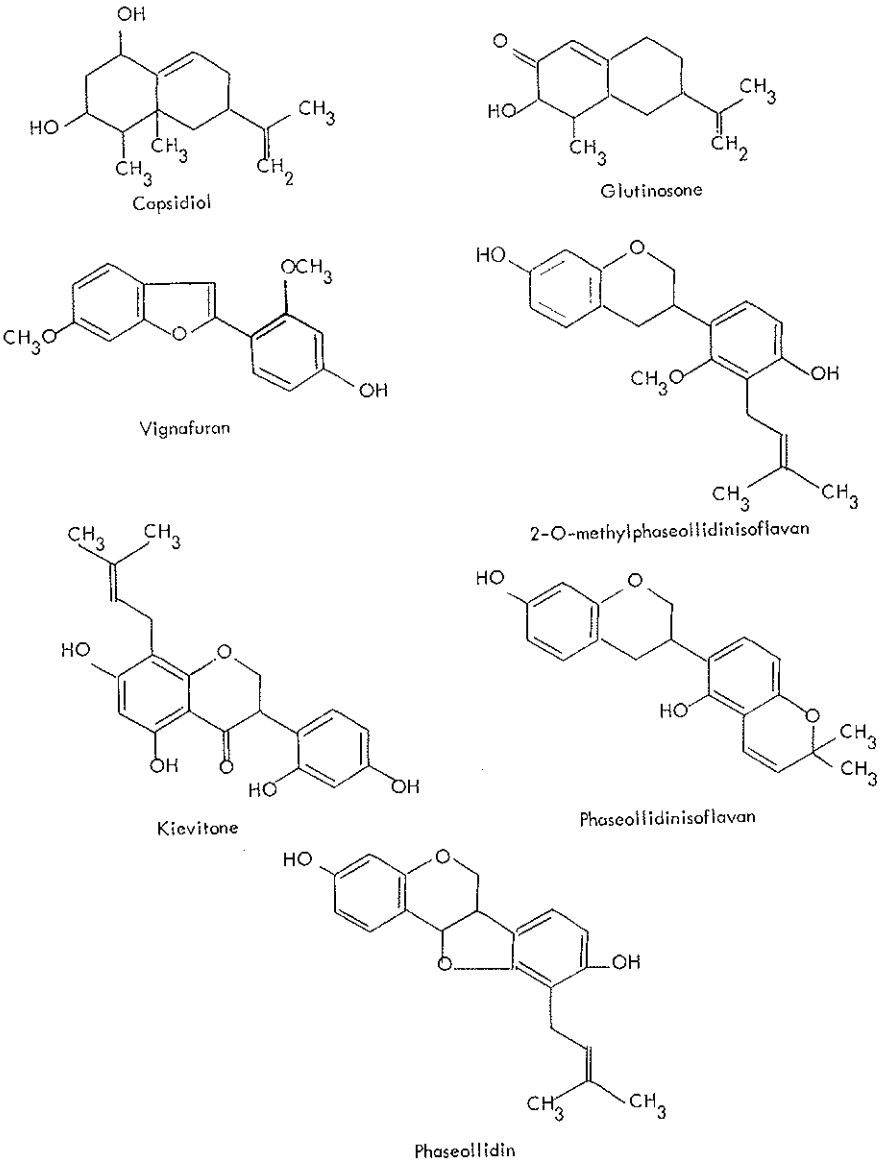
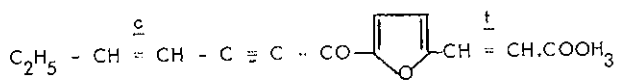


FIG. 1 — Formulae of some phytoalexins.

in determining resistance and susceptibility of plants towards disease. Whatever their precise function may be, however, they can only represent one of the complex of factors operating in disease resistance and immunity. Any protective substances present in the healthy plant, for example, must be important in this connection.

In an investigation carried out in our laboratory, a potent antifungal compound was isolated from healthy seedlings of broad bean (*Vicia faba*). The first indication that antifungal compounds are present in these seedlings arose from an observation that fungal growth on nutrient agar was inhibited by the presence of segments of healthy stem or root tissue [35]. Evidence was obtained that the antibiotic effect observed was due to a phenolic substance and a hydrophilic compound containing nitrogen, sulphur, and phosphorus. A highly antifungal lipophilic substance was also shown to be present and our main investigation was directed towards the isolation and identification of this substance. Its isolation from 8-day old seedlings was eventually achieved using the following procedure. The macerated seedlings were extracted with benzene at 20°C and the extract was transferred to columns of acid-washed neutral dry silica gel. Fractions obtained by a graded elution procedure were monitored in a spore germination test with *Alternaria brassicicola*. The active material from the columns was obtained pure by repeated crystallization from hexane. The compound (10 mg per Kg. of seedlings), had a melting point of 63.5-64°C and ran as a single spot on a thin layer chromatogram. It was identified as methyl-β-2-(hept-4'-enyl-1'-oxo-2'-ynyl)-furan-5 acrylate and given the name wyerone. Full details of its chemical analysis, together with data relating to its ultra violet, infra red, proton magnetic resonance, and mass spectra have been reported [18, 19]. The compound has also been synthesized.



Wyerone

Wyerone was shown to have a wide fungicidal spectrum when tested against phytopathogens in spore germination tests [17]. It

showed high *in vitro* activity against a range of fungi. Against *Alternaria brassicicola*, for example, the ED_{50} was only 3 ppm. However, it was some 40 times less active than this against *Botrytis cinerea* (ED_{50} 125 ppm.). Thus *A. brassicicola*, which does not affect broad bean plants, is readily controlled by wyerone, whereas *Botrytis spp.*, which cause the well known chocolate spot disease, are not.

Can we conclude then that wyerone, a preformed natural fungicide, affords protection to broad bean seedlings from many of the fungi to which they are exposed in the field? While such a protective mechanism might operate, the situation is much more complex. Firstly, there is the possibility that mechanical and physical factors operate in determining whether resistance is shown. The leaf cuticle of *Vicia faba*, for example, might impede or prevent the penetration of certain fungi; the role of pectolytic and other enzymes at the infection site or cellular membrane changes must also not be overlooked. Protective chemicals, either exuded from healthy leaves into the thin film of moisture on the leaf surface or produced there as antibiotics from leaf saprophytes, might also provide a defence mechanism by inactivating enzymes and inhibiting metabolic reactions of the invading pathogen.

That fungicidal compounds are present in the washings of healthy leaves of a number of plant species has been demonstrated [38] and these might be a factor in determining whether the spores of some fungal species can germinate on the leaf surface. A further means by which natural protection of broad bean might arise is by the formation of the phytoalexin when the plants are in contact with the spores of certain fungi [12, 14].

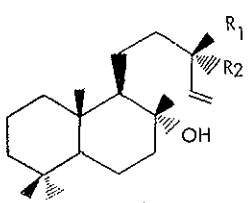
In addition to all these possible defence mechanisms we have wyerone and the unidentified phenolic and hydrophilic compounds present within the tissues, all of them having marked antifungal properties. Not only this, but small quantities of a dihydroderivative and the corresponding secondary alcohol of wyerone are also present in bean seedlings [18]. Both of these are fungicidal, the former showing similar properties to wyerone, the alcohol being less active.

Since *Vicia faba* alone gives rise to such an array of naturally occurring fungicides it is clear that the number of defensive chemicals occurring within the plant kingdom must be phenomenally large.

Recent work at Wye College has opened up another potential source of such compounds. This came from speculations by the writer on why plant roots growing in soil or compost usually remain healthy although they are exposed to a wide range of bacteria and fungi which can readily destroy dead root tissue. Such considerations led us to investigate the chemicals exuded by pea and bean seedling roots into the water in which the seedlings were growing. It was found in both cases when the water extracts were run on t.l.c. plates that antifungal compounds were present [9]. Thus it is clear that the living root operates to defend itself against attack within the hostile environment of the soil.

Amongst the known phytoalexins, one finds considerable variations in chemical structure, legumes usually producing compounds of the isoflavanoid type and solonaceous species tend to produce sesquiterpenes. Some of these naturally occurring fungicides taken from one plant have been shown to protect other plants against fungal attack. This has been done, for example, with the solonaceous phytoalexin capsidiol which is highly active against *Phytophthora infestans*. When the compound was applied to tomato plants at 100 ppm prior to inoculation with *P. infestans*, it provided almost complete protection, the control plants being completely destroyed after seven days [39].

Another example of the use of a naturally occurring fungicide in plant protection is provided by "sclareol" — an epimeric mixture of sclareol and 13-epi-sclareol. We have shown that this mixture of diterpenes, which is present in the exudate which occurs on the surface of healthy leaves of a tobacco (*Nicotiana glutinosa*), will prevent the germination of rust spores at low concentrations [5].



Sclareol

Sclareol: R_1 = OH, R_2 = Me

13-epi-sclareol: R_1 = Me, R_2 = OH

This finding led us to examine its use as a rust fungicide. When applied as a spray to broad bean, dwarf bean or wheat plants at 100

ppm it provided almost complete protection against certain rust diseases [3].

These examples illustrate possible agricultural uses for these sophisticated organic fungicides which occur naturally within the plant kingdom. Whilst it is unlikely that sufficient of these materials could ever he obtained from plant sources for extensive field application, these naturally occurring compounds can often be synthesised. This has been done, for example, with pisatin [6], wyerone [18], rishitin [24], vignafuran [32] and orchinol [36].

The synthetic approach can also be used to prepare analogues closely related to the naturally occurring fungicide. Not only might these be of agricultural significance but they might he of value in controlling fungal pathogens of man and animals. *Aspergillus fumigatus* for example, can infect the lung and outer ear and this same pathogen is one of a range of *Aspergillus* species which, like *Candida albicans* and *Fusarium solani*, can cause fungal infection of the eye. Such cases of keratomycosis are fortunately not common hut they are extremely difficult to treat with existing fungicides because these chemicals are often irritant or toxic to the delicate tissues of the eye. Ocular treatment with naturally occurring fungicides, however, might be more successful and it is for this reason we have arranged to have our new compounds of this type, together with some of their synthetic analogues, examined for their capacity to control fungal infections of the eye.

It can be seen from this account that studies on the chemical basis of plant disease resistance are considerations not only of academic but also of practical significance. With the modern techniques now available and the attention being given to this area of research we can look forward to interesting developments.

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DISCUSSION

HEIMPEL

I have a comment, a suggestion and an offer to make to you. It occurred to me that I have not heard, for some time, so much enthusiasm or such imaginative research.

I go to my specialty: *Bacillus thuringiensis*, an insect pathogen, has varieties that produce a toxin called the β-exotoxin. As Dr. Somerville told you, it is a nucleotide, with a phosphorus hooked on to it, and this toxin is completely soluble in water and is autoclavable. The toxin only affects multiplying or dividing cells. For example, a Swedish scientist demonstrated that this toxin completely inhibits the bacterium *Sarcina flavus*. I suggest you try this toxin, as a systemic poison, against rapidly multiplying fungi. I am willing, if you wish, to supply you with experimental lots of the toxin to test in your system.

WAIN

Thank you very much for this suggestion.

WILLIAMS

May I ask how these things work? Do any of them perhaps inhibit the synthesis of chitin?

WAIN

No, but this a very good question. Now if you want to seek a systemic fungicide you try to synthesise a chemical which will interfere with some biochemical mechanism which is vital to the fungus, but which is not vital to the plant. You ask yourself what are the differences between fungi and plants? Take one example, plants undergo photo-

synthesis — so if your chemical interferes with chlorophyll biosynthesis, you would kill the plant not the fungus — but that is the wrong way around, unfortunately! But your question, Professor Williams, is appropriate in another direction because fungal cells all contain chitin. So if you can interfere with chitin biosynthesis you should control the fungus. However chitin as far as I know, is not important in the green plant which should therefore remain unharmed. This has been a logical approach in the search for systemic fungicides for some time. Your question as to how our new compounds act, cannot yet be answered. There are many of them and their mode of action, metabolism, biosynthesis and so on, are receiving attention in various parts of the world. However this involves much work and each chemical has to be investigated separately.

WILLIAMS

I do hope that you will screen these materials routinely for effects on chitin synthesis because as was mentioned I think this morning, these antichitin materials like 60-40 and so on have vast potential as insecticides.

NAKANISHI

What is the structure requirement, generally speaking, for the action of these compounds?

WAIN

For entry into the plant and systemic activity it would seem that you must have both lipophilic and hydrophilic groupings in the molecule. One of the best systemic compounds, a very simple molecule, is 2-4-D. This compound has got both lipophilic and hydrophilic groupings and this applies also to most of the growth promoting benzoic and phenylacetic acids. One cannot generalise because there are systemic compounds which do not have these properties but these considerations are useful in the search for new systemic chemicals.

NAKANISHI

I should like to know more about the plant's growth hormones and their relations with the antifungal substances produced by the plants.

WAIN

As you probably know, my research group in England is concerned with two main projects: one is studies on the hormonal control of plant growth, which occupies about two thirds of our effort — what I have been talking about today, that is the chemical basis of plant disease resistance makes up the other third. So we are very much in the hormone field, and I can assure you that much is known about the complex of hormones and hormone inhibitors which control plant growth and development. The main hormones, as you know, are auxins, gibberellins and cytokinins. Then you have two endogenous hormone inhibitors — abscisic acid and xanthoxin; xanthoxin was discovered in my laboratory.

NAKANISHI

Excuse me, I was speaking of the mode of action.

WAIN

Oh, I see. Now if you are talking about the mode of action, studies have heen made with each of these hormones and inhibitors but about how the complex operates at cell level, very little is known.

KARLSON

I was very intrigued by your example about the coffee, where you have different varieties, one synthesizing phytoalexins.

I think tbat combining genetical and biochemical techniques we may have a new approach for the production of resistant strains of plants.

In effect it should not be difficult using tissue culture methods to develop in test tubes plant strains which contain phytoalexins, thus avoiding field tests.

WAIN

Thank you for those remarks. Yes, our approach could be extended more widely. For example, suppose you are growing a plant to obtain some compound of pharmacological importance — taking a simple example; suppose you wanted nicotine from tobacco. You can easily grow undifferentiated callous tissue from tobacco stem explants in tissue culture. If nicotine is synthesized in the callous tissue then why bother to grow tobacco in the field?

Talking about tissue culture research I must tell you about a recent discovery we have made. Firstly, I would remind you that when a plant grows, hormones are produced in the growing tip and these diffuse downwards and cause the cells below to get bigger. As the cells get bigger, the plant gets bigger... and this is growth. The hormones which do this are the auxins and gibberellins, both of which promote extension growth. Other hormones, the cytokinins which are nearly all 6-substituted adenine derivatives, promote cell division. If you place a suitable piece of plant tissue on a sterile culture medium, with all the necessary nutrients, hormones, sugars and so on but no cytokinin, then no growth occurs on incubation because cell division cannot occur in absence of cytokinin. If however, you add cytokinin to the medium, growth occurs and you get a mass of undifferentiated callous tissue. We have recently made a cytokinin which is not an adenine derivative which, when used in this way with tobacco, promotes the tobacco tissue to grow and develop into small plants. So here you get morphological differentiation, as well as cell division.

CANONICA

Concerning the biosynthesis of these phytoalexins, this can be interesting also from a practical point of view. The infection stimulates the formulation of phytoalexin; this means it is able to form a chain of enzymes which synthesize the phytoalexin. We can imagine two different hypotheses about the starting point of this chain. We can imagine that the stimulation can be ab initio stimulation. So the biosynthesis can be stimulated starting from very simple precursors. Another hypothesis can be: can the biosynthesis utilize compounds like flavonoids, which are normally present in the plant tissues?

WAIN

I think research in the phytoalexin field at the biochemical level is not keeping pace with the discovery of new phytoalexins. I showed this afternoon 4 or 5 new ones and Dr. Cruickshank will no doubt be able to add to the list. It is so easy now, you see, to isolate them using the thin layer chromatography technique which I described. I have always been convinced that defensive compounds are present in plants but our early attempts to separate them from leaf tissue using ordinary extraction and non-chromatographic procedures gave disappointing results. The simple technique using thin layer plates has revolutionized this whole area of research. We have recently shown in our laboratory that plant roots can also be a source of defensive chemical. All roots in the soil are surrounded by a vast range of bacteria and fungi — but except in rare cases, the living root is immune from attack. Now suppose we remove the plant from the soil and kill its roots by dipping them in boiling water for a short time. If we now put the plant back in the soil, the dead root tissue will be rapidly destroyed. As I explained in my talk, this led to experiments in which bean and pea seedlings were grown with their roots in water. When the water was extracted and the extract examined by the thin layer chromatography technique, several antifungal compounds were isolated. Thus, the living root is able to defend itself in the soil environment.

GILBERT

I just wanted to ask a little bit more about sclareol. You said that sclareol could be obtained from tobacco but that you would not grow tobacco to make it. But is there not sufficient plant material left from the tobacco industry to be used for preparing it as a by-product?

WAIN

Yes, when we published our results in Nature, I received several letters from tobacco manufacturers, and as a result I have on my shelves about 3 kg of sclareol sent by them. Certainly they get sclareol as a by-product and they did not know what to do with it. But there is still not enough to treat the wheat belt of Canada for example!

SIDDAL

I simply meant to follow on Prof. Nakanishi's question, wondering why there is relatively little knowledge of the molecular mode of action of plant hormones. I suspect that the answer is the complexity of the system in plants because five hormones generally interact with each other and the study of one of them in isolation could be relatively meaningless. Is this true, or is there another reason?

WAIN

In studying mode of action, you have got to study firstly, whether your chemical can get into the tissue; to do this it has got to penetrate various membrane barriers. We have a small biophysics group studying nothing but these problems. Once it gets into the cell, the molecule has got to survive the barrage of plant biochemistry that is imposed upon it — it must not be metabolized and broken down until it reaches its site of action; having got there, we presume that the molecule must possess certain structural requirements to produce its biological effect. We have been working on chemical structure/biological activity relationships since 1945. As a result, we can design new active plant growth regulators by incorporating features which are essential for activity into the molecule. However, we still know very little about the basic mode of action of these substances at cell level. All studies on the control of plant growth by chemicals have enormous agricultural importance as well as being of great academic interest and significance. If I may say so, a study week such as this, devoted to this area of hormone research would be most stimulating and useful.

BALLIO

I am referring to the point raised by Professor Nakanisbi and further commented by Dr. Siddal, namely the mode of action of plant hormones. I feel that one of the reasons why research in this field lags behind that on animal hormones is to be found in the fact that, in general, physiological and biochemical studies with plant tissues started later than with animal tissues. Nevertheless, we are now witnessing a period of very active interest in this area, and the rate at which results

are produced is steadily increasing. For instance, it is now established that auxins, cytokinins and abscisic acid induce large effects on the electrogenic transport of protons and monovalent cations. The manner in which these hormones interact with membrane transport is presently under very active consideration and important results have been recently obtained. I should like to know your opinion on that.

WAIN

I would like if I may, in answering that question, to take an example not related to plant diseases, but again, back into the auxin field. When the plant is growing, its growth is controlled by hormones — auxins, gibberellins, and cytokinins are the three known at present. Also influencing growth are two hormone inhibitors: xanthoxin and abscisic acid. Now the more you study a growing plant the more you marvel at the ordered way in which things happen. When a plant is subjected to water stress, or to physiological stress of any kind, it takes defensive action, just as it does in regard to disease. If you take a tomato plant, growing with an adequate water supply, it will have say, one unit of abscisic acid in its leaves. If however you withhold water, as soon as the wilting syndrome is evident that is, it is visibly wilting, the level of abscisic acid in the leaves has increased over 50 times.

This abscisic acid inhibits auxins, gibberellins, and cytokinins and growth is suppressed. The plant does not utilize its energy in growing during this critical period of its existence — its energy is conserved. Furthermore, this same abscisic acid closes the leaf stoma so that loss by transpiration is cut down. In Mexico they have given a good deal of attention to breeding new varieties of maize. Some of these are rich in their content of lysine and therefore more nutritious. Another of their maize varieties, which they have called *Latente*, shows resistance to drought.

I brought back some of the *Latente* seed from Mexico and one of my Mexican research students has been working with it in our laboratory. He has shown that seedlings of *Latente* have a much greater capacity to produce abscisic acid when subjected to water stress, than do other maize varieties which are not drought resistant. A similar finding has been obtained with sorghum — the drought resistant variety we studied produced abscisic acid much more rapidly when wilted than did the

varieties which are not tolerant to drought. So here is a defense mechanism which a plant uses when subjected to physiological stress — in this case shortage of water. Our more recent investigations have shown that the abscisic acid defense mechanism also operates when the plant roots are subjected to waterlogging. We have already seen that plants can defend themselves against diseases and all these effects arise from the production of specific chemicals within the plants.

A REVIEW OF THE ROLE OF PHYTOALEXINS IN DISEASE RESISTANCE MECHANISMS

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MARSHALL WARD [201] in England and NOEL BERNARD [15] in France first recognized the possibility of a plant's response to an invading fungal pathogen as being partially responsible for the restricted growth of the pathogen in resistant plant tissues. These ideas were formalized some 30 years later with the publication by MÜLLER and BÖRGER [122] of the Phytoalexin Theory of Disease Resistance which emphasized the dynamic nature of host-pathogen interactions and the involvement of fungal-elicited rather than constitutive host metabolites in the toxicity syndrome concerned with the control of fungal growth *in vivo*. MÜLLER [121] in a subsequent discussion of the above theory defined phytoalexins as "antibiotics which are produced as a result of the interaction of two metabolic systems, host and parasite, and which inhibit the growth of microorganisms pathogenic to plants". Several authors have redefined the term. A recent one is that of INGHAM [81] which states that phytoalexins are "post-infectional metabolites whose formation involves either gene de-repression or activation of a latent enzyme system". As the original theory was written in terms of inhibition of fungi in hypersensitive (resistant) tissue, I prefer to define phytoalexins as fungal-elicited host-metabolites with antifungal activity formed in hypersensitive tissue which play a primary role in the inhibition of fungal growth *in vivo*. This definition, in my view does not limit formation of phytoalexins to hypersensitive tissue,

but does put the emphasis on their occurrence in host tissues which are resistant to fungal diseases.

In this review I shall limit my discussion as far as possible to papers which have been published in the last 5 to 10 years on host-pathogen interactions involving fungal pathogens only. In my view there is as yet little evidence for the inhibition of viruses or bacteria by known phytoalexins even though the same plant metabolites have been demonstrated in tissues infected by these microorganisms [11, 109, 110]. I shall further limit my review to studies undertaken within the Leguminosae; the Solanaceae and the Convolvulaceae as genera from these families have been extensively studied and the collective information is more complete than in other families.

Chemical nature of phytoalexins

Pisatin (XVI), rishitin (XXX) and ipomeamarone (XXVII) were the first compounds in the above families of the phytoalexin class to be isolated, characterized and chemically identified. They occur in fungal infected tissues of pea, white potato and sweet potato respectively. Many post-infectionally formed compounds have now been isolated and chemically identified from several genera within the families from which these early examples have been drawn (Table I, II, Fig. 1). Sufficient data for some but not all of these compounds have been presented to justify their inclusion in the phytoalexin class. Some may be precursors of phytoalexins, others may be fungal metabolites of phytoalexins, others again which have been detected only towards the end of the normal incubation period of the disease in susceptible hosts may be merely compounds associated with symptomology. Some of these compounds may have little functional role as compounds primarily associated with the inhibition of fungal growth in disease resistance. It is of interest that most phytoalexins are chemically and probably biogenetically related to known classes of constitutive secondary metabolites of the botanical family to which their host genera belong. For example, in the Leguminosae the pterocarpanoid phytoalexins are chemically related to isoflavonoids which are well-known to occur in healthy tissues of many

TABLE I — *Post-Infectional Compounds: Leguminosae.*

Fungal Species	Disease Reaction *	Compound	Compound Number	References Isolation	References Structure
(a) Birdsfoot trefoil (*Lotus corniculatus* L.)					
Helminthosporium turcicum Pass.	I	Sativan	I	83	18
» »	I	Vestitol	II	83	18
(b) Broad bean (*Vicia fabae* L.)					
Botrytis fabae Sard.	C	Wyerone	III	65	51
» »	C	Wyerone acid	IV	45	107
» »	C	Wyerone epoxide	V	66	66
B. cinerea Fr.	I	Wyerone	III	65	
» »	I	Wyerone acid	IV	45	
» »	I	Wyerone epoxide	V	66	
Phytotophthora megasperma Drechs. var. *sojae* A.A. Hildb.	I	Wyerone	III	93	
B. cinerea	I	Medicarpin	VI	67	68
(c) Cowpea (*Vigna unguiculata* (L.) Walp. s. lat.)					
Phytophthora vignae Purss.	I & C cv.	Kievitone	VII	139	22
Colletotrichum lindemuthianum (Sacc. & Magn.) Bri. & Cav. (races)	I & C cv.	2'-Methoxyphaseollidin isoflavan	VIII	147	147
» »	C	Medicarpin	VI	106	
» »	C	Vignofuran	IX	148	148
(d) French bean (*Phaseolus vulgaris* L.)					
Monilinia fructicola (Wint.) Honey	I	Phaseollin	X	37	142
C. lindemuthianum (races)	I & C	Phaseollin	X	12	
M. fructicola	I	Phaseollidin	XI	42	145
C. lindemuthianum	C	Phaseollidin	XI	42	
» »	I & C cv.	Phaseollinisoflavan	XII	11	22

* I = Incompatible host-pathogen interaction (resistant).
C = Compatible host-pathogen interaction (susceptible).

TABLE I — *Continued.*

Fungal Species	Disease Reaction *	Compound	Compound Number	References Isolation	References Structure
Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. & Hans.	C	1a-Hydroxyphaseollone	XIII	73	75
» »	C	2'-Methoxyphaseollidin isoflavan	XIV	193	193
C. lindemuthianum	C	6a-Hydroxyphaseollin	XV	42	23
Rhizoctonia solani Kühn	C	Kievitone	VII	172	
(e) Jack bean (*Carnavalia ensiformis* (L.) DC.)					
P. megasperma var. *sojae*	I	Medicarpin	VI	93	
(f) Pea (*Pisum sativum* L.)					
Ascochyta pisi Lib.	C	Pisatin	XVI	34	143
M. fructicola	I	Pisatin	XVI	34	
F. solani (Mart.) Sacc. f. sp. *pisi* (F. R. Jones) Snyd. & Hans.)	C	4-Hydroxy-2,3,9-trimethoxypterocarpan	XVII	151	150
» » » »	C	3-Hydroxy-2,9-dimethoxy pterocarpan	XVIII	151	150
» » » »	C	2,3,9-Trimethoxypterocarpan	XIX	151	150
M. fructicola	I	Maackiain	XX	175	183
(g) Lucerne (*Medicago sativa* L.)					
Stemphylium botryosum Wallr.	C	Medicarpin	VI	78	
H. turcicum	I	Medicarpin	VI	78	
» »	I	Sativan	I	83	
(h) Red clover (*Trifolium pratense* L.)					
H. turcicum	I	Medicarpin	VI	79	
» »	I	Maackiain	XX	79	
(i) Soybean (*Glycine max* (L.) Merr.)					
P. megasperma var. *sojae* (races)	I & C cv.	Glyceollin	XXI	100,140	21,108

* I = Incompatible host-pathogen interaction (resistant).
C = Compatible host-pathogen interaction (susceptible).

TABLE II — *Post-Infectional Compounds: Solanaceae and Convolvulaceae.*

Fungal Species	Disease Reaction *	Compound	Number Compound	References Isolation	References Structure
(a) Jimson weed (*Datura stramonium* L.)					
M. fructicola	I	Lubimin	XXII	208	91,179
» »	I	4-Hydroxylubimin	XXIII	208	91,180
» »	I	Capsidiol	XXIV	208	54,17
» »	I	2,3-Dihydroxygermarene	XXV	208	180
(b) Sweet pepper (*Capsicum frutescens* L.)					
M. fructicola	I	Capsidiol	XXIV	177	
F. oxysporum Schlecht f. sp. *vasinfectum* (Atk.) Snyd. & Hans.	C	Capsidiol	XXIV	177	
» » »	C	Capsenone	XXVI	178	180
(c) Sweet potato (*Ipomea batatus* L.)					
Ceratocystis fimbriata Ell. ex. Halst.	I & C cv.	Ipomeamarone	XXVII	1	104
» »	I	Ipomeamaronol	XXVIII	28	29
» »	I	Dehydroipomeamarone	XXIX	132	133
(d) Tomato (*Lycopersicum esculentum* Mill.)					
Phytophthora infestans (Mont.) de Bary	I	Rishitin	XXX	165	89
(e) White potato (*Solanum tuberosum* L.)					
P. infestans	I	Rishitin	XXX	164	
» »	C	Rishitin	XXX	119	
» »	I	Rishitinol	XXXI	90	90
» »	I & C	Lubimin	XXII	121	
» »	I	4-Hydroxylubimin (syn. Oxylubimin)	XXIII	90	
» »	I & C	Phytuberin	XXXII	198	26
C. fimbriata	I	Phytuberin	XXXII	198	

* I = Incompatible host-pathogen interaction (resistant).
C = Compatible host-pathogen interaction (susceptible).

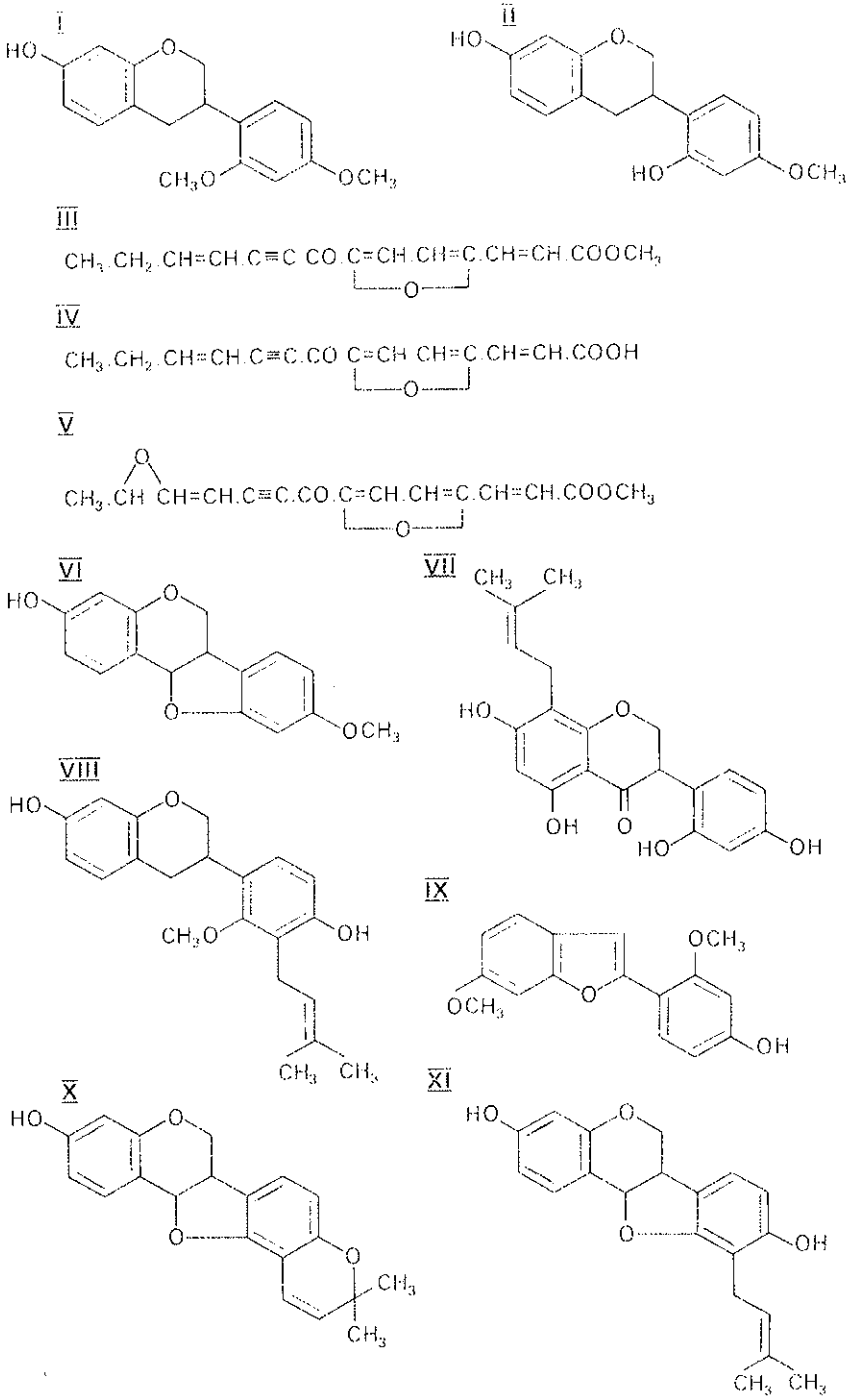


FIG. 1 — Chemical structures of post-infectionally formed compounds in the Leguminosae, Solanaceae and Convolvulaceae.

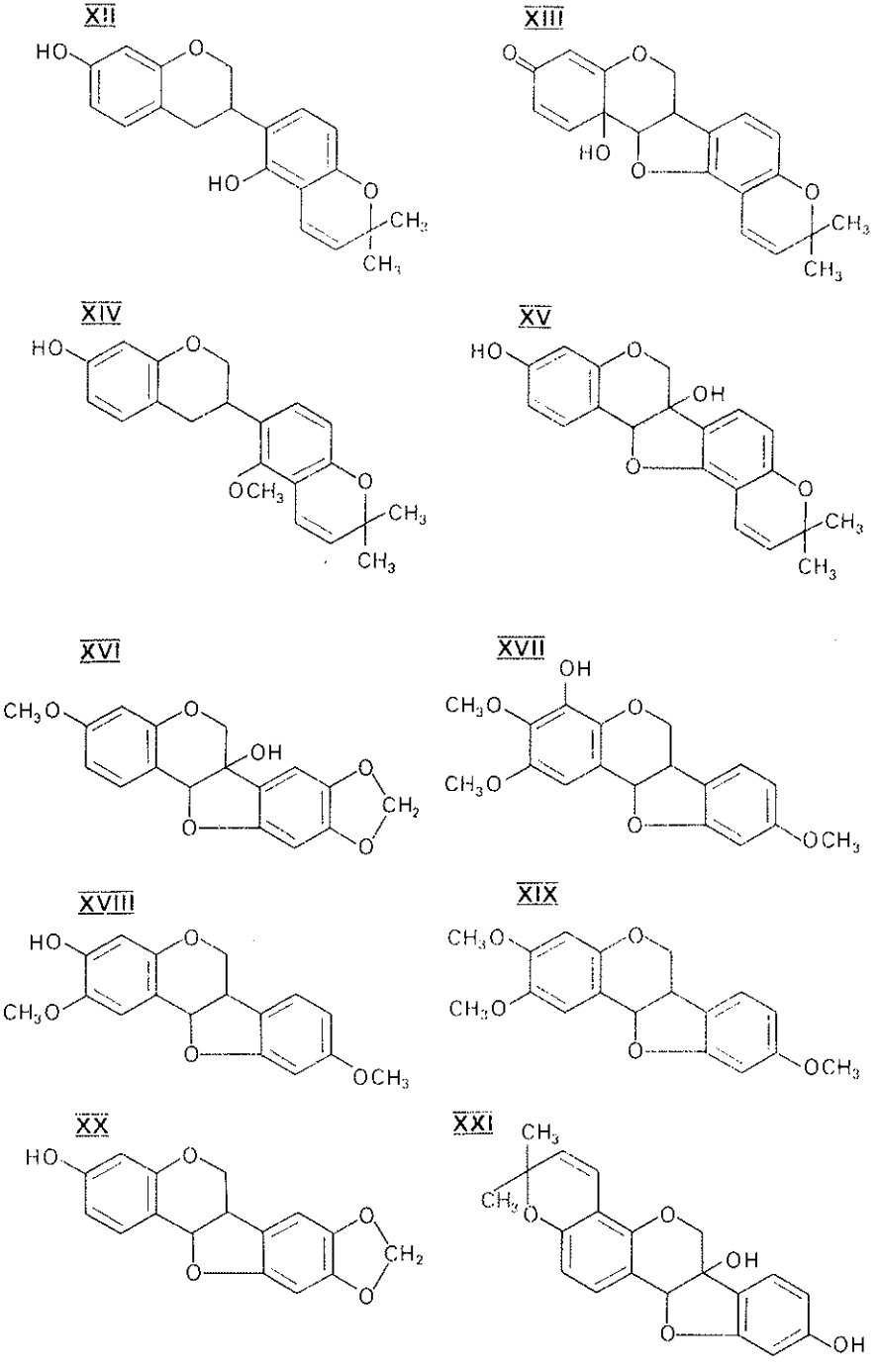
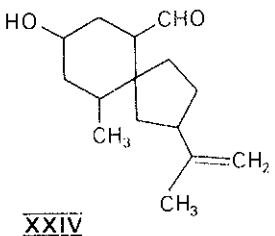
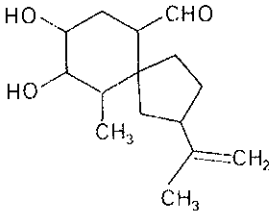


FIG. 1 — (*Continued*).

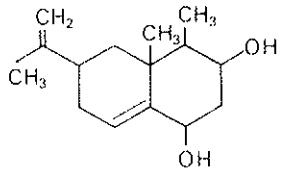
XXII



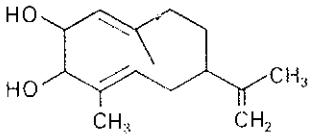
XXIII



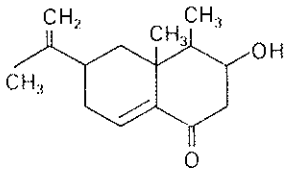
XXIV



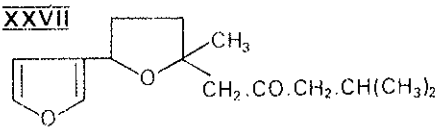
XXV



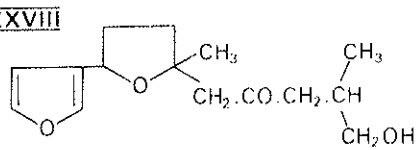
XXVI



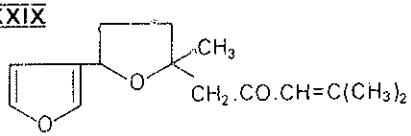
XXVII



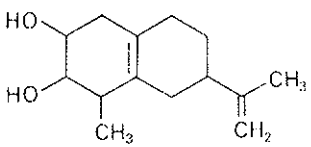
XXVIII



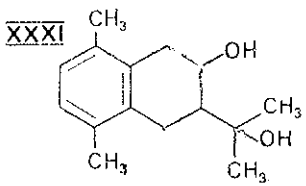
XXIX



XXX



XXXI



XXXII

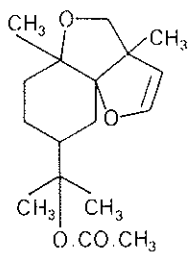


FIG. 1 — (*Continued*).

legumes. Similarly, terpenoids to which most of the phytoalexins of the Solanaceae belong, are well-known constitutive compounds of the potato family.

It was originally believed that phytoalexins may be host-specific; however, as more genera have been studied, it has become apparent that this can no longer be substantiated. To the contrary, the same compounds have been isolated in several instances from several genera. It is now clear that although one compound may be the dominant phytoalexin associated with a particular host-pathogen interaction, for example phaseollin (X) in French bean tissues infected with *Colletotrichum lindemuthianum* and rishitin (XXX) in white potato tissues infected with *Phytophthora infestans*, other compounds may also occur. The quantitative relationships between such groups of compounds however are not constant under all conditions. They may change with time and treatment [42] as shown in studies of the time-course of phaseollin and phaseollidin (XI) accumulation following either fungal inoculation or abiotic treatments of French bean endocarp tissue. They may also change as a function of the pathogen [195, 196]. For example, a comparison of the isoflavonoid compounds in French bean hypocotyl tissue infected with *Fusarium solani* f.sp. *phaseoli* with those accumulated in identical tissue infected with *Rhizoctonia solani* revealed a marked contrast. Phaseollin, phaseollidin and 2′-methoxyphaseollinisoflavan (VIII) accumulated to high levels in the former situation, while kievitone (VII) was not detected. In the latter case, kievitone accumulated soon after infection and both it and phaseollin reached high concentrations. Phaseollidin and phaseollinisoflavan (XII) occurred in small amounts while 2′-methoxyphaseollinisoflavan was not detected.

These studies emphasize the dynamic nature of the relationships and inter-relationships between not only the several antifungal products but also the metabolic processes involved in their biosynthesis. They also indicate the unique nature of each interaction. As stated in an earlier review [30] the occurrence of more than one phytoalexin within a single host species makes the potential complexities of such systems infinite.

CHEMICAL NATURE OF FUNGAL METABOLITES INVOLVED IN STIMULATION OF PHYTOALEXIN BIOSYNTHESIS

In spite of the importance in host-pathogen interactions of the role of fungi and products elaborated and secreted by them during the infection process, the role of fungal metabolites has received relatively little attention from workers in this field. Several early reports [36, 129, 186] indicated that cell-free extracts either from germinated conidia or fungal cultures elicited the formation of phytoalexins in the tissues to which they were applied. The property of crude and partially purified components of cell-free extracts in this regard has been confirmed in recent studies [44, 52, 94, 97, 119, 198]. However, the isolation and purification of the biologically active compounds in the extracts has proved to be a more difficult problem.

Simple treatments in some of the earlier papers such as heat inactivation and acetone precipitation of biologically active fractions suggested that perhaps macromolecules were involved. A partially purified preparation from mycelium of *Phytophthora megasperma* var. *sojae* race 1 was reported [52] to have a molecular weight of 1 to 3×10^4. Chemical tests were consistent with its being a glycopeptide. METLITSKII *et al.* [119] demonstrated that the maximum activity in an extract of germinated zoospores of *P. infestans* in relation to rishitin (XXX) and lubimin (XXII) formation in potato tissues was associated with a protein fraction. In more recent studies [44], the pisatin (XVI) elicitor activity of fractions obtained from filtrates of *F. solani* cultures was shown to be stable to deoxyribonuclease (DNase) and ribonuclease (RNase) but considerably reduced by pronase suggesting that at least some of the activity was due to proteinaceous components. KEEN [94] attempted purification of glyceollin (XXI) elicitors from culture fluids of three races of *P. megasperma* var. *sojae* by gel-filtration. Several partially purified fractions were obtained of relatively low molecular weight. With the possible exception of this report, macromolecules such as polypeptides, glycoproteins or proteins were implicated.

In studies involving cell-free extracts of *Monilinia fructicola*, two fractions with elicitor activity were isolated [39]. The major

fraction after further purification was shown to be a highly water-soluble sulphur-containing polypeptide with *ca.* 65 amino acid residues (mol. wt *ca* 8×10^3). This compound, designated monilicolin A, was obtained using French beans and phaseollin (X) as the indicators of phytoalexin activity. Monilicolin A was active at low concentrations [Minimum Effective Dose (M.E.D.) = 2.5×10^{-9} M, Medium Effective Dose (ED_{50}) = 8×10^{-9} M] as an elicitor of phaseollin. However, it was not active as an elicitor of phytoalexins of the pea or broad bean. This property, namely phytoalexin elicitor specificity is of special interest as it was not exhibited by *M. fructicola* or by crude cell-free extracts of this fungus. It would be of interest to know if a closely related polypeptide capable of eliciting pisatin but not phaseollin would have been isolated if peas and pisatin had been used as the indicator of phytoalexin activity (cf. 182).

Extensive biological and chemical studies in the area of elicitors have been reported recently by ALBERSHEIM and co-workers [2, 3, 6, 7, 8]. These authors discuss isolation and bioassay procedures associated with the extraction, identification, and biological activity of fungal metabolites potentially involved as elicitors of phaseollin and glyceollin from *C. lindemuthianum* and *P. megasperma* var. *sojae*, respectively. In the studies on *C. lindemuthianum* [2, 3] the most purified preparations contained polysaccharide molecules ranging in mol. wt from 0.7 to 1.5×10^5. The predominant glycosyl residues in preparations from both culture filtrates and mycelial walls were 3- and 4-linked glucose units. The M.E.D. of this polysaccharide able to elicit phaseollin on sliced surfaces of bean cotyledons was *ca.* 10^{-7} M. In the studies on wall-released metabolites of *P. megasperma* var. *sojae* four polysaccharide fractions were shown to have glyceollin elicitor activity [8]. The polysaccharides present were heterogeneous in size ranging in mol.wt from 1×10^3 to greater than 1×10^5. Fraction I was composed of branched β-1,3 glucan units. Fractions II and IV were composed of a highly branched mannan-containing glycoprotein while fraction III contained a mixture of the two polysaccharides attached to protein. The M.E.D. for fraction I able to elicit glyceollin on cut surfaces of soybean hypocotyl, calculated from the data of AYERS *et al.* [6], was 2×10^{-9} M (ED_{50} = 4×10^{-9} M), an activity level very similar to that previously reported

for monilicolin A as a phaseollin elicitor. The average relative elicitor activities of fractions I-IV were 100, 16, 25 and 7 and the percentage glucose contents were 95, 13, 30 and 10. Based on these studies, it appears that both proteinaceous compounds and polysaccharides extracted from pathogenic fungi can be effective elicitors of phytoalexins.

Host-pathogen specificity and phytoalexin elicitors

Host-pathogen specificity is a well-known, but little understood phenomenon in plant pathology. In an experiment reported by Metlitskii *et al.* [120], seven potato cultivars of known genotype were inoculated with *P. infestans* race 0. Data obtained from an analysis of the inoculated tissues for rishitin (XXX) and lubimin (XXII) showed a good correlation between the concentration of these two phytoalexins and the disease reaction of the cultivars. In a second experiment, the potato cultivar Lyubimets (R_1-genotype) was inoculated with race 1 and race 1.2.3.4. of *P. infestans* and similarly treated with cell-free filtrates of these two races obtained from zoospore suspensions. In both situations, a good correlation between phytoalexin concentration and the resistance of the cultivars to the two races of *P. infestans* was obtained. Similar correlations between the disease reactions of the soybean cultivars Harosoy and Harosoy 63 to races 1, 2 and 3 of *P. megasperma* var. *sojae* and the concentrations of glyceollin (XXI) formed in hypocotyl tissues of these cultivars treated with cell-free extracts of these races have been observed [94]. In another study [44] extracts of culture filtrates of *F. solani* isolates, which differed in pathogenicity towards peas, were shown to differ in their pisatin (XVI) eliciting potential.

In the above examples, with one possible exception [94], there appears to be little qualitative difference among the elicitor fractions prepared from the various races within a pathogenic species. This conclusion is supported by recent studies [6, 7] which have shown that polysaccharide elicitors prepared from culture filtrates and cell walls of races 1, 2 and 3 of *P. megasperma* var. *sojae* are chemically and biologically identical. They appear to be neither race nor cultivar specific.

In research on the isolation and identification of elicitors, fungi have been cultured on conventional media designed for optimal fungal growth for incubation periods of 3 days to 3 weeks. No attempt has been made to take into consideration the fact that in an infection-droplet at the infection-court on a plant surface the conventional conditions used for growth of fungi *in vitro* do not apply. Firstly, nutritional conditions in the infection-droplet are minimal relative to that of conventional growth media. Secondly, the nutritional conditions in infection-droplets are partially due to nutrients which leach out of the plant epidermal cells; and, thirdly, the fungal mass during the 3 to 24 hours incubation period prior to detection of phytoalexins in the infection-droplet and in infected plant tissues is of quite a different order of magnitude to that of the fungus grown for several days or weeks in conventional cultures. In my view, the studies that have been reported in this area are important as "signposts" to indicate to future investigators the classes of fungal metabolites which may play a role *in vivo* as elicitors of phytoalexins. However, it is doubtful that any of the compounds reported to date have such a role.

The host-specific activity of monilicolin A, which is not exhibited by *M. fructicola* or crude cell-free extracts of this fungus, is relevant to this discussion. Do fungi when they germinate and grow produce an almost limitless array of compounds which function as elicitors of different phytoalexins on each of a wide range of their hosts or nonhosts which they may chance to infect? Energy considerations suggest that the answer should be no. As a working hypothesis, it is suggested that while the potential number of fungal metabolites is large, the actual number is small, and that host nutrients both in the infection-droplet and in infected host tissues are actively involved as substrates in their formation. This idea was originally described as part of a "double-induction" concept [31] to emphasize that both host and pathogen are actively involved in the stimulation and regulation of phytoalexin biosynthesis.

Alternative hypotheses to explain specificity have been discussed recently by several authors. METLITSKII *et al.* [120] have postulated that elicitors (inducers) of phytoalexins are released by a patbogen in response to contact with cells of a resistant cultivar. Presumably "recognition factors" (perhaps lectins) are involved (cf. 101). In the

case of contact with cells of a susceptible cultivar, the elicitors (inducers) are not released or are released in smaller amounts. KOJIMA and URITANI [102] postulated that spore-agglutinating factors in the host tissues may be involved. They envisaged that spores germinate and come into contact with the spore-agglutinating factor(s) at an early stage of infection and that a stimulus generated through this contact is transmitted to both pathogen and host which stimulates physiological changes in the pathogen and triggers off the defence mechanism in the host. AYERS *et al.* [8] have postulated a complex system involving "specificity factors" and "inhibitors" which occur primarily in incombatible host-pathogen interactions to control the release of the cell wall polysaccharides which they have shown may function as elicitors of phaseollin (X) and glyceollin.

BIOLOGICAL AND PHYSIOLOGICAL ASPECTS OF PHYTOALEXIN FORMATION

Phytoalexins are not detected in fresh healthy plant tissues nor do they normally occur as wound responses although under special conditions some have been detected after certain physical treatments [20, 152]. It is of interest to discuss some conditions under which phytoalexins are formed in plant tissues and whether any plant organ specificity occurs.

Fungal species

Phytoalexins have been shown to be formed following inoculation of plants by fungi either pathogenic or nonpathogenic to the host species involved [32]. Similarly, both virulent and avirulent races of pathogenic fungi may elicit similar qualitative responses. The responses are not limited to any special group of fungi. They may be obligate or facultative parasites (biotrophs or necrotrophs) or true saprophytes. The net accumulation of phytoalexin with time follows a fairly characterstic sigmoid curve after a lag phase of a few hours following inoculation in most pod, hypocotyl, and leaf systems that have been studied. The slope of this curve appears to be important

in relation to the particular host-pathogen (nonpathogen) interaction. In general, the phytoalexin response of plants inoculated with nonpathogens may be represented by a curve with a fairly steep angle of slope, while similar responses towards pathogens may be represented by curves with lower angles of slope. However, exceptions occur. The control of fungal growth appears to depend not only on the rate of net accumulation of phytoalexin but also on its selective toxicity, which will be discussed later.

Organ of plant

In most of the early studies on pea and sweet potato, endocarp of the pea pods and parenchyma of the sweet potato roots were employed to demonstrate and analyse the plant's response to infection. More recently, the seed cavities of sweet pepper fruits [177] have been used to study capsidiol (XXIV) formation. Leaf tissues of lucerne [78], red clover [79], white clover [43], potato [118], and sweet peppers [202] have been shown to respond to fungal infection with the formation of medicarpin (VI) and maackiain (XX) in the legumes and rishitin (XXX), lubimin, (XXII) and capsidiol in the solanaceous plants.

Glyceollin (XXI) formation has been demonstrated in inoculated cotyledon and hypocotyl tissues of soybean [6, 92]. Phaseollin (X) and several related isoflavonoids have been reported from infected hypocotyl tissues of French bean [10, 173, 196]. Rishitin, lubimin, and phytuberin (XXXII) have been demonstrated in infected tissue slices of potato tubers [119, 164, 198]. In two recent papers [25, 151] pisatin (XVI) has been reported in crown-roots and epicotyls of peas infected with two *formae speciales* of *F. solani* while pisatin, phaseollin, phaseollinisoflavan (XII) and kievitone (VII) have been detected in exudates from roots of pea and French bean grown in a non-sterile aerated aqueous medium [24]. This culmulative evidence supports the conclusion that there is little organ specificity in relation to phytoalexin formation. However, owing to the physical nature of some plant surfaces, physiological contact between host and pathogen may be difficult to establish and quantitative aspects may be difficult to reproduce.

Tissue culture

Pisatin has been isolated from pea callus grown under axenic conditions on solid media in the presence of coconut milk [9]. Similar studies [50] on the response of suspension cultures of soybean cells to treatment with a polysaccharide isolated from *P. megasperma* var. *sojae* have demonstrated the formation of glyceollin under axenic conditions. This culture technique may provide bacteria-free model systems, which are otherwise difficult to maintain, suitable for studies on the biosynthesis of phytoalexins in plant cells.

Necrosis

Cellular browning, which is often equated with cell death, is a common symptom of fungal infection. It is especially associated with hypersensitive host reactions. In many plant species, phytoalexin formation following fungal infection is also associated with cellular browning. This was observed by MÜLLER and BÖRGER [122] who postulated that phytoalexins may be regarded as end-products of necrobiosis of infected host cells. In more recent studies using bean hypocotyls infected by *C. lindemuthianum*, RAHE *et al.* [153] reported that phaseollin formation was preceded by cellular browning. These observations were confirmed by BAILEY and DEVERALL [12] who claimed that phaseollin accumulation was in fact limited to infected tissue which was visibly brown. However, this interpretation was later modified [170], when it was pointed out that the degree of visible browning varied greatly from cell to cell and that it seemed likely that similar variations would occur in cellular contents. These workers concluded that it remained to be seen whether phaseollin is found only within necrotic cells and how closely its production is related to the symptom picture. A second well studied example of this situation is the association of rishitin and related terpenoids with infection of cut potato tuber slices with *P. infestans*. SATO and TOMIYAMA [162] stated that rishitin accumulated exclusively in the brown lesions and suggested that cell death may possibly be a trigger for the synthesis of rishitin. This view was strengthened by the data of Sato, KITAZAWA and TOMIYAMA [163] which demonstrated that some 20% of infected host

cells had died prior to the initiation of rishitin formation. A third example relates to the accumulation of wyerone acid (IV) following infection of broad bean leaves with *Botrytis cinerea*. Although it was originally suggested that wyerone acid might be produced by metabolically stimulated but otherwise normal cells [45], further work appeared to indicate that wyerone acid production may be confined to cells undergoing necrobiosis and browning [113]. On the basis of the observations discussed above a causal relationship between cellular browning and phytoalexin formation seems very much a matter of interpretation.

Germination and infection by fungal spores involves the formation and excretion of many fungal metabolites. As discussed above, some are active as elicitors of phytoalexins. Some have also been shown to be enzymatically active in relation to cell wall degradation [211]. In host-pathogen interactions where both the fungus and its host are metabolically active, it is obviously difficult to isolate changes associated with a single aspect of the metabolism of the complex system involved. VARNS, CURRIER and KUĆ [198] attempted to simplify the situation in relation to rishitin formation in potato slices by stimulating its synthesis using sonicates and boiled aqueous extracts of three races of *P. infestans*. Necrosis typical of the hypersensitive response and rishitin formation, occurred after treatment of the tissue slices with unboiled extracts; however, when heated sonicates were applied, necrosis was much less but accumulation of rishitin was unchanged. Two further interesting observations related to necrosis were also made by these authors. On the one hand, *Helminthosporium carbonum* Ullstrup, a nonpathogen of potatoes, was reported to stimulate levels of rishitin comparable with those obtained with *P. infestans* without marked necrosis. On the other hand, flecking (restricted necrosis) without detection of rishitin or other related terpenoids was observed following inoculation of potato sprouts with avirulent strains of *P. infestans*.

PAXTON, GOODCHILD and CRUICKSHANK [141] have also attempted to simplify the *M. fructicola*/French bean endocarp model host-pathogen system by substituting *M. fructicola* with monilicolin A, a metabolite of this fungus which is an active elicitor of phaseollin as discussed above. In these studies, while comparable amounts of

phaseollin were formed in endocarp diffusates when *M. fructicola* and monilicolin A treatments were used, cellular browning was observed only following fungal inoculation. Fine-structure, plasmolysis and uptake of vital strains were also not affected by monilicolin A treatments [141]. These findings were consistent with those of RATHMELL and BENDALL [156] who concluded on the basis of analyses of phenolic compounds in French bean hypocotyls that phaseollin formation may represent a specific stimulation of isoflavonoid metabolism which is separate from the general increase in phenol metabolism associated with cell necrosis. The lack of cellular browning following monilicolin A treatment contrasts with the severe browning caused by the polysaccharide isolated from *C. lindemuthianum* [2]. In the latter case, cellular browning was used as a parameter for the estimation of phaseollin concentration.

NAKAJIMA, TOMIYAMA, KINUKAWA [125] have reexamined the question of the distribution of rishitin and related terpenoids in potato tissue infected with an avirulent strain of *P. infestans*. It was demonstrated by incorporation of acetate-2-^{14}C into rishitin that this compound was synthesised mostly in healthy tissue adjacent to infected cells. It appeared that fungal metabolites involved in stimulating rishitin formation diffused ahead of the growth of fungal mycelium and that rishitin diffused and accumulated both in and outside browned cells within the browned tissue zone. A new approach based on the use of fluorescent microspectrography of broad bean tissue infected with *B. cinerea* [115] also supports the argument that the phytoalexin of this tissue, wyerone acid, is produced by live cells. Similarly, capsidiol in sweet pepper inoculated with *P. infestans* has been shown to accumulate prior to cytoplasmic disorganisation of the infected cells and may well be a product of adjacent uninfected cells [85]. Further, typical symptoms associated with hypersensitivity in soybeans and the changes in levels of several constitutive phenolics, commonly associated with these symptoms, may occur in the absence of glyceollin formation [95]. The collective evidence strongly indicates the possible metabolic independence of phytoalexin biosynthesis from cellular browning and necrobiosis. This is very significant if phytoalexins are to be used either as protectants or defence agents in agricultural practice.

ANTIFUNGAL ACTIVITY

By definition phytoalexins are post-infectionally formed compounds. Their main effect *in vivo* would be expected to be on mycelial growth after physiological contact has been established between the pathogen and its host. If this is true, mycelial growth assays should be more meaningful as a measure of the toxicity of phytoalexins than spore germination assays which were designed to evaluate protectant fungicides. In terms of present estimates, the median effective dose values of phytoalexins towards fungi, which are strongly inhibited by them such as nonpathogens of their host of origin, are of the order of 0.5 to 5×10^{-4}M [40, 84, 193].

Many known phytoalexins, especially the pterocarpanoids from the Leguminosae are sparingly soluble in water after purification and recrystallization. This is a problem well-known to natural product chemists; it may bear little relationship to the solubility of these compounds in the lipophilic environment of cell membranes. Phytoalexins also may be metabolized *in vitro* by test fungi under some culture conditions [210]. This may be important when assays are carried out over incubation periods of several days. These and other matters associated with bioassays have given rise to differences of opinion as to the most appropriate *in vitro* assay systems and the validity of the results obtained as discussed later. As all *in vitro* bioassays represent oversimplified, semi-static systems which lack the "metabolic support systems" which occur *in vivo*, it is not surprising that they have their weaknesses. They provide problems of technique which are a continuing challenge.

Selective toxicity

Where mycelial growth assays have been used to assess toxicity, as in the case of pisatin (XVI) [29], ipomeamarone (XXVII) [128], phaseollin (X) [40], and wyerone acid (IV) [112], these compounds have been shown to be less toxic towards pathogens than nonpathogens or weak pathogens of the plant from which the phytoalexin was isolated. Where germination assays have been used, no characteristic selective toxicity pattern has been observed which is related to the

known pathogenicity of the fungal species tested. Similarly, results based on germination assays have shown little difference in sensitivities among races of *P. infestans* towards rishitin (XXX) [84] or races of *C. lindemuthianum* towards phaseollin [12] even though the virulence of these strains is quite different on certain host cultivars.

The pathogen-nonpathogen selective toxicity pattern for pisatin and phaseollin discussed above has been confirmed in mycelial growth studies [193] although there were some exceptions. It was found that the pea pathogen *Aphanomyces euteiches* Dres. was markedly inhibited by pisatin at concentrations of 3.2×10^{-4}M and *F. oxysporum* f. sp. *pisi* was as sensitive to pisatin as most of the nonpathogens of peas. Pathogen-nonpathogen selective toxicity was also a characteristic of phaseollidin (XI) although again there were some exceptions [146]. Extensive studies on the inhibitory activity of capsidiol (XXIV), the major phytoalexin of sweet pepper, and some 20 related sesquiterpenes to several fungal species however have shown little correlation between pathogenicity and sensitivity towards the compounds [205].

In contrast to the reports discussed above on fungal races, HARROWER [69] has reported on the basis of mycelial growth studies, that different races of *Ascochyta pisi* have differential tolerances to pisatin. Similarly it has been shown that seven races of *Ceratocystic fimbriata*, which have different host specificities, show differential sensitivity towards extracts containing ipomeamarone and several related furanoterpenoids [103].

VANETTEN [193] has suggested that some of the anomalous results from different laboratories using different biossay systems brings into question the validity of *in vitro* assays for fungitoxicity. The same matters have been discussed by BAILEY, CARTER and SKIPP [13] wbo showed that growth of sporelings of *C. lindemuthianum* in agar media containing phaseollin was more sensitive to this compound than more mature mycelium obtained from the periphery of growing cultures [40]. In spite of the criticisms made by these authors of the conventional technique, the pathogenicity of *C. lindemuthianum* can be best explained by its tolerance to phaseollin and not by its sensitivity to this compound. VANETTEN [193] concluded from his studies that we need some alternative to

the comparison of *in vitro* measurements of antifungal activity and *in situ* concentrations of phytoalexins. This is true but it is difficult to devise such an alternative.

Metabolic basis of selective toxicity

NONAKA [127] and DE WITT-ELSHOVE [209] demonstrated that under certain conditions of culture *A. pisi* and other pathogens of peas can convert pisatin to less toxic metabolites. The observations lead to the concept that differential detoxification could offer a metabolic basis for the pathogen-nonpathogen pattern of selective toxicity of phytoalexins discussed in the previous section. Most of the evidence (Table III) in support of this idea is derived from *in vitro* studies, however some *in vivo* data have become available which need further evaluation.

When *Phoma herbarum* West *medicaginis* Fckl. and two other pathogens of lucerne were incubated in broth with medicarpin (VI) the concentration of this compound dropped to zero in 24 hours and several conversion or degradation products were formed [77]. When lucerne leaves were inoculated with these fungi, while similar compounds were detected in infection-droplets collected after 24 hours, they were not detected in leaf lesion tissue 7-14 days after inoculation when symptoms were visible, even though the quantities of medicarpin in leaf tissue were relatively high ($0.29 - 2.1 \times 10^{-7}$ mol/g fr.wt of leaf).

In a second example, *Stemphylium botryosum*, a nonpathogen of beans and peas, converted phaseollin into phaseollinisoflavan (XII) [80] and pisatin into 3,6a-dihydroxy-8,9-methylenedioxypterocarpan [197] *in vitro* [70]. However, these conversions can hardly be regarded as detoxification as the conversion products were at least as inhibitory as the parent compounds. It is again of interest to note that, while the new compounds could he detected in diffusate solutions, they were not detected in inoculated tissue even though high amounts of pisatin were measured (1.9×10^{-5} mol/g fr.wt of pea endocarp). No analysis of hean tissues was reported.

In a third example [42], it was observed that when French bean endocarp was inoculated with mycelial macerates of several fungi

TABLE III — *Fungal Metabolites of Post-Infectional Compounds.*

Post-Infectional Compound	Fungal Metabolite	Fungus species	*In vivo/ In vitro*	References Isolation	References Structure
Capsidiol	Capsenone	*F. oxysporum* f. sp. *vasinfectum*	*In vitro* & *In vivo* (I&C)*	203 204	54
Pisatin	3,6a-Dihydroxy8,9-methylene dioxypterocarpan	*F. solani* f. sp. *pisi*	*In vitro*	197	197
Phaseollin	Phaseollinisoflavan	*S. botryosum*	*In vitro*	70	22
»	»	*C. lindemuthianum*	*In vivo*	10	
»	1a-Hydroxyphaseollone	*F. solani* f. sp. *phaseoli*	*In vitro* & *In vivo* (C)	73,198	75
»	6a-Hydroxyphaseollin	*C. lindemuthianum*	*In vitro* & *In vivo* (I&C)	23,42	23
»	1,6a-Dihydroxyphaseollin	»	*In vitro*	76	76
»	6a,7-Dihydroxyphaseollin	»	*In vitro*	76	76
Wyerone acid	Reduced Wyerone acid	*B. fabae*	*In vitro* & *In vivo* (C)	114	116

* I = Incompatible (resistant)
C = Compatible (susceptible)

(pathogens and nonpathogens of French beans) two compounds were detected by thin layer chromatography which were not observed when conidial inoculations were carried out. When mycelial macerates of *C. lindemuthianum* were incubated separately with phaseollin and phaseollidin, each parent compound was partially converted into one or the other of the new compounds. The compound derived from phaseollin bas been identified as 6a-hydroxyphaseollin (XV) [23]. It is of interest again to note, firstly, that both the conversion products were detected *in vitro* and in infection-droplets but not in *C. lindemuthianum*-infected bean tissue although relatively large amounts of both phaseollin and phaseollidin were isolated (9.3 and 0.2×10^{-6} mol/g fr.wt respectively) and secondly, that the toxicity of the conversion products was similar to the parent compounds. The studies indicate that when leaf or endocarp surfaces are inoculated with mycelium using the infection-droplet inoculation technique the results may simulate those obtained *in vitro* more than *in vivo*. As chemical changes *in vitro* are culture medium dependent [210] they do not necessarily reflect the true *in vivo* situation.

VAN DEN HEUVEL and VANETTEN [73] and VAN DEN HEUVEL and GLAZENEN [76] have studied the detoxification of phaseollin *in vitro* and have reported that incubation of this compound in shake cultures of actively growing mycelium of *F.solani* f.sp.*phaseoli* results in the disappearance of phaseollin and the appearance in solution of several metabolic products. The most prominent of these has been identified as 1 a-hydroxyphaseollone (XIII) [74]. Another has been identified tentatively as 1,6a-dihydroxyphaseollin [76]. 1 a-hydroxyphaseollone has been shown to occur in large amounts (1.1×10^{-5} mol/g fr.wt) within 48 hours after inoculation, along with phaseollin and phaseollinisoflavan in both bean pod diffusates and in *F. solani* f.sp. *phaseoli* infected hypocotyl tissue [196]. However, 1,6a-dihydroxyphaseollin was only found *in vitro*. The *in vivo* evidence reported in tbis example is in contrast to tbe earlier reports. It may reflect better analytical techniques, or simply the differential ability of some fungi to metabolise phytoalexins *in situ*. The relative antifungal activity of 1a-hydroxyphaseollone was much lower than that of phaseollin towards eight species of fungi; thus, this appears to be a true example of detoxification.

MANSFIELD and WIDDOWSON [114] have compared the metabolism of wyerone acid (IV) by *B. fabae* and *B. cinerea in vitro* and observed that loss of wyerone acid from *B. fabae* cultures was associated with the appearance of reduced wyerone acid [116]. Wyerone acid was also modified by *B. cinerea* as judged by loss of ultra violet absorbance. Extracts of *B. fabae*-infected leaf tissue have been shown to contain reduced wyerone acid, and this appears to be coincident with a decrease in wyerone acid concentration [112, 113]. This example appears to be a case of detoxification *in situ* as reduced wyerone acid is less toxic than wyerone acid to *B. fabae*. These studies provide further evidence of the ability of phytopathogenic fungi to metabolize phytoalexins both *in vitro* and *in vivo*. However, they do not give an adequate explanation for the differing abilities of *B. fabae* and *B. cinerea* to infect and cause disease in broad beans.

Finally it is of interest to discuss two examples from the Convolvulaceae and Solanaceae. *In vitro* studies indicated that incubation of ipomeamarone with *Corticium rolfsii* Sacc. resulted in the almost complete disappearance of ipomeamarone from the medium over a period of 6 days [188]. WARD and STOESSL [203] reported the rapid loss of capsidiol and the appearance of capsenone (XXVI) in shake cultures when capsidiol was incubated with *B. cinerea*, and *F. oxysporum* f.sp. *vasinfectum* but not with *Phytophthora capsici* Leonian or *M. fructicola*. Analyses of diffusates obtained from the interaction of sweet pepper fruit following the separate injection of conidial suspensions of these fungi into the fruit cavities were consistent with these results. Capsidiol and capsenone were detected in the former situation while only capsidiol was detected when *P. capsici* or *M. fructicola* were the infecting fungi. When six species of *Fusarium* representing pathogens and nonpathogens of sweet pepper were tested, the results confirmed that the extent of capsidiol-capsenone conversion was not closely correlated with pathogenicity [178].

Mode of action

Few studies have been reported on the biochemical basis of the mode-of-action of phytoalexins. Since the term phytoalexin refers to a functional class of compounds of diverse chemical structure, it is

unlikely that any generalizations as to their modes-of-action could be expected. However, it may be of interest to summarize the available data which has been published as it may provide a stimulus for further research, which is urgently needed in this area.

a) Pterocarpan phytoalexins — Pisatin at inhibitory concentrations (1.9×10^{-4}M) appears to be fungistatic rather than fungicidal towards germination of *M. fructicola* [35]. At similar concentrations, pisatin was shown to be weakly phytotoxic to wheat root growth but not toxic to pea endocarp or the epidermal cells of pea or tobacco leaves. More recent studies however have shown that, when higher concentrations of pisatin (0.8 to 1.3×10^{-3}M) were applied to whole epidermal cells and isolated protoplasts of mesophyll cells of pea, damage to plasma membranes was observed [169].

In studies on the mode-of-action of phaseollin [195] it has been shown that phaseollin was rapidly taken up from solution by *R. solani*. Dry weight increase was inhibited at low concentrations (0.5×10^{-4}M) while dry weight of the fungus actually decreased at higher concentrations (1.5×10^{-4}M). The ability of *R. solani* to take up glucose-U-^{14}C from solution was decreased while mycelium which had previously incorporated ^{14}C rapidly released the labelled materials into solution. Exposure of hyphae to phaseollin resulted in reduction in respiration rate and immediate cessation of protoplasmic streaming and shrinkage of the protoplast from the hyphal tip. In spite of these observed effects, when *R. solani* was transferred to fresh broth, new growth occurred which indicated that at least at the concentration used (1.5×10^{-4}M) phaseollin was fungistatic and not fungicidal.

Further observations indicated that phaseollin had no effect on either endogenous or exogenous respiratory rates of hypocotyl tissue of beans; however, rapid release of $^{86}Rb^{+}$ from hypocotyl tissue previously treated with this ion occurred suggesting that phaseollin does have the potential to induce a physiological response in plant tissue by acting on the plasma membrane or on some process needed for membrane function [195]. Subsequent comparative studies of phaseollin, pisatin, medicarpin and glyceollin suggested that the membrane damaging potential of phaseollin may be due to the phenol group in its structure, and by analogy, a portion of the antifungal activity of the phenolic pterocarpans may be attributed to the phenol

moiety [191]. In the case of substituted phenols such as pisatin this explanation obviously cannot apply.

b) Terpenoid phytoalexins - URITANI and OSHIMA [190] examined the effect of ipomeamarone on respiratory enzyme systems in rat liver mitochondria and found that this sesquiterpene inhibited oxidation and phosphorylation at concentrations of 1 to 4×10^{-3}M. Oxygen up-take was higher when either p-hydroxybutyrate or α-ketoglutarate was supplied as substrate than when succinate was used. The ATP-inorganic phosphate reaction and 2,4-dinitrophenol-induced oxygen up-take were also inhibited; however, ipomeamarone did not act as an uncoupling reagent.

In an account of some biological properties of the norsesquiterpene alcohol rishitin (XXX), it has been reported [54] that adventitious root formation in urd (*P. mungo* L.) is promoted at concentrations of 1 to 5×10^{-4}M; however, at concentrations greater than 1×10^{-3}M injury was observed. Gibberellin (GA_3)-induced elongation of wheat leaf sections was inhibited at 1×10^{-4}M, while indole-acetic-acid-induced elongation of isolated *Avena* coleoptiles was completely inhibited at 10^{-3}M. While the observations are a little oblique in relation to the mode-of-action of rishitin in host-pathogen interactions, they do indicate that rishitin is phytotoxic only at relatively high concentrations.

Mode-of-action studies do not appear to have been reported for capsidiol; however, it has been shown that both *P. infestans* and *P. capsici* can survive extended periods of exposure to concentrations of 0.5 to 1.0×10^{-3}M [85]. As is the case with many of the other phytoalexins which have been studied, capsidiol appears to be fungistatic and not fungicidal. Other secondary or tertiary factors, which come into effect at later stages of infection than phytoalexin formation, must be presumed responsible for the subsequent death of hyphae in hypersensitive host cells where this occurs.

Structure-activity relationships

Considerations based on the three-dimensional structures and the toxicity *in vitro* of eight pterocarpans and related compounds towards *M. fructicola* lead PERRIN and CRUICKSHANK [144] to propose that

biological activity of these compounds towards this fungal species was dependent on the aplanar stereochemical relationships of the antifungal compounds such that these molecules could slip into curved bioreceptor sites on the fungal cell membranes. Recently the structure-activity relationships between several newly identified pterocarpans and their toxicity towards *A. euteiches* and *F. solani* f.sp. *cucurbitae* have been examined [194]. Mainly on the basis of the antifungal activity of 3-hydroxy-8,9-methenedioxy-6a,1 1a-dehydropterocarpan and the lack of activity in the analogous pterocarpan, 3,6a dihydroxy-8,9-methylenedioxypterocarpan, the original proposals have been challenged.

The antifungal activity of rishitin and 15 closely related compounds [84] suggests that for terpenoid compounds to exhibit antifungal activity a hydroxyl group at C-3 in required. Saturation of the double bond between the rings and/or that of the isopropenyl group does not reduce antifungal activity. Aromatization of the A ring also does not reduce antifungal activity but introduction of oxygenated functions into the side chain does have this effect. No unambiguous structure-activity relationships were observed in studies on capsidiol and related sesquiterpenes [204]; however, it was concluded that the structural and steric features may be of the utmost importance and that the relative importance of such features may differ for different species of fungi. The apparently anomalous situation in relation to the pterocarpan studies discussed above could be due to the use of different fungi in the bioassays on which the interpretations of structure-activity were based.

Disease reaction: susceptibility versus resistance

A unified concept of disease reaction involves compatibility and incompatibility between host and fungus species following plant-fungus interactions. These two situations describe the two extremes of a phenomenon in which there are many intermediate situations due to differences in the host and fungus genotypes involved. Environment also may influence gene expression and through it the phenotypic expression of compatibility and thus disease reaction. Com-

patible interactions result in host susceptibility and the pathogen is considered to be virulent. Incompatible interactions result in host resistance and the pathogen is considered avirulent. It must be remembered however, that the same cultivar may be either susceptible or resistant depending on the race of the pathogen with which it interacts, and that similarly a pathogen may be virulent or avirulent depending on the cultivar with which it interacts. Thus, the terms susceptible and resistant cultivar and virulent and avirulent race have little meaning except in reference to specific host-pathogen interactions and as a shorthand for the purposes of agronomic description.

In many of the studies discussed above, the extreme situations of compatible and incompatible interactions represented by the use of known virulent pathogens and non-pathogens of the host plants concerned, have been used as they represent relatively simple situations which are clearly defined. In this section situations involving the interaction of cultivars of host plant species and races of fungal species will be discussed.

The pea was the first legume species to be examined from the above viewpoint [38]. Quantitative studies of pisatin (XVI) formation using several cultivar-race combinations showed that when one race of *A. pisi* was used to inoculate several cultivars of peas the concentration of pisatin varied with the cultivar. On the other hand, when one pea cultivar was inoculated with several races of *A. pisi* the concentration of pisatin was related to the strain of *A. pisi* [38]. In time-course studies comparing the relative rates of net accumulation of pisatin in endocarp tissues of the pea cultivar Little Gem inoculated with two races of *A. pisi* to which Little Gem was susceptible and semi resistant respectively, the rate of pisatin accumulation was greater in the case of the less compatible interaction. BAILEY and DEVERALL [12] have measured the accumulation of phaseollin (X) in hypocotyl tissue of the French bean cultivar Kievit Koekoek following inoculation with gamma and delta races of *C. lindemuthianum* and have found that accumulation was more rapid where the avirulent delta race was used than where the virulent gamma race was employed. As cultivar race combinations are available within this host-pathogen system which show reciprocal compatibility reac-

tions, it would be of interest to analyse these systems to determine whether they support the findings already observed.

When the rates of accumulation of glyceollin (XXI) in hypocotyls of the two near-isogenic soybean cultivars Harosoy and Harosoy 63 infected with races 1 and 2 of *P. megasperma* var. *sojae* were compared, it was found that amounts of glyceollin were 10 to 100 times greater in the incompatible combinations than in the compatible combinations [92]. These data support the concept that glyceollin is involved as a primary chemical factor in the expression of single gene resistance in soybean. Similar studies on near-isogenic cultivars of cowpea infected with an isolate of *P. vignae* gave support to the hypothesis that the chemical basis for the expression of monogenic resistance in the cowpea cultivar Caloona is directly related to the rate of net accumulation of kievitone (VII) in inoculated tissues [139].

Similar examples of differential rates of ipomeamarone (XXVII) accumulation have been reported [1] in four sweet potato cultivars infected with *C. fimbriata.* Comparisons of R-gene and r-gene cultivars of potatoes showed that rishitin was formed in the former but not in the latter following inoculation by *P. infestans* race 0[163]. However, other compatible and incompatible potato cultivar-*P. infestans* interactions have been analysed [120, 199] in which only quantitative differences in amounts of rishitin (XXX) have been reported. In the extreme situations of susceptibility and resistance, the results may be " black and white ", but in most comparisons they appear to be " shades of grey ". Quantitative differences apparently are more important than qualitative ones where cultivar/race interactions are concerned.

Biosynthesis

On the basis of studies on potato-*P. infestans* interactions it has been stated that hypersensitivity and the formation of phytoalexins are a consequence and not the cause of plant resistance to infection [98]. The opposite view has been expressed on the basis of studies on the resistance of lettuce (*Lactuca sativa* L.) to the downy mildew fungus *Bremia lactucae* Regel [111]. In my view plant

resistance to infection cannot be argued to depend on a single event. It must be looked upon as a process involving a series of interdependent metabolic events of equal importance. Viewed from the point of view of the " double-induction " concept discussed earlier in this review, plant metabolites which leach into the infection-droplets on plant surfaces serve as growth substrates for the germination of fungal spores and the formation of fungal metabolites. These compounds then diffuse into the plant tissues and stimulate the changes in plant metabolism which result in the formation and accumulation of phytoalexins in hypersensitive tissue with resultant inhibition of growth of the pathogen. In terms of this concept the qualitative and quantitative nature of the fungal metabolites are both very important in relation to phytoalexin formation. Unless appropriate fungal metabolites are formed and released into the infection-droplet at the infection-court, and within the infected plant tissues, phytoalexins would not be expected to be formed. Unless phytoalexins are formed and accumulate, inhibition of fungal growth would not be expected to occur. Both these events and the series of events associated with the regulation of the intermediary metabolic pathways concerned with phytoalexin formation are all critically involved in disease resistance.

In plants, as in other living systems, the formation of new compounds following the application of external stimuli may involve either single step metabolic changes such as occur in the hydrolysis of glycosides to aglycones or complex biosynthetic pathways. Considerations based on the structures of the isoflavonoid phytoalexins in the Leguminosae and the wide occurrence of isoflavonoids as glycosides in healthy tissues of many legume species, suggest that it is theoretically possible that some or all of these pbytoalexins may occur naturally as glycosides. Some support is given to this possibility by reports that maackiain (XX) exists as a glucoside in red clover roots [19] and leaves [185] and that medicarpin-β-D-glucoside has been found in lucerne roots [168]. However, most reports, as discussed later in this section state or infer from biosynthetic studies that *de novo* synthesis of inhibitory compounds occurs whicb involve either *de novo* synthesis of enzymes or isozymes, or activation and regulation of enzymes already present in healthy plant tissue.

De novo *formation of enzymes*

A gene-activation hypothesis for pisatin (XVI) and phaseollin (X) biosynthesis based on the Jacob-Monod model for the lac operon of *Escherichia coli* has been proposed [60, 63]. Evidence for this theory was based on the observation that actinomycin D-induced (elicited) pisatin formation was accompanied by an increased synthesis of certain fractions of rapidly labelled RNA. However as discussed previously [41], the increase appeared mainly associated with the transfer RNA region (4S-6S) whereas incorporation was minimal in the messenger RNA region (10S-30S) and appeared to be only marginally above the controls. As evidence for enzyme synthesis it was reported that phytoalexin production was correlated with increases in protein synthesis and phenylalanine ammonia-lyase activity and that both increases could be inhibited by high concentrations of cycloheximide [57]. In a series of more recent papers [58, 59, 61, 62, 71] a wide range of compounds at high concentrations (1 mg/ml) have been used to study the induction of pisatin and phaseollin formation and phenylalanine ammonia-lyase activity. These authors believe that in the abiotic stimulation of these two pterocarpanoid phytoalexins the test compounds bind specifically to or in some other way associate with double stranded DNA and change its *in vivo* conformation resulting in new messenger RNA formation and *de novo* protein synthesis.

In similar studies on endocarp tissue of French bean no evidence was obtained for an increase of RNA synthesis during stimulation of phaseollin formation by low concentrations of actinomycin D [16]. It was demonstrated using low temperature infiltration of endocarp tissue with actinomycin D for six hours prior to elicitor (monilicolin A) application at 20°C, that actinomycin D did not stop phaseollin biosynthesis although RNA synthesis was inhibited by more than 95 %. Stimulation of phaseollin formation by actinomycin D at 20°C was thought to be due to transient effects, which were circumvented under conditions of low temperature infiltration when active plant metabolism would be minimized. It was also observed that the elicitor, monilicolin A, alone did not appear to stimulate the incorporation of ^{3}H-uridine into total RNA; indeed, in spite of the large differences in phaseollin formation between the monilicolin A

treatment and the water controls, incorporation of ^{3}H-uridine was slightly lower in beans treated with the peptide. These data suggest that stimulation of phaseollin formation may be more closely related to inhibition than to stimulation of total RNA synthesis or specific classes of RNA.

As discussed in an earlier review [41] the status of phenylalanine ammonia-lyase in the " induction process " is somewhat anomalous as this enzyme is present in significant quantities in healthy pea and bean tissue [63]. Production of new messenger RNA would seem unnecessary, as the control of enzyme formation could be exerted at the level of protein synthesis. Doubt has also been cast on the rate-controlling role of phenylalanine ammonia-lyase in isoflavonoid biosynthesis by RATHMELL [155] who observed a spatial and temporal separation between phaseollin biosynthesis and phenylalanine ammonia-lyase activity in French bean. Similar spatial separation of this enzyme and kievitone (VII) biosynthesis in cowpea has been reported [123]. The case for a gene-activation hypothesis based on the lac operon model appears to need re-examination.

Activation or activity control of preinfectionally formed enzymes

As no evidence in this area is available from host-pathogen interaction or related studies this section must be very speculative. SMITH [171] in discussions of the regulatory mechanisms in the photocontrol of flavonoid biosynthesis has pointed out that since phenylalanine ammonia-lyase is known to be synthesized at significant rates in darkness, it is no longer possible to envisage the role of light being able to "switch on" the synthesis of this enzyme and it is therefore unlikely that on/off control of messenger RNA transcription is involved. While the influence of light and of fungal infection on flavonoid and isoflavonoid biosynthesis are unlikely to be the same, they do involve a common enzyme, namely phenylalanine ammonia-lyase. SMITH [171] concluded that light may either stimulate the rate of phenylalanine ammonia-lyase synthesis or depress the rate of inactivator synthesis; the same comments could be said of infection. In both situations a specific enzyme inactivator may be involved. While the number of known reversible enzyme

inactivators in plants is small there is considerable evidence for the *in vivo* operation of activation/inactivation enzyme control mechanisms in several plants [117, 158]. Inactive forms of phenylalanine ammonia-lyase have been isolated from radish (*Raphanus sativa* L.) seedlings [4] and gherkin (*Cucumis sativus* L.) seedlings [5]. More recently an inactivator of this enzyme has been isolated from gherkin hypocotyls [53] and a high molecular weight fraction extracted from sunflower (*Helianthus annuus* L.) leaves has been reported to inactivate phenylalanine ammonia-lyase from sunflower [28]. D-phenylalanine has been shown to be an effective inhibitor of inactivation of this enzyme [27].

As one possible working hypothesis to explain the host-plant response as expressed by phytoalexin synthesis following fungal infection, it is postulated (a) that in the healthy plant the enzyme system involved is normally inactive due to the presence of a naturally occurring "inactivator" of that enzyme existing as an enzyme-inactivator complex; and (b) that fungal metabolites which act as phytoalexin elicitors are effective "inhibitors" of inactivation due to a differential binding affinity between the inactivator and the inhibitor. Such action would result in the differential neutralization of the inactivator and the activation and quantitative regulation of the pathway involved in phytoalexin biosynthesis. A recent report [49] on *in vitro* interactions between macromolecules of *P. infestans* and macromolecules of potato tissue is not inconsistent with the above concepts, however many problems remain to be solved before the biochemical basis of this phenomenon is elucidated.

Biochemical pathways

The details of the biosynthesis of phytoalexins are at present only beginning to be understood. Because of its central importance to the problem of disease reaction, it is worth summarizing the results of reports that may contribute to its clarification.

As an example from the Leguminosae, Grisebach and Barz [55] indicated that flavonoid biosynthesis in healthy tissues was as follows: Phenylpropane intermediates from the shikimic acid pathway condense with acetate units to form chalcones; ring closure

of the chalcones leads to the flavones, while ring closure plus aryl migration leads to the formation of isoflavones and coumestans. The existence of a flavonoid pool in healthy tissues has implications for the understanding of the biogenesis of phytoalexins. In infected tissues of peas, it was shown [56] that simple precursors such as phenylalanine and cinnamic acid were readily incorporated into pisatin (XVI). STOESSL [175], who reported maackiain (XX) in infected pea tissue, has suggested that this compound may be on a biosynthetic route very close to that leading to pisatin and that it may in fact he the immediate precursor of this compound. In similar studies on infected French bean, phenylalanine, cinnamic acid, acetate, and daidzein (7,4'dihydroxyisoflavone) have been shown to be incorporated into phaseollin (X) [72]. These data are consistent with the involvement of the acetate and shikimic acid pathways in the biosynthesis of this compound. More recently isoliquiritigenin (2',4',4-trihydroxychalcone) has heen established as a precursor of glyceollin (XXI) [96]. The incorporation of phenylalanine and isoliquiritigenin into glyceollin and the concomitant accumulation of daidzein, coumestrol (3,9-dihydroxycoumestan) and sojagol (3-hydroxy-9,10-dimethylchromancoumestan) during pterocarpan production was used to propose a biosynthetic pathway for glyceollin similar to the one previously suggested for coumestrol [46]. Pterocarpan biosynthesis has also been studied in red clover following ahiotic treatments of very young seedlings [47]. This work has shown that isoliquiritigenin and formononetin (7-hydroxy-4'-methoxyisoflavone) were readily incorporated into medicarpin (VI) and maackiain. Isoliquiritigenin was an excellent precursor of formononetin. The author suggested that when the aryl migration step characteristic of isoflavonoid biosynthesis takes place in the production of formononetin, it must be associated with 4'-methylation. The more complex 2,4 and 2,4,5-oxygenation patterns in ring D of medicarpin and maackiain respectively would appear to he built up subsequently from the simple 4'-0-methyl group of formononetin. Further studies of the same system [48] have shown two further compounds, namely 2'-hydroxyisoflavone and 2'-hydroxyisoflavanone to be excellent precursors of medicarpin hut not of maackiain.

URITANI [189] suggested that ipomeamarone (XXVII) pro-

duction in infected sweet potato was due to the stimulation of an abnormal pathway in carbohydrate metabolism leading to furanoterpenoid synthesis. Studies using tracer techniques have shown that ipomeamerone is synthesized via the acetate-mevalonate pathway [130, 131, 134, 137]. In an extension of these studies [132], the incorporation of acetate-2-^{14}C into ipomeamarone was shown to be markedly inhibited by the presence of dehydroipomeamarone (XXIX). This latter compound appeared earlier than ipomeamarone in time-course studies of the production of this compound and when labelled the label was efficiently incorporated into ipomeamarone. These data provide support for the conclusion that dehydroipomeamarone is the immediate precursor of ipomeamarone. On the basis of these results and earlier data, the authors indicate a possible biochemical pathway from acetyl CoA to ipomeamarone via farnesylpyrophosphate and dehydroipomeamarone (Fig. 2). A structural comparison of farnesol ($C_{15}H_{26}O$), dehydroipomeamarone ($C_{15}H_{20}O_3$) and ipomeamarone ($C_{15}H_{22}O_3$) indicates that reduction occurs after the formation of the furan and hydrofuran ring. Some of the enzymes in the earlier steps of this proposed pathway have been described; SUZUKI, OBA and URITANI [184] believe that β-hydroxy-β-methylglutaryl coenzyme A may be the rate-determining enzyme in ipomeamarone biosynthesis.

OZERETSKOVSKAYA *et al.* [138] have shown that in undamaged parenchyma cells of potato tubers there are only traces, if any, of either the steroid glycoalkaloids α-solanine and chalconine or the terpenoids rishitin (XXX) and lubimin (XXII). In periderm formed after slices of potato tubers were cut, α-solanine and chalcoline were observed in large amounts while little, if any, rishitin or lubimin was detected. However, in cut tissues which were immediately inoculated with an avirulant race of *P. infestans* the reciprocal situation was observed. These studies have been confirmed and extended [168] to show a similar change in metabolism following the application to cut tissues of either a cell-free sonicate of *P. infestans* or a spore suspension of a nonpathogen of potato. Where different races of *P. infestans* were used, the suppression of the accumulation of steroid glycoalkaloids was greater in incompatible than in compatible host-pathogen interactions. As in the earlier study, the marked

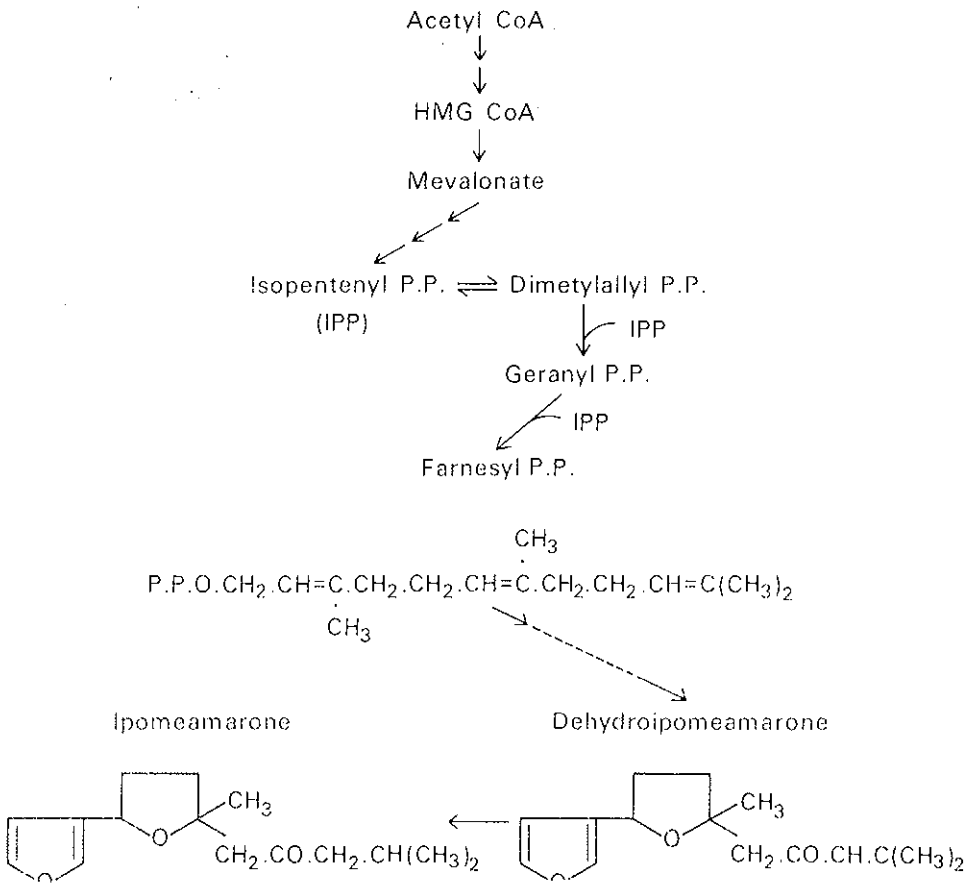


FIG. 2 — Possible mechanism of biosynthetic pathway of ipomeamarone. Acetyl CoA: acetyl coenzyme A; HMG CoA: β-hydroxy-β-methylglutaryl coenzyme A; P.P.: pyrophosphate; IPP.: isopentenyl pyrophosphate (redrawn after OGUNI and URITANI [132]).

suppression of steroid alkaloid accumulation was associated with the accumulation of high levels of terpenoid phytoalexins.

On the basis of studies of the incorporation of ^{14}C-labelled acetate and mevalonate into rishitin and steroid glycoalkaloids it was suggested that their synthesis involves the acetate-mevalonate pathway and occurs *de novo* [167]. In more recent studies on the formation of rishitin and lubimin following abiotic treatments of potato tuber tissues, isolubimin and oxylubimin (XXIII) were considered to be intermediates in lubimin and rishitin biosynthesis, respectively [86].

Finally the biosynthesis of capsidiol (XXIV) has been studied by BAKER and BROOKS [14]. Mevalonolactone [2 - ^{14}C] was administered at various time intervals before and after fungal inoculation of fruits of sweet pepper. Introduction of the labelled precursor six hours before inoculation resulted in its highest incorporation into capsidiol. Increasing the incubation period following inoculation from 2 to 3 days resulted in an increased concentration of capsidiol but little increased incorporation of mevalonolactone indicating that the transport of this compound is slow in relation to the time taken for activation of the biosynthetic system by the fungus.

Acetate [2 - ^{14}C] and acetate [1,2 - $^{13}C_2$] were also shown to be successfully incorporated into capsidiol [14] confirming and extending an earlier report that the acetate-mevalonate pathway is involved in capsidiol synthesis [177]. The mode of incorporation of acetate [1,2 - $^{13}C_2$] into capsidiol supports the hypothesis that the angular methyl group of the capsidiol skeleton arises by migration from the C-10 position of a eudesmane-type intermediate. From considerations of the biogenetic relationships between several terpenoid phytoalexins, which have been isolated from several genera of the Solanaceae, it has been concluded [179] that while they may utimately be derived from the same precursors never-the-less they are products lying on divergent hiosynthetic routes. While little experimental data is available a numher of speculative schemes have been proposed for the biosynthesis of several bicyclic terpenoids. These have been summarized in a recent review [181].

Conclusions and some potential practical applications

The major efforts of workers in this area of physiological plant pathology have been directed towards the analysis in chemical and biochemical terms of the plant's response to infection in the hope that an understanding of this response will lead to a better appreciation of the basic physiological mechanisms underlying disease resistance. It is now clear, that in spite of a small number of apparently anomolous examples which have been reported [149], a *prima facie* case can be made for the involvement of phytoalexins as primary factors in disease resistance. The schematic flow-diagram represented in Fig. 3 summarizes the biological steps involved in phytoalexin formation and the role of phytoalexins in disease reaction.

As might be expected where two organisms are interacting with each other, the metabolic changes with which research workers are concerned are hy no means simple ones. In infected tissue, the pathogen secretes low and high molecular weight molecules and enzymes into the host tissue as it germinates in the infection-droplet, penetrates the plant's external surfaces, and grows or attempts to grow either inter or intracellularly in the host tissues. In response to fungal infection the host plant's metabolism is altered in many ways including the formation and accumulation of phytoalexins and related compounds. Concurrently with these changes, and in some cases associated with them, are increases in the concentration of some constitutive host metabolites. Morphological and other changes such as cellular browning may also be part of the infection syndrome. These changes may occur at different rates. On present data, it appears that one of the major factors which affects the symptomatic outcome of infection is the rate of net accumulation of phytoalexins in infected tissues in relation to the threshold concentration required for inhibition of the pathogen's growth. Compounds which are precursors of phytoalexins may also be present. Similarly, and especially in the case of susceptibility, fungal metabolites of phytoalexins may also occur. At any given point in time during infection and disease development samples taken for analysis represent a specific static situation — a sample of the " disease state " which has its own biochemistry. A full understanding of the dynamic situation of anabolism and catabolism which represents the " disease

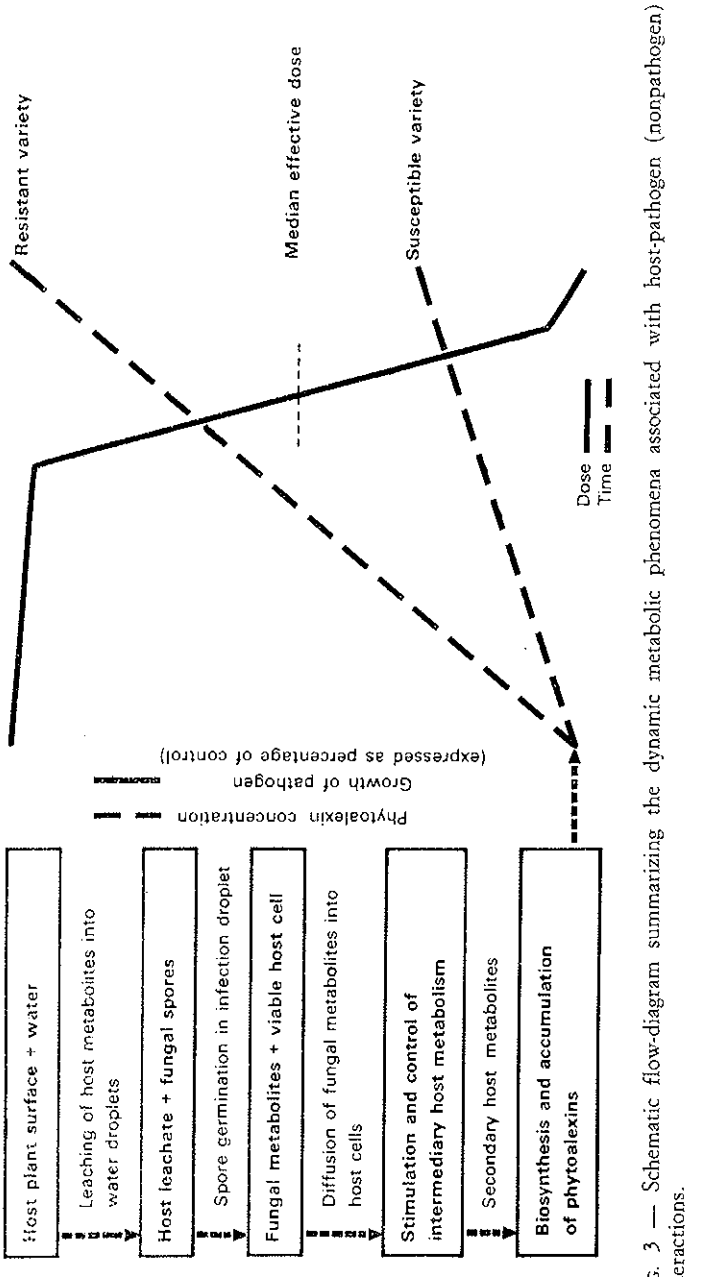


FIG. 3 — Schematic flow-diagram summarizing the dynamic metabolic phenomena associated with host-pathogen (nonpathogen) interactions.

state " can only be obtained by careful and detailed time-course and kinetic studies.

During the past 20 years, but especially in the last five, very significant progress has been made in the isolation and identification of many new natural products which are formed *de novo* and accumulate following inoculation of excised plant organs by fungi under laboratory conditions. Studies on organs attached to growing plants, where they have been reported, in general confirm the laboratory experiments although quantitative details may vary. The nature of the diseased tissue involving infected, affected and healthy cells, makes accurate quantitation of specific metabolites in the diseased tissue very difficult. The recently reported techniques for the chemical measurement of fungal mass in infected tissue [159, 160, 174, 212] offer promise for the future in that it may soon be possible to express amounts of phytoalexins on a more satisfactory basis than total fresh or dry weight of tissue. This is especially important when comparing the responses of resistant and susceptible cultivars where the biomass of the fungus present in the tissue may differ by several orders of magnitude. It is also important in studies of the rate of accumulation of phytoalexins following infection.

Over the same period, our knowledge of the nature of the pathogen's metabolites involved in the plant's response and phytoalexin formation, has almost stood still. The very recent reports on the chemistry of mycelial-wall polysaccharides and their properties in relation to the stimulation of phytoalexin biosynthesis are certainly of value. However, their non-specificity of action and their constitutive origin which require the invocation of " specificity factors " and " fungal growth inhibitors ", as yet unknown, detract from their interest as an explanation of the role of the pathogen in this phenomenon. Obviously, there is still much to be learnt before the role of the pathogen is understood.

Our understanding and elucidation of the biosynthesis of the complex pterocarpanoid structures of the legume phytoalexins is still in its infancy. The recent studies on the biosynthesis of medicarpin and maackiain in red clover represent an important forward step, but much remains to be learnt of the enzymes involved and what controls the rate of synthesis. The detailed studies on the

biosynthesis of ipomeamarone (XXVII), and more recently of capsidiol (XXIV), have increased our knowledge of the biogenesis of these compounds. However, further research will be required before the enzymes involved in these pathways are fully known and their regulation understood.

Traditionally plant pathologists have been primarily interested in improved methods for disease control. This has often been achieved in a very empirical manner with little understanding of the physiological principles involved. In the work reviewed in this paper, the opposite approach has been taken. Workers have tried to analyse and understand the nature of pathogenesis and disease resistance without being too concerned, in the first instance, with disease control. It may be of interest to conclude this review with a few examples bearing on possible applications to disease control which may accrue from some aspects of phytoalexin research.

Plant protection

Phytoalexins have comparable antifungal activity to conventional compounds used in plant protection. Surveys of post-infectionally formed compounds such as those currently being carried out for chemotaxanomic purposes [82] if supplemented by assessments of the biological activities and pharmacological properties of such compounds, could lead to the discovery of important new drugs with uses either in agriculture or medicine. However, the properties of some of them, for example, the instability of pisatin (XVI) to sunlight, combined with their fungistatic rather than fungicidal and weak antibacterial action, do not make them attractive for direct application to leaf surfaces in the field. In most cases their complex ring structures do not lend themselves to low synthetic processes from cheap and readily available raw materials. In spite of these general comments, individual compounds must be assessed in their own right. A promising start has been made by WARD, UNWIN and STOESSL [207] who have reported some control of late-blight of tomatoes (*P. infestans*) with capsidiol. An efficient and practical synthesis of the orchid phytoalexin orchinol and related phenanthrenes (overall yield *ca.* 16 %) has been described [176] and recently the synthesis of rishitin (XXX) has been reported [129].

Breeding for disease resistance

The most successful method of obtaining disease resistant cultivars is by the incorporation into agronomically acceptable cultivars of host resistance genes to a particular pathogen by plant breeding and selection procedures. Data discussed earlier in this review on soybean, potato and some other crops clearly indicated that the net rate of accumulation of phytoalexins is greater in resistant cultivars than susceptible ones even though it may not be the only change involved. Although no progeny trials in relation to the inheritance of phytoalexin formation following crosses of resistant and susceptible cultivars have been published, it is reasonable to suggest, on the basis of the consistency of the cultivar data, that phytoalexin concentrations for individual plants within a progeny are genetically inherited and that selections could be made on this basis. While this parameter may not be an absolute measure of all factors involved in resistance, it may be a very reliable index value that could be used to evaluate the relative disease resistance of that part of the progeny which shows no visible symptoms and are merely classified as " resistant ". It is suggested that tests for phytoalexin concentration should follow normal screening procedures and should be applied only to those plants showing no macroscopic symptoms of disease. Sophisticated tests of this sort, while adding an additional parameter to assist the plant breeder to select the best of his plants for further crosses or for seed increase, would require better analytical plant chemistry support services than normally available in many plant breeding institutes. They potentially would rank along with computer techniques and phytotron facilities currently used in some institutes to increase the efficiency of the evaluation of breeding lines.

A second approach used in plant breeding is the induction of mutants and their screening for disease resistance. A recent report [64] has indicated that treatment of pea seed with the chemical mutagen sodium azide (1×10^{-3}M) in potassium phosphate buffer at pH 3 resulted in mutants of the pea which accumulate pisatin as a constitutive metabolite. These tests have been carried through to the M-3 generation. This and related procedures for the induction of mutants for the accumulation of pisatin show pro-

mise but require confirmation. It is of interest to note that while some of the mutants were dwarfed some appeared normal. Unfortunately no evidence on the relative resistance of the mutants to disease was presented. However, it is physiologically significant that pisatin may accumulate, even if at relatively low concentrations, in otherwise normal plants as a constitutive metabolite. It will be interesting to watch further developments in this area.

Chemotherapy and induced resistance

If an overview is taken of plant pathology it is clear that most plants are susceptible to infection by most fungi but resistant to most diseases. This suggests that the operation of innate resistance mechanisms in plants is normally very efficient. If this were not true then consideration of the relative reproduction rates of plants and fungi together with our dependence on plant products either directly or indirectly for food, clothing and shelter would make our existence on planet Earth very tenuous. The gene pools available to plant breeders of the world's major crops are limited and concern has been expressed as to their vulnerability by a committee of the U. S. National Academy of Sciences [126]. It is an open question under existing plant improvement and crop management practices as to how long it may be before these gene pools will approach exhaustion. In the light of the above situation, disease control methods based on the activation and control of the plant's own defence systems must be considered as a serious possibility for the future.

Pro-drugs capable of stimulating phytoalexins fall into several categories: *a*) heavy metal ions, especially silver, mercury and copper; *b*) some metabolic inhibitors, especially iodoacetic acid and *p*-chloromercuribenzoate; and *c*) antagonists of nucleic acids and protein synthesis, especially actinomycin D and cycloheximide. These have been shown [41] to stimulate directly or indirectly pisatin and phaseollin (X) formation. Many other compounds reported by HADWIGER and coworkers [58, 59, 61, 62, 71] also have this property. It is of interest to note that some organo-mercuric [187] and other synthetic organic fungicides [135, 136, 157] along with butylamine, the non fungitoxic portion of the systemic fungicide

benomyl, are active stimulants of phytoalexins. Treatments of the roots of soybean plants with butylamine prior to hypocotyl inoculation with *P. megasperma* var. *sojae* have been reported [99] to result in up to almost three times the amount of glyceollin (XXI) than in similar control plants treated with water. In this situation butylamine appeared to act not as a stimulant of phytoalexin synthesis but as a " conditioner " of the plant cells which responded to infection by producing the phytoalexin at a greater rate than they would normally.

Changes in host reaction towards increased resistance when plants have been inoculated by avirulent races of a specific pathogen or attenuated pathogens or nonpathogens of the host under study prior to inoculation with a virulent strain of a pathogen have been reported [154]. Although phytoalexins have been implicated, very little experimental evidence for this has been forthcoming, Kuć [105] in a recent study of induced resistance in beans to anthracnose (*C. lindemuthianum*) obtained both local and systemic protection and concluded that this was due to the " conditioning " of the cells to accumulate phaseollin and related pterocarpanoid compounds when challenged by the pathogen rather than the stimulation of phytoalexins as a result of the original treatment. There appears to be some similarity between the divergent approaches to this problem by Klarman [99] and Kuć [105]. This may also be the explanation for the induced resistance of stem-infected tobacco plants to blue mould (*Peronospora tabacina* Adam) [33].

Finally, Ayers *et al.* [7] have studied the effect of adding " Fraction I " of the polysaccharide elicitor of glyceollin, which they isolated from mycelial walls of *P. megasperma* var. *sojae*, to hypocotyls of the soybean c. v. Harosoy 63. When the treatment was simultaneous with fungal inoculation by a virulent race of *P. megasperma* var. *sojae*, the tissue remained susceptible; however, if the elicitor was applied 10 hours prior to inoculation, fungal growth was inhibited and the tissues exhibited only the minor symptoms associated with resistance. In this situation it appears that the polysaccharide acted as a pro-drug for the stimulation of glyceollin and that this resulted in the adequate defence of soybean hypocotyls to infection by a virulent race of the fungal pathogen. By either

indirect or direct stimulation of phytoalexin formation, or by future techniques based on a knowledge of the rate-controlling steps in phytoalexin biosynthesis, the way could be opened to improved methods of disease control by manipulation of plant metabolism through chemotherapy to activate defence systems in genetically susceptible cultivars of plants.

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DISCUSSION

GRANITI

Professor Wain mentioned bananas with a turnip taste. These are the result of a breeding program in Central America for resistance of Banana to the "Panama disease" caused by *Fusarium oxysporum* f. sp. *cubense*. Similarly, many resistant varieties of various crops were made in the last few decades and some are commercially available. There is, however, some concern about what plant breeders get from their crosses. Nobody can say what factors have been incorporated along with the genetic information for resistance when crosses are made with wild or inedible species. Toxic substances may be present in new resistant varieties. For example, the potato cultivar "Lenape" has been withdrawn because of its high content of steroid glycoalkaloids. Many papers called the attention of researchers to the problem of the toxicity of phytoalexins towards animals and humans. In a recent review, Kuć and Currier (*) stated that the presence of phytoalexins and related compounds in foods obtained from new resistant varieties, stressed plants, plants treated with pesticides and infected plants is a potential hazard to human health. Would you please comment on this point?

CRUICKSHANK

Yes. I think that the problem, if there proves to be one, must be kept in perspective. For many years plant breeders have been developing disease resistant cultivars of crop plants. The fact that we now know a little about the chemistry of some of the toxins which may be involved in the expression of resistance should not alter the acceptability or otherwise of the cultivars involved. A further aspect however, must also be considered. Host-pathogen interactions

(*) KUĆ J. and CURRIER W., « Adv. Chem. Ser. », *149*, 356-368 (1976).

in plant tissues are very localized phenomena. Incompatible interactions normally involve very small numbers of host cells for any single infection. Thus, the absolute amount of toxic products formed as a result of infection of a potato tuber of a resistant variety by *Phytophthorá infestans* would be very small in relation to the total mass of uninfected potato tissue which would be consumed when this vegetable is eaten as part of our diet. If however, disease control measures involved either the growing of plants selected for some constitutive fungitoxic compound, which also had mammalian toxicity, or the spraying of plants with a compound with phytoalexin-elicitor activity which resulted in the formation in plants of compounds with both fungal and mammalian toxicity, the situation in relation to potential problems of the type raised would have to be watched very carefully. I believe such potential problems should be kept in mind if this area of crop protection develops, but I do not think we should adopt defeatist attitudes as I believe the problems will be self correcting.

NAKANISHI

Excluding the well known cases like rishitin, for example, there are lots of antibiotic principles found in plants from seemingly healthy plants. Would you call these phytoalexins or are they simple antibiotic and antifungal compounds isolated from plants? The formation of phytoalexins is a self-defense mechanism. However if the strain is very resistant it may already have it even if it is not infected. I thought that the third from the last slide was somewhat contradictory of your basic definition of phytoalexins, because it is said that it slows down the growth of the pathogen.

CRUICKSHANK

By definition phytoalexins are post-infectionally formed compounds. Toxic compounds found in healthy plant tissues fall into a wider class of compounds referred to as phytoncides.

In reference to your second question I must emphasize that the discussion of host-pathogen specificity with reference to the involvement of "inhibitors" and "specificity factors" in phytoalexin formation is

purely speculative. It concerns a system postulated by Ayers *et al.* [8] (*), to be involved in the control of the release of cell wall polysaccharides which have been shown to be elicitors of phaseollin and glyceollin in French bean and soybean hypocotyl tissue.

KARLSON

Well, the difference between substances being elicited by infection and being produced normally is some what arbitrary. You can always have a constitutive strain of the same plant, where you do not need the elicitor — the compound is made all the time. That is the same as constitutive strains of bacteria.

A second point. Are there any measurements of the amount of phytoalexins in susceptible plants and in non-susceptible plants for the same species. Is it really proven, that the amount of phytoalexin is higher in the resistant case?

CRUICKSHANK

Results of studies by Keen [92] on the soybean cultivars Harosoy and Harosoy 63 in relation to the time-course of the net accumulation of glyceollin following hypocotyl inoculation with races 1 and 2 of *Phytophthora megasperma* var. *sojae* clearly establish this point. Other similar examples have been reported by several groups who have studied this aspect of the problem [1, 38, 120].

WAIN

I would just like to comment in regard to whether wyerone is just a natural antifungal or a phytoalexin.

Wyerone occurs in healthy broad bean plants. We extracted it at Wye and determined its structure. It is a methylester. One possibility which we considered was whether wyerone becomes hydrolysed to wyerone acid by a hydrolase enzyme operation within the fungal cell. If this were to occur, then wyerone acid and not wyerone could be the

(*) The numbers refer to the bibliography of this paper.

active fungicide. To examine this possibility further, we exposed fungal spores to wyerone and then looked to see if wyerone acid was produced. None was found. We then examined broad bean plants infected with *Botrytis cinerea* to see whether under these conditions wyerone acid was produced. No wyerone acid was detected but to our surprise we found that the diseased plant contained some twenty times more wyerone than a healthy plant.

It would seem therefore that although wyerone does not readily control *Botrytis cinerea* the plant produces wyerone when infected in an attempt to stem the infection. As I have already said, we isolated wyerone from healthy broad bean seedlings — had we known we would have extracted diseased plants where the yield would have been much higher. Whether wyerone should be considered a preformed antifungal or a phytoalexin is debatable.

CRUICKSHANK

On the basis of the published studies on interactions between *Botrytis* species and broad beans I would consider wyerone [51] to be a phytoncide and wyerone acid [45] to be a phytoalexin. However, studies by Keen [93] on the interaction between *Phytophthora megasperma* var. *sojae* and broad beans suggest that wyerone may be classified as a phytoalexin. More recent information [66] indicates that a further compound, wyerone epoxide is also involved in the same complex as a post-infectionally formed compound with some antifungal activity. In broad beans, as in several other host plants studied, one tends to get a family of chemically related compounds formed following fungal infection of plant tissues.

NAKANISHI

My point was just simple-minded. I mean of course if you do proper comparative studies with healthy and infected plants as you have just explained to us you can come out with a compound which fits exactly the definition of phytoalexins. But — as organic chemists — you find antifungal compounds and I presume we should not call them phytoalexins; we just call them a compound with such and such an activity unless proper studies are done. Is that right?

CRUICKSHANK

I entirely agree. In my view the term phytoalexin is being used too loosely by many people at the moment. Although it is important, it is insufficient to merely note that the compounds are post-infectionally formed. Comparative time-course and toxicology studies should also be reported. Many of the papers, especially those published as "Notes" do not provide sufficient information for a reviewer to evaluate with confidence even the potential functional role of the compounds reported.

SIDDAL

Just a small point — it may be that half of the literature containing natural products is derived from infected plants unknown to the organic chemist. It may also be that a lot of the variation in levels of content of natural products in plants is due to this kind of phytoalexin-elicitor mechanism. I just wonder how much of the literature on phytoalexins is already written but we do not know this because differential assay has never been carried out.

NAKANISHI

Are there any cases in which a fungal metabolite has been shown to be incorporated into a so-called phytoalexin? Are such examples known or not? I was just thinking — besides the gene turnoff and so on — could there be a possibility that they are further developed secondary products derived from fungi — not necessarily the metabolite itself — but one intermediate leading to a fungal metabolite induced by that type of thing?

CRUICKSHANK

This type of experiment has been done and the evidence shows that the fungal metabolites which act as elicitors of phytoalexins are not incorporated into the phytoalexin molecules. The mode of action of elicitors, however, is largely unexplored and I would prefer not to comment further on this question.

MARINI-BETTÒLO

We have so far discussed about phytoalexins belonging to two main groups: flavonoids and furans. But may I recall that in plants there are also other substances formed only during infection which belong to completely different structures. The mansonones, which are sesquiterpene quinones, have been found in the elms when the plants are attacked by *Ceratostomella ulmi* (*) whereas the same mansonones are present normally in *Mansonia altissima* bark and wood (**). These substances are fungistatic but I doubt if you could call them phytoalexins or stress products.

CRUICKSHANK

I think that each compound that is found must be evaluated in its own right. I don't think I could answer your question without knowing more about the experiments that were involved.

KARLSON

One last comment. What do you think about the possibility that the hypha of the fungus which grows into the tissues is in part broken down, gives off high molecular material, carbohydrates polymers proteins or breakdown products of proteins and that these things which originate from the fungus would then elicit the synthesis of phytoalexins?

I'm still looking for a mechanism in which the fungus would use the compounds which elicit the effect for its own purpose, not for the purpose of the defense of the plant.

CRUICKSHANK

I think that is a very interesting comment and it would be nice to investigate it, but at this stage I would not like to comment.

(*) OVEREEM J. C., EIGERSMA D. M., « Phytochem. », *9*, 1949 (1970); CHEN F. C., LIN Y. M. and CHEN A. H., « Phytochem. », *11*, 1190 (1972).

(**) MARINI-BETTÒLO G. B., CASINOVI C. G., GALEFFI C., DELLE MONACHE F., « Ann. Ist. Sup. Sanità », *2*, 327 (1966).

WAIN

May I just comment on that just to point out very briefly that it is not necessary to have the fungus or the fungal spore to promote the formation of phytoalexin. You can do this in many ways; you can take spores in water and then filter them off, and this water in the form of a droplet would also lead to the production of the phytoalexins. So it cannot be anything in the way of breakdown products from the fungal hypha.

THE POSSIBLE SIGNIFICANCE OF UNCOMMON AMINO ACIDS IN PLANT-VERTEBRATE, PLANT-INSECT AND PLANT-PLANT RELATIONSHIPS

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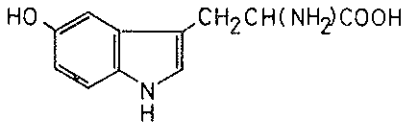
INTRODUCTION

Some twenty-five to thirty amino acids are commonly found in all living organisms either as protein constituents such as glycine and glutamic acid or as metabolic intermediates such as citrulline and homoserine. In addition to these "common" amino acids a large number of other amino acids are known to occur in micro-organisms, plants and animals. In plants, from which over two hundred have been isolated, these "uncommon" amino acids usually occur in the free state or as simple condensation compounds of low molecular weight such as the acetyl, γ-glutamyl and oxalyl derivatives. In addition to the amino acids themselves a numher of imino acids such as proline and pipecolic acid are found as protein constituents or in the free state in living organisms. In this chapter, for the sake of simplicity, I shall use the term "amino acid" to include these.

The distribution patterns of the "uncommon" amino acids in plants are very variable. Certain of the amino acids are restricted, as far as we know, to species of a single family or genus whilst others enjoy a wider distribution and may be found in species of totally unrelated taxa.

Some of the most interesting information that has emerged from the study of "uncommon" plant amino acids concerns the extremely high concentrations in which they sometimes accumulate. In the West African legume *Griffonia simplicifolia*, 5-hydroxy-L-tryptophan [I] can account for as much as 14% of the seed weight [1], while L-3,4-dihydroxyphenylalanine (L-dopa) [II] and canavanine [III] reach concentrations approaching 10% in the seeds of *Mucuna* and *Dioclea* species respectively [2, 3]. Such high concentrations are not necessarily restricted to the storage organs however, for while the rhizomes of *Polygonatum multiflorum* contain 6% of the imino acid azetidine-2-carboxylic acid [IV] the shoots of a second Liliaceous species *Convallaria majalis* contain 3% dry weight of the same compound [4].



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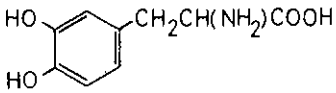

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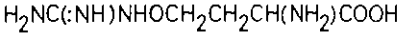

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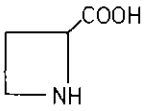

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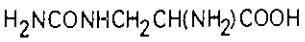


V

When an "uncommon" amino acid is found in very high concentrations in a seed or a rhizome it is tempting to suggest that its role is one of storage, and indeed it has been shown that some "uncommon" amino acids in seeds disappear rapidly when the seeds begin to germinate. This is not always true however and albizzine [V] which occurs as a major free amino acid in the seeds of *Albizzia julibrissin* continues as a major free amino acid in the developing seedlings while azetidine-2-carboxylic acid, already mentioned as a constituent of two Liliaceous species, is synthesized rather than degraded in the developing seedlings of the legume *Delonix regia* [5]. These findings suggest that the primary role of "uncommon" amino acids in all plants is not necessarily one of storage, and even when

these compounds do have a storage function it is unlikely that this is their only role. If, for instance, a plant accumulates high concentrations of canavanine in its seeds, and that canavanine is metabolised rapidly on germination, it can be argued that nitrogen storage is the role of this nitrogen-rich amino acid. This argument however ignores the fact that arginine appears to fill this role equally well in some other species (unpublished observation) and as all plants possess the necessary complement of enzymes to synthesize and metabolise arginine the use of the common amino acid for storage would relieve the plant of the necessity of providing an entirely new complement of enzymes to handle canavanine. Looking at the problem in a slightly different way we can imagine an arginine-accumulating species undergoing a mutation which gives rise to a form that accumulates canavanine instead of arginine. If the two amino acids serve no other purpose than that of storage and are equally efficient in this respect the mutant form of the species will not survive in competition with the unmutated form whose genetic resources and raw materials are not being diverted to the synthesis of superfluous enzymes. The fact is however that some plants which accumulate canavanine have survived and do compete successfully with species which do not make this "uncommon" amino acid. It must be concluded therefore that even if canavanine is no better than arginine as a nitrogen store its presence must be advantageous in some other respect or respects.

I should like to suggest that during the course of evolution many thousands or hundreds of thousands of "uncommon" amino acids have arisen in living organisms due to mutational changes in enzymes concerned with amino acid synthesis or utilisation, but that only those which have improved the "fitness" of an organism to survive in a particular environment have survived themselves, and are available for our study to-day. Environmental factors have in effect acted together, during the course of evolution as a universal biological screening programme which has preferentially selected plants containing secondary compounds, including the "uncommon" amino acids, which are physiologically active in other organisms which occur in the plant's environment.

The two most obvious ways in which an "uncommon" amino acid or other secondary compound can improve the "fitness" of a plant in a given environment are by affording protection against

potential predators on the one hand and by discouraging potential competitors on the other.

In the following sections I have presented evidence which encourages me to believe that some "uncommon" amino acids can benefit the plants which synthesize them in hoth of these ways.

"Uncommon" Amino Acids in plant-animal relationships

It has been suggested in the Introduction that environmental pressures acting on chemically variable plant populations have led to the natural selection of plants containing "uncommon" amino acids which are beneficial to the plants which synthesize them. One such environmental pressure is that exerted on the plant populations by herbivorous animals. The stripping of bark from trees by deer, the defoliation of shrubs by goats and the destruction of seeds by rodents being examples of this.

Most plants are immobile and cannot escape from potential predators by "running away". They are therefore dependent on physical adaptations, such as thorns and hard seed coats, or chemical adaptations, such as unpalatahle or toxic secondary compounds for their protection. Here we shall consider what part "uncommon" amino acids may play in this protection.

Virtually nothing is known of the palatability or otherwise of "uncommon" amino acids to animals at the present time. The toxicity of certain of them to man, his domestic animals and to experimental animals and birds in the laboratory is well established however. While it is not suggested that man or these particular animals and birds necessarily exercised the original pressures which led to the selection of plant species containing the toxic compounds, the ohservation that some of these "uncommon" amino acids are toxic to a broad range of domestic and experimental animals strongly suggests that they have a protective role in the wild.

α-*Amino-β-oxalylaminopropionic acid* (oxalyldiaminopropionic acid, ODAP) [VI]

This amino acid was isolated independently by two groups of workers in India from seeds of *Lathyrus sativus* [6, 7]. The in-

$$HOOCCONHCH_2CH(NH_2)COOH$$

VI

gestion of these seeds causes classical neurolathyrism, a disease which in man is characterised by paralysis of the legs and in extreme cases, death. In the laboratory the purified amino acid is toxic in primates, rodents and birds. The nature of the toxicity varies however with the method of administration and the age of the animal [8]. The higher homologue of ODAP, α-amino-γ-oxalylaminobutyric acid

$$HOOCCONHCH_2CH_2CH(NH_2)COOH$$

VII

[VII] is found together with ODAP acid in a number of *Lathyrus* species and is also neurotoxic in experimental animals as are the synthetic oxalyl derivatives prepared from glycine, α and β alanine and lysine [9]. A recent survey has shown that ODAP acid is not restricted to the genus *Lathyrus*, but also occurs in seeds of *Crotalaria* and *Acacia* species, some of which are known to be toxic to grazing animals [10].

Canavanine (2-Amino-4-(guanidinoxy) butyric acid)

Canavanine was one of the first "uncommon" amino acids to be isolated from a plant source (*Canavalia ensiformis*) and it has attracted considerable attention from biochemists as a natural arginine analogue which can be incorporated into proteins by micro-organisms [11]. Less is known of its toxicity in animals, but TSCHIERSCH [12] has demonstrated that it is lethal to mice when included into their diet at a concentration of 5%, a concentration which is frequently exceeded in legume seeds. The toxicity of the amino acid to a variety of mammalian cells grown in culture has also been shown, a recent example being the demonstration of MILLER and CONSIGLI that canavanine reduces cellular proliferation in embryonic mice cells [13].

Indospicine (L-2-Amino-6-amidinohexanoic acid) [VIII]

$$H_2NC(:NH)CH_2CH_2CH_2CH_2CH(NH_2)COOH$$

VIII

Indospicine is another close analogue of arginine which was isolated from the seed and leaves of the legume *Indigofera spicata* which is toxic to grazing animals [14]. Subsequent studies using the purified amino acid have shown that it is hepatotoxic in chicken, mouse, rabbit, guinea pig, sheep, pig and ox. It is also teratogenic in the rat (the only animal tested) [15].

L-α,γ-Diaminobutyric acid [IX]

$$H_2NCH_2CH_2CH(NH_2)COOH$$

IX

The principal free amino acid in the seeds of at least 13 species of *Lathyrus* [16] is α,γ-diaminobutyric acid [17], the lower homologue of ornithine. This amino acid is not found in those species of *Lathyrus* which are responsible for human lathyrism, but RESSLER has demonstrated that the compound produces convulsions and death when administered to rats. O'NEAL and his colleagues [18] showed later that this compound disrupts the Krebs-Henseleit urea cycle in the mammalian liver by inhibiting the action of ornithine transcarbamylase. This disruption causes the concentration of ammonium ions in the blood to rise to toxic levels.

L-α-Amino-β-methylaminopropionic acid [X]

$$CH_3NHCH_2CH(NH_2)COOH$$

X

This isomer of L-α,γ-diaminobutyric acid occurs as a major free amino acid in the seeds of several *Cycas species* [19, 20]. Experiments

using both isolated and synthetic material showed that the L but not the D isomer was toxic in rats, mice and chicks [21].

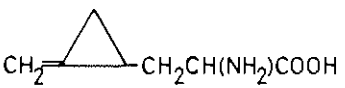
β-Cyanoalanine [XI]

$$NCCH_2CH(NH_2)COOH$$

XI

β-Cyanoalanine, first isolated from *Vicia sativa* [22] occurs in the free state and as the γ-glutamyl derivative in the seeds of many *Vicia species* [23]. It produces convulsions and death when injected into or fed to rats and chickens (LD_{50} in young rats, 13.5 mg/100 g by subcutaneous injecton [24]). In rats one of the effects of β-cyanoalanine appears to be the inhibition of liver cystathionase, the enzyme which converts cystathionine to cystine [25].

Hypoglycin A (β-Methylenecyclopropyl-propionic acid) [XII]

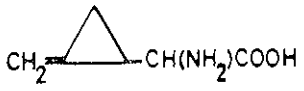


XII

Hypoglycin A is found in the unripe fruit of *Blighia sapida* (ackee) a tree which is grown for its fruit in Africa and the West Indies. The amino acid can cause acute hypoglycaemia and occasionally death in man. Von Holt and Benedict [26] have suggested tbat it is not the amino acid itself but an unsaturated carboxylic acid formed from it in the mammalian system which blocks fatty acid metabolism and this in turn leads to a depletion of carbohydrates. The amino acid also acts as a teratogen when injected into pregnant rats [27].

α-(*Methylenecyclopropyl*)-*glycine* [XIII]

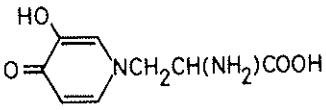
The lower homologue of hypoglycin A has been isolated from the seeds of *Litchi chinensis* by Gray and Fowden [28]. As litchi



XIII

seeds, unlike the fruit of *Blighia sapida*, are not a common article of human diet the toxicity of this "uncommon" amino acid has not created a medical problem. Nevertheless the authors found it to be toxic to mice in the laboratory.

Mimosine (β-[N-(3-hydroxypyridone-4)]-α-aminopropionic acid [XIV]



XIV

Mimosine has been detected in various species of the Mimosoideae, one of the three sub-families of the Leguminosae, but has attracted most attention as a constituent of *Leucaena leucocephala* [29]. This tropical legume has been grown widely as a cover crop, but the foliage is toxic to grazing animals. The most striking feature of mimosine poisoning in mammals is its depilatory effect. Sheep which have eaten the plant lose their wool and the same effect is produced by injection or oral administration of the purified amino acid [30]. Mimosine also produces liver damage however [31] and this hepatotoxicity appears to be the principal hurdle in the attempts which have been made to utilise this compound as a chemical shearing agent.

Selenocystathionine [XV]

A second depilatory amino acid is selenocystathionine [32] which accumulates in the seeds of *Lecythis ollaria* (monkey's coconut), a tree of the order Myrtales which grows in Central and South America. The toxicity of these seeds is well-known to people in

$$HOOCCH(NH_2)CH_2SeCH_2CH_2CH(NH_2)COOH$$

XV

the areas concerned but poisonings still occur [33]. The pure amino acid has been shown to inhibit the growth of mouse fibroblasts in culture — an effect reversed by L-cystine. The same seleno amino acid has also been identified in *Astragalus pectinatus* [34] (Leguminosae), in *Stanleya pinnata* [35] (Cruciferae), and in *Neptunia amplexicaulis* [36] an herbaceous legume of Queensland, Australia which is toxic to livestock.

Se-Methyl-selenocysteine [XVI]

$$CH_3SeCH_2CH(NH_2)COOH$$

XVI

VIRUPAKSHA and SHRIFT have shown that the principal toxin of the selenium accumulating species of *Astragalus* (the loco weeds), *Oonopsis condensata* and *Stanleya pinnata* [37, 38] is se-methyl-selenocysteine. These plants which grow on selenium-rich soils in different parts of the world are extremely poisonous to grazing animals, particularly horses.

"UNCOMMON" AMINO ACIDS IN PLANT-INSECT RELATIONSHIPS

While vertebrates clearly exercise major selectionary pressures on some plant populations their overall effect is probably less than that of insects.

The devastating effects of *Locusta* species are proverbial and the trivial name "army worms" given to the larvae of *Prodenia* species acknowledges the destructive ability of the "serried ranks" of these caterpillars. Those of us who collect seeds will also testify to the fact that all, or nearly all, the seeds which we have gathered from a particular species are frequently infested by the larvae of bruchid beetles.

It is a matter of common observation however that most phytophagous insects have their likes and dislikes concerning the plant species on which they and/or their larvae feed. These likes and dislikes must clearly be related to the chemistry of the plants and indeed it has been known for a great many years that some plants are able to synthesize compounds such as the pyrethrins and nicotine which are powerful insecticides.

In the previous section I have reviewed our current knowledge concerning plant amino acids which are toxic to vertebrates, and I have suggested that the presence of these amino acids may have improved the plant's "fitness" to survive in an environment inhabited by vertebrate herbivores. This argument is based on circumstantial evidence and the assumption that a compound which is toxic to man and laboratory animals will in all probability be toxic to monkeys and wild rodents. Where medical and agricultural interests have been involved, compounds such as ODAP and mimosine have been tested against and found toxic to a broad range of higher animals but this is not true of most "uncommon" amino acids. The reasons for this are firstly the difficulty and expense of obtaining "uncommon" amino acids in the quantities required for biological assays using large animals and secondly, a lack of support for research which is not immediately concerned with the improvement of human health or wealth.

Much of the circumstantial evidence provided in the previous section has arisen from medically oriented research. The toxic amino acids which have been studied are those which are toxic to man or to his domestic animals. No comparable volume of information on compounds which are toxic to insects exists. Fortunately it is possible to obtain evidence if we can enlist the active co-operation of field ecologists, biologists and phytochemists. I personally have been fortunate in having the help of Professor D. H. Janzen for many years, and more recently to have enjoyed the collaboration of Professor Chapman and Dr. Bernays, who are themselves contributors to this Symposium. Janzen and I have been primarily interested in the possible role of "uncommon" amino acids in protecting legume seeds against attack by the larvae of bruchid beetles. This interest originated from an observation by Janzen that the seeds of some legume species in a particular tropical habitat were attacked by beet-

les while others were not and my recognition of the resistant seeds as being ones which contained high concentrations of "uncommon" amino acids. Our problem was then to prove or disprove the hypothesis that the "uncommon" amino acids could protect plants from insect attack. Quantitative analyses of L-dopa in individual seeds of different *Mucuna* species [2], which were free from insect predation, showed that the concentration of this amino acid was remarkably constant from seed to seed, and species to species. This finding was consistent with a protective role for the amino acid in that "the seeds of genotypes producing lower concentrations would be susceptible to attack by a wider range of insects and animals" while "seedlings from seeds with excess concentrations of L-dopa would on the other hand, be at a disadvantage when in competition with seedlings using a higher proportion of their seed reserves for normal vegetative purposes". Another factor which appeared to support this hypothesis was the relationship which Janzen found between seed size, crop weight and bruchid predation [39]. Using a sample of 36 species of woody Central American Leguminosae he showed that the seeds of unattacked species were larger than those of attacked species, but the large seeded species produced not only fewer seeds but a smaller weight of seeds per seed crop per unit of photosynthetic ability. These legumes appear in fact to have adopted two distinct strategies to avoid destruction by bruchid beetles, the first strategy being to produce a large number of small seeds to ensure that the beetles do not find them all, and the second being to make a smaller number of large seeds and provide them with chemical protection. Seen in terms of the plant's overall economy this second strategy justifies, as a mere storage function does not, the diversion of resources to the synthesis of special enzymes for the production of "uncommon" amino acids. In terms of primary metabolic requirements, the presence of 10% of canavanine in a seed would seem to represent an extravagant use of enzyme resources. If however the presence of 10% of canavanine saves 20% of the seed crop from destruction then its presence is clearly advantageous to the plant, and the resources diverted to the synthesis of canavanine may very well be less than those which would otherwise be required to make good losses due to predation.

The first direct attempt to determine the toxicity of "uncommon"

plant amino acids to insects was made with the larvae of *Prodenia eridania* (the southern army worm) [40], which is a polyphagous species. The larvae were fed on artificial diets to which were added, in various proportions, the ground seed of different legume species, and also purified "uncommon" amino acids isolated from legume species. These experiments showed that canavanine and 2(S), 3(S), 4(R) β-hydroxy-γ-methylglutamic acid [XVII] which had been isolat-

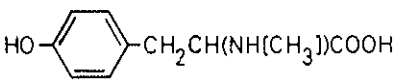
$$HOOCCH(CH_3)CH(OH)CH(NH_2)COOH$$

XVII

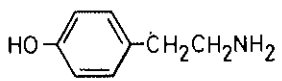
ed from the bruchid-free seeds of *Gymnocladus dioicus* [41, 42] were repellent to the larvae producing 100% mortality when supplied in artificial diets at concentrations equivalent to those found in the seeds themselves. At low levels 5-hydroxytryptophan, the "uncommon" amino acid of *Griffonia simplicifolia* seeds [1], proved toxic to the larvae and at the concentrations found in the seed it was repellent. A similar variability in toxicity was observed with the L-dopa of *Mucuna* seeds. In these experiments the larvae were repelled by concentrations of L-dopa equivalent to those found in the seeds, but appeared to thrive on diets which contained approximately one-thirtieth of the L-dopa found in the seeds. On metamorphosis however the normal hard black pupal case failed to form, suggesting that the dietary L-dopa even at low concentration could significantly effect tyrosinase activity in the insect [43].

Although these findings were of great interest, the larvae of *Prodenia eridania* are not seed eaters and it was clearly necessary to test the toxicity of "uncommon" seed amino acids against insects which were. The insect chosen for this purpose was *Callosobruchus maculatus* (Bruchidae) [44] which is popularly known as the southern cowpea weevil because its larvae constitute a major economic pest in eating seeds of *Vigna unguiculata*, the cowpea. Synthetic "seeds" were manufactured by compressing finely ground cowpea flour in a pharmaceutical pill press to provide cylindrical "seeds" of 17 mm diameter and 7 mm depth. The adult female insects have no objection to laying their eggs on these "seeds" and for the purpose of the experiments ten eggs were allowed to remain on each "seed".

The larvae hatching from the eggs develop in the "seeds" and the eventual number of adult insects emerging from each "seed" can be counted. The toxicity of "uncommon" amino acids from the seeds of other legume species to the larvae of *C. maculatus* was determined in the following way. The "uncommon" amino acids were mixed in increasing proportions with the cowpea meal prior to manufacturing the "seeds" and the numbers of adult insects emerging from "seeds" with added amino acids compared with the numbers emerging from control "seeds". The results of these experiments showed that 5-hydroxytryptophan, N-methyltyrosine [XVIII], mimosine, β-cyanoalanine, azetidine-2-carboxylic acid and the amines, tyramine [XIX] and β-aminopropionitrile [XX] were lethal at 1% concentration. At this concentration canavanine, α,γ-diaminobutyric acid, γ-methylglutamic acid [XXI] and m-carboxyphenylanine [XXII], produced a significant effect on the number of adults emerging and all proved lethal at a concentration of 5%. At 1% homoarginine [XXIII],



XVIII



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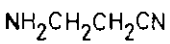

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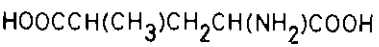
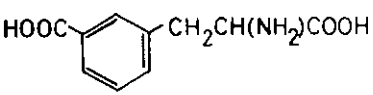
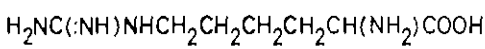


XXI

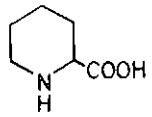


XXII



XXIII

pipecolic acid [XXIV], S-carboxy-ethylcysteine [XXV], albizzine and L-dopa produced no significant effect on the numbers of adults emerging, at 5% however the effect of homoarginine and pipecolic acid was significant and that of the remaining three lethal.



XXIV

$HOOCCH_2CH_2SCH_2CH(NH_2)COOH$

XXV

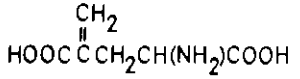
Control experiments using common amino acids showed that the addition of these to the diet could also decrease the number of adults emerging. No common amino acid proved lethal at a concentration of 1% however. At a concentration of 5%, 32% of the common amino acids were lethal compared with 89% of the uncommon amino acids. While clearly a major imbalance of normal nutrients is able to affect beetle production there is a very significant difference in the effect produced by adding common and uncommon amino acids to the diet.

Plants and insects are both evolving however and there is no guarantee that the ability to synthesise a defensive compound will protect a plant indefinitely. Mutant forms of insects as well as mutant forms of plants are continuously arising. If one of these mutant insects is capable of metabolising or in some other way neutralising the effect of the plant's defensive compound then it has increased its own chances of survival in that it has a source of food which is not available to forms not adapted in this way. For some time I believed that all *Mucuna* seeds were resistant to attack by bruchid beetles and kept on saying so. Now I know that 99% of *Mucuna* seeds may be resistant, but not all, for I have been given a *Mucuna* seed from the East Indies which contained L-dopa just like all the rest, but also an unmistakable bruchid. A bruchid which has solved the problem of living with 10% of L-dopa. In the same way the presence of canavanine appears to protect seeds from most, but not all, bruchids. An example of canavanine's failure in this respect is seen in the infestation of *Dioclea megacarpa* seeds by *Caryedes brasiliensis*. The seeds of this species contain over 8% of canavanine yet the larvae of *C. brasiliensis* thrive in them. Investigating this apparent anomaly ROSENTHAL and co-workers [3] have shown that the insect possesses both an arginyl-tRNA synthetase which discriminates against canavanine (thereby preventing its incorporation into

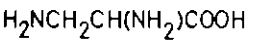
insect protein) and enzymes capable of degrading the amino acid. That an insect species has evolved mechanisms for discriminating against and detoxicating an "uncommon" amino acid suggests once again that some at least of these secondary compounds play a significant role in protecting the plants which make them from insect attack.

In preliminary experiments E. A. BERNAYS and A. NAVON have shown (private communication) that free amino acids fed to 3 grasshopper species can either stimulate or inhibit feeding. Ten "common" amino acids and 14 "uncommon" amino acids were used in the experiments. In each experiment a solution containing the amino acid (0.01M) and sucrose (0.1M) was evaporated on a glass fibre filter providing a solid mixture of sucrose and amino acid equivalent to approximately 10% of the weight of the filter for the insects; a solution of sucrose (0.1M) was used in preparing "controls". It was found that 3 "common" amino acids (serine, proline and α-aminoadipic acid) slightly stimulated feeding (11-20%) in the polyphagous insect *Schistocerca gregaria* (Fovskal) while 2 "common" amino acids (asparagine and methionine) produced an inhibition (20-50%). Of the "uncommon" amino acids tested 3 (canavanine, homoarginine and α,γ-diaminobutyric acid) produced an inhibition of 20-50%, while the effect of L-dopa (51-90% inhibition) was greater. None of the "uncommon" amino acids stimulated feeding in *Schistocerca*.

The insect *Chortoicetes terminifera* (Walker) which feeds on a more restricted diet of grasses and legume leaves showed a greater response to the amino acids. Feeding was increased by over 40% by serine and proline but inhibited by 51-90% by the "common" amino acids methionine, α-aminoadipic acid and citrulline, and by 20-50% by β-alanine. At the same concentration 2 "uncommon" amino acids (canavanine and L-dopa) produced 91-100% inhibition, and all the other "uncommon" amino acids used (γ-methyleneglutamic acid, [XXVI], albizzine, β-cyanoalanine, pipecolic acid, mimosine, homoarginine, α,β-diaminopropionic acid [XXVII], α-amino-β-oxalylaminopropionic acid, α,γ-diaminobutyric acid, α-oxalylamino-γ-aminobu-

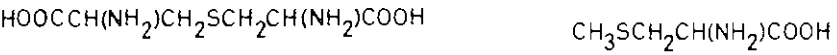


XXVI



XXVII

tyric acid, djenkolic acid [XXVIII] and S-methylcysteine [XXIX] with the exception of mimosine produced lesser degrees of inhibition. Mimosine neither stimulated nor inhibited feeding.



XXVIII

XXIX

The feeding of the third insect *Locusta migratoria* (L.) which is strictly graminivorous, was stimulated over 41% by α-aminoadipic acid and β-alanine and to a lesser extent by serine and proline. It was inhibited (20-50%) by arginine and unaffected by the remaining "common" amino acids (asparagine, cysteine, methionine, homoserine and citrulline).

On the basis of the restricted data available it is apparent that there is considerable variation in the reaction of the three insect species studied to free amino acids at the arbitrary concentrations used. The polyphagous insect was least affected, 20 % of the "common" amino acids and 28% of the "uncommon" amino acids being inhibitors and 30% of the "common" amino acids stimulators. With *C. terminifera* 40% of the "common" and 93% of the "uncommon" amino acids were inhibitors, while 20% of the "common" amino acids were stimulators.

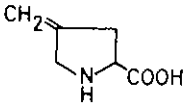
With *L. migratoria* 10% of the "common" amino and 93% of the "uncommon" amino acids were inhibitors and 40% of the "common" amino acids stimulators. No "uncommon" amino acid had a stimulating effect in any one of the insects.

These findings suggest that some "uncommon" amino acids may be repellent as well as toxic to insects.

"Uncommon" Amino Acids in plant - plant relationships

In the two previous sections I have considered the possibility that "uncommon" amino acids may have a defensive role and protect the plants which accumulate them from animal and insect predators. In this section I shall discuss the possibility that these compounds

may also improve a plant's chances of survival by discouraging the growth of competitive plant species. Fowden in an early paper [45] described the effects of a number of naturally occurring "uncommon" amino acids on the growth of mung bean (*Vigna* (formerly *Phaseolus*) *aureus*) seedlings. Of the amino acids which he used, azetidine-2-carboxylic acid had the most powerful inhibitory effects while α-(methylenecyclopropyl)glycine and 4-methyleneproline [XXX] were less inhibitory. Pipecolic acid, γ-methyleneglutamic acid and β-pyrazol-1-ylalanine [XXXI] produced no inhibition at the concen-



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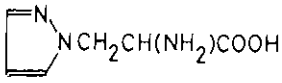
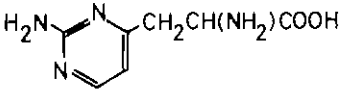


XXXI

trations used. Further studies showed that azetidine-2-carboxylic acid was replacing proline in the protein of the mung bean seedlings, the replacement being almost complete in newly synthesized protein after dormant seeds had been allowed to imbibe relatively high concentrations of azetidine-2-carboxylic acid in solution before germination. Similar inhibition was produced in the seedlings of other species such as barley, peanut and cucumber, and carrot tissue in culture [46]. A highly significant result, however, was the finding of FOWDEN and co-workers [46, 47, 48], that azetidine-2-carboxylic acid was not incorporated into the proteins of *Convallaria majalis* (Lily of the valley) and *Polygonatum multiflorum* (Solomon's seal) which are Liliaceous species which synthesise this "uncommon" amino acid. It was found moreover that the proline activating enzyme of these two species did not activate azetidine-2-carboxylic acid while the enzymes of mung bean and pea (*Pisum sativum*) were able to do so. The two Liliaceous species are protected from the potentially lethal compound which they synthesise, by the greater specificity of their proline activating enzymes (prolyl-transfer ribonucleic acid synthetases) which can distinguish between proline and its lower homologue and discriminate against the smaller molecule.

Mimosine also inhibits seedling growth in the mung bean [49] while canavanine has been shown to inhibit growth in numerous plant species when supplied either to the whole plant or to cultured

cells [50, 51, 52, 53, 54]. Canavanine has also been found to inhibit pollen tube development in some species, as has lathyrine [XXXII] in species of *Lathyrus* which do not synthesise this heterocyclic amino acid, providing it would seem a chemical barrier to hybridisation between certain species within the genus [55]. More recently another amino acid, N-dimethyltryptophan [XXXIII] has been identified as the compound in seeds of *Abrus precatorius* which inhibits seedling growth in other species [56].



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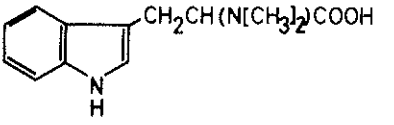

XXXIII

Under laboratory conditions it is now clear that an "uncommon" amino acid produced by one species of plant may be toxic to another species, and it is tempting to speculate that the presence of such an amino acid may be advantageous to the plant containing it by restricting competition from other species. In the natural environment however conditions are more complex than in the laboratory and much more information on the release of "uncommon" amino acids into the soil, their uptake by other species and their degradation by microorganisms is needed before we can be certain that they can act as plant growth inhibitors in a natural environment.

Relevance of "uncommon" amino acids to world food supplies

From the foregoing sections it will be clear that there is great variability in the capacity of different species of plants to synthesise "uncommon" amino acids. There is also great variability in the capacity of plants and other organisms to metabolise tbe "uncommon" amino acids which the plants have made. We know that a particular "uncommon" amino acid may act as an essential nutrient in one organism and yet be toxic to another. We also know that an "un-

common" amino acid may accumulate in one organ of a plant and be absent from another organ.

Because certain of these compounds are toxic to mammals (including man) and because certain, but not always the same ones, are toxic to insects and other forms of life a knowledge of their distribution and physiological activity is very relevant to the selection and development of plant species as human and animal food.

Possibly the greatest problems facing mankind at the present time are the problems of limiting the world population in the long term and feeding increased numbers in the short term.

Agricultural output can be increased in two ways. Established crop species can be improved, modified and introduced into new areas of the world, or wild species and species grown on a restricted scale or used only in times of famine may be developed as new major crops.

Agricultural philosophy has tended in the past to favour the improvement of old well-established crops and their modification for use in a wide range of environments. I believe that more attention should be given to the second alternative. There may be many instances where it would be easier and cheaper to improve a native crop or bring a wild species into cultivation, rather than attempt to grow crops from another region in a less than ideal environment.

My work has been largely concerned with one plant family, the Leguminosae, and I believe that a thorough understanding of the distribution and characteristics of "uncommon" amino acids in species of this family will provide invaluable information as to the potential of species and genera as food for man and animals.

The legumes seem at first sight to be ideally suited for agricultural development. With their associated nodular bacteria they can fix atmospheric nitrogen which allows them to grow without added inorganic nitrogen on nitrogen-poor soils. Many species of the family are well adapted to the semi-arid regions of the world and provide fodder for wild and domestic animals when drought has deprived the animals of almost all other foods. The seeds of legumes are usually much richer in protein than are the seeds of the cereals.

Why then do we find only about 20 legume species grown as important world crops and perhaps 100 more as local crops which

are used regularly or occasionally as "famine" foods? The answer seems to be that most legumes contain secondary compounds such as alkaloids, cyanogenic glycosides and certain of the "uncommon" amino acids which are toxic to man or his domestic animals.

In old established cultivated varieties we frequently find that the concentrations of toxins are low, reflecting conscious selection by man of more palatable and acceptable strains over a prolonged period of time. Time however is not on our side and if we are to double a country's food supply in 25 years (the population of Pakistan will double in that time) we must consider wild species and little used crops which have been neglected in the past. If these have been neglected because of the presence of unidentified toxins we may, when we have identified the toxins, be able to use our knowledge to develop toxin-low or toxin-free strains which will contribute to the world's food supplies. A good example of this approach is seen already in reduction of HCN yield from 312 mg per 100 grms in the coloured lima bean (*Phaseolus lunatus*) grown in Java to 10 mg per 100 gm in the white variety of the same species grown in America [57]. Another example is the reduction of ODAP concentrations in *Lathyrus sativus* seeds in India. In an early paper [16] I recorded the occurrence and distribution of various toxic amino acids in tbe seeds of 53 species of *Lathyrus* and suggested that human lathyrism in India might be eradicated by replacing *Lathyrus sativus* with one or more of the *Lathyrus* species in which I had found no ODAP. Rather than attempt this rather radical solution Indian agricultural research workers have heen applying the analytical techniques developed for the identification of ODAP to screen varieties of *Lathyrus sativus* and select low-toxin strains which can be used to replace the high-toxin strains responsible for the disease. The rationale of this approach is that *Lathyrus sativus* grows well in poor areas, it is rich in protein and there is no need to persuade the farmers to change to a different species which is unfamiliar to them and possibly less well adapted to the local conditions.

This type of solution can only be applied if the nature of the toxicity is understood and the distribution of the toxin known. At King's College our efforts have been directed towards the accumulation of a "bank" of information on "uncommon" amino acids in legumes. To date we have analysed the seeds of some 1500 species

representing 300 of the 700 genera, which have been collected with the aid of Dr. B. A. KRUKOFF, and are continuing. With adequate support we hope to extend this work to the seed proteins and screen routinely for the presence or absence of alkaloids and cyanogenic glycosides. We have adapted an automatic analyser to recognise "uncommon" as well as "common" amino acids and the quantitative data from this analyser is stored on magnetic tape in a computer bank. Eventually when the "bank" is complete we shall be able to retrieve without delay basic information on the essential amino acid content of any species and also information on the nature and concentration of any toxic amino acid present. Knowing the potential nutritive value of a seed and the variability of a toxin in the same species, a judgment will then be possible on the feasibility of developing a toxin-low variety of that species for growing in a given area.

Basic knowledge of the toxicity of these compounds to insects is no less important. The development of a toxin-free crop would be totally impractical if the reduction in toxicity to man or domestic animals was accompanied by an equal or greater reduction in toxicity to predatory insects which might destroy the crop before it could be harvested. Such a result is not inevitable however, because as we have seen, an amino acid which is toxic to one form of life may not be toxic to another. Within the past month, for example, we have found that the leaves of some *Acacia* species are rich in homoarginine which can replace lysine in the mammalian diet. The preliminary findings of Bernays and Navon indicate nevertheless that this same guanidino amino acid is a feeding inhibitor in *Locusta*.

In discussing the nutrient value of plants (in particular legume seeds) I have spoken of toxic amino acids which occur in the free state. It is usual to find other free amino acids "common" and "uncommon" in plants and these may account for more than 10% of available nitrogen. In the cooking of food much of this free amino acid pool is lost. Where processing is done on a commercial scale thought should be given to the extraction of "essential" amino acids from the effluent of the processing plants. Here again however basic information on the nature and content of the amino acid pool is required before the feasibility of recovering essential nutrients in this fashion is considered.

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DISCUSSION

MARINI-BETTÒLO

Thank you, Professor Bell, for this excellent presentation. Now I open the discussion. I just wondered about the last observation which you made. I was told recently that there is a program in the Andean highlands to reduce the alkaloid content of lupins which are used for food. Unfortunately when the geneticists remove all the alkaloids the alkaloid-free lupins very seldom reach maturity as they are destroyed by insects. I think that this is an example of what we were considering, the presence of secondary substances preventing attack by insects.

BELL

Yes, I understand that similar effects with lupins have been observed in Australia. Clearly if a secondary compound protects a crop against insect attack but is also toxic to man we have a potential problem. If the compound is more toxic to insects than mammals it may be possible to develop a strain of the crop species which contains enough secondary compound to repel insects but not enough to poison man. Alternatively, as in the case of the seeds of *Lathyrus sativus*, no toxic symptoms may develop in man unless the seed constitutes a major part of the diet. The seeds may therefore be perfectly satisfactory as human food if they constitute less than a certain proportion of the diet. I suspect that the toxicity of "uncommon" amino acids such as occur in *L. sativus* is enhanced when there is a deficiency of their common analogues (as during periods of famine) in the diet. Under these circumstances the "uncommon" amino acids may be able to compete successfully for enzyme sites normally concerned with the metabolism of the common analogues. We know, however, that some secondary compounds are toxic to insects but not to mammals and we should perhaps look for others and consider how such natural protection can be introduced into or selected for in crop plants.

NAKANISHI

There are two questions. One is a simple one; that is, do you happen to know the pK of the guanidoxy group of canavanine?

BELL

It is 6.6, according to the literature.

NAKANISHI

Next questions are: Have you looked for the presence of D-amino acids, and have you tried their effect on insects or plants — because these would be also uncommon.

BELL

No, D-amino acids have been reported in higher plants — usually as their N-malonyl derivatives — but I have no information about their activity in insects or other plants.

NAKANISHI

I would like to call your attention to a simple reagent to distinguish between D and L amino acids. It's a Hoffmann La Roche reagent and is a derivative of fluorescamine. It gives a much stabler derivative and simply by measuring the rotation or circular dichroism spectra one can determine whether the amino-acid is D or L (*).

I just want to give this as a general information. It only reacts with primary amino-groups and in the presence of other functional groups. We have done this on 40 micrograms of a very complicated nucleic acid and determined its absolute configuration (unpublished).

BELL

When I said we had not looked for D-amino acids or studied their effects I overlooked one example. We synthesised both forms of

(*) The reagent is called MDPF (cf. WEIGELE *et al.*, Tetrahedron, October, 1975).

α-amino-β-methylaminopropionic acid. The L-form was toxic in mice and chicks, the D-form was not.

STAAL

I disagree somewhat with your interpretation concerning the abnormalities in the lepidopteran pupae. The transparent cuticle, visible in the treated pupae, is present in every pupa, but it is usually covered by the wings. It appears that the wings of your pupae were not fully expanded. This is a phenomenon commonly observed if the diet lacks unsaturated fatty acids and it is easily corrected by adding linoleic and linolenic acid to the diet. I have no idea what caused the defect.

BELL

Thank you. I was just guessing that the apparent lack of pigmentation might be due to an inhibition of polyphenoloxidase by excess L-dopa.

GILBERT

I just wanted to ask whether homoarginine has ever been used for treatment of staphylococcal infections. You said that it was toxic to Staphylococcus and not to mammals.

BELL

That is correct. Synthetic L-homoarginine was found to be toxic to *Staphylococcus aureus* in the laboratory, and also to *Candida albicans*. I do not know if it has been used in medicine.

SOMERVILLE

I would just like to ask a simple question. What happens to these amino acids when the seed germinates? There seems to be a very high level in the seeds.

BELL

There isn't a single answer to this question. Once upon a time I thought they all disappeared and were incorporated into the protein amino acids. Serial experiments showed, for example, that canavanine was rapidly metabolised in germinating seeds and seedlings of *Medicago sativa*. In *Albizzia julibrissin*, however, albizzine occurs as a major free amino acid in both the seed and the leaves of the plant.

WILLIAMS

I should think those *Mucuna* seeds with all the L-dopa might provide cure for people with Parkinson's disease.

BELL

Yes. I suggested this medical use for *Mucuna* seeds in the original publication.

WILLIAMS

Was it cheap enough?

BELL

I suspect the economics vary from country to country. It could be cheaper to synthesise L-dopa in California but cheaper to grow *Mucuna* seeds and extract them in countries where labour is relatively cheap.

GILBERT

Can I answer that one? Merck-Darmstadt is getting L-dopa in Brazil from the plants.

KNÜSLI

I was impressed by the picture of the Australian sheep. Am I right in thinking that the Australian government once looked at this as a means for shearing sheep?

BELL

Yes. The cost of mimosine, and toxic side-effects are the main difficulties.

ABO-KHATWA

Your discovery regarding the pipecolic acid which is a homologue of proline is a rather interesting one. As you may know, some insects utilize proline in their flight muscles as a source of energy, particularly tsetse flies. This reminds me of work back in the 60's when O'Brien and Sacter described some analogs of alpha glycerophosphatase. They developed several compounds related to alpha glycerophosphatases in attempts to inhibit the α-glycerophosphate shuttle. These compounds were quite important *in vitro* but they were not studied *in vivo*. So if you can kindly provide me with some of these amino acids like pipecolic acid, I might try them against tsetse flies and see how effective they are.

BELL

If I might say so I think there is a better chance of it working with a lower homologue than with a higher homologue. In general where you have homologues, the lower ones tend to be more active, presumably because they can get into enzyme sites. If I may make a guess, I'd say you have a better chance with azetidine-2-carboxylic acid than with pipecolic acid. There is a point I should perhaps have made earlier and that is the proline-t-RNA-synthetase of *Convallaria majalis* (Lily of the Valley) discriminates against azetidine-2-carboxylic acid, that is to say, the plant which makes the lower homologue does not incorporate it into its own protein.

NAKANISHI

Could you give a general comment on this problem of cross-breeding plants so that you get a higher constituent of what you're after? The reason I ask this is that recently I heard that Professor David Lavie's group who are working on cucurbitacins — there are more than 30 congenors known — have by crossbreeding many northern and southern Israeli

species been able to in a sense wipe out differentiation between species.

I was just wondering whether you might make a general comment on such crossbreeding.

BELL

Yes. I think this would depend on whether you are talking about crossing species or strains of the same species?

NAKANISHI

Species.

BELL

When we studied *Lathyrus*, a genus with clearly defined sub-genera based on biochemistry, I was anxious to discover what happened to the amino acid patterns when hybrids between species of different sub-genera were made. We found that in no case was it possible to cross species that had different amino acid patterns.

THE CHEMICAL RESISTANCE OF PLANTS TO INSECT ATTACK

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All phytophagous insects, including polyphagous species, exhibit some degree of preference in the plants which they select to eat. This selection implies some degree of resistance to insect attack on the part of the non-host plants, and it is in this broad sense that we consider resistance in this paper.

Selection may be influenced by the physical character of the plant, but there is evidence to show that chemical factors are of very great, and often of overriding importance. Even before an insect bites a leaf it is subject to stimulation by volatile chemicals derived from the leaf and by the chemicals on the leaf surface; as soon as it bites it is exposed to an even larger number which are normally contained within the leaf. The insect is capable of perceiving many of these chemicals which individually may act as phagostimulants, or as deterrents of feeding, or which may induce no overt behaviour. In practice whether or not the insect feeds depends on the balance of phagostimulants and deterrents; the decision is made within the central nervous system on the basis of all the information received. Sometime one chemical may be particularly important in governing host-plant selection, but even in these cases the insect is affected by the overall chemical milieu.

Although much has been written about the responses of phytophagous insects to specific chemicals or particular plants, it is difficult to form any general impression of the role of chemicals in host-

plant selection by any one group of insects. The most comprehensive studies have been on the species *Leptinotarsa* of JERMY (1961) and HSIAO (HSIAO and FRAENKEL, 1968 a, b, c; HSIAO, 1969, 1974) and in larval Lepidoptera by *e.g.* GUPTA and THORSTEINSON (1960), MEISNER and ASCHER (1972) and HAMAMURA (1970). In this paper we have attempted to make a synthesis of the known chemical interactions of acridids with their host-plants, considering how these interactions may be modified by behavioural, physiological and ecological phenomena. The relevance of this work to the development and use of resistant varieties of crop plants is then discussed.

Chemicals acting at a distance from the plant

Plant odours may attract or repel insects. Laboratory experiments have shown that nymphs of *Schistocerca gregaria* (Forskål) and other species are attracted over a distance of about 1m by the odour of crushed grass (MOORHOUSE, 1969, 1971). The principal attractant chemicals are found in the carboxylic acid and neutral fractions derived from sequential chemical extraction of *Lolium perenne*, but the specific chemicals were not identified (C. L. GREEN, unpublished reports). KENNEDY and MOORHOUSE (1969) showed that the upwind response in the wind-tunnel was non-specific, the insects responding in the same way even to stimuli not emanating from the plant.

GOODHUE (1962) observed that nymphs of *Schistocerca* turned away from leaves of *Lavendula* without touching it with the mouthparts; an olfactory repellent is implied. To date there is no evidence of specific chemical attraction of acridids to host-plants but some strongly odoriferous plants may be repellent.

Phagostimulants and antifeedants

There are numerous examples of the normal host-plant ranges of acridids (*e.g.* MULKERN *et al.*, 1964; GUIDO and PERKINS, 1975), but in few instances has the basis of selection been investigated and in even fewer have short-term feeding effects, phagostimulation or deterrence, been differentiated from long term physiological effects involving nutrition or antibiosis. We consider that in any feeding

experiment lasting more than 24h it is impossible to differentiate physiological from behavioural effects of chemicals and in this section we have included only experiments of short duration, usually less than 6h.

Locusta migratoria (L.) is normally graminivorous (BERNAYS *et al.*, 1976). In a series of experiments, fifth instar nymphs were offered 187 different species of plants from many different families: full meals of more than 80mg were only on species of Gramineae and the closely related Juncaceae and Cyperaceae. Extracts of 98 species were made with chloroform, acetone, methanol and water and the plants which were not eaten yielded one or more extracts which inhibited feeding on wheat flour discs (Table 1). This was true of those grasses which were relatively unpalatable as well as of the dicotyledons, but the plants which were eaten in large amounts produced no fractions which inhibited feeding; often their extracts were phagostimulatory (BERNAYS and CHAPMAN, 1977).

A similar result was obtained from a less extensive survey of potential host-plants of *Chorthippus parallelus* (Zetterstedt) which is also graminivorous (BERNAYS and CHAPMAN, 1970a, 1975). Eleven dicotyledons were rejected by the grasshoppers and all yielded deterrent fractions. Of four grasses which were normally eaten in large amounts, only one produced a deterrent fraction, but other extracts from the same species were phagostimulatory. *Anthoxanthum odoratum* was less acceptable than other grasses and it produced a deterrent extract.

Schistocerca is polyphagous, eating a wide range of plants, but again the plants which were not eaten generally yielded fractions which reduced feeding on starch discs (Table 1). Many extracts promoted feeding, including some which inhibited feeding by *Locusta*.

It is possible, in some cases, to enhance the palatability of a plant by removing the deterrent chemicals. For instance, the average size of a single meal of *Lactuca* eaten by nymphs of *Locusta* was increased from 36mg to 108mg if the leaves were soaked in chloroform to remove lipids before being offered to the insects.

In these three species, feeding on different ranges of plants, it is certain that chemicals in the plants play a dominant role in the discrimination of host from non-host plants. Chemicals which in-

TABLE 1 - *Correlation of meal size on different plants with the production of extracts which inhibited or promoted feeding. A large meal for* Locusta *is greater than 80mg, for* Schistocerca *greater than 100mg. The category "small meal" includes many plants which were not eaten at all.*

Plant group	Meal size	*Locusta*				*Schistocerca*			
		Number of species	Number with extracts			Number of species	Number with extracts		
			inhibiting	no effect	promoting		inhibiting	no effect	promoting
Compositae	large	0	—	—	—	6	—	—	6
	small	12	12	—	—	1	1	—	—
Euphorbiaceae	large	0	—	—	—	0	—	—	—
	small	4	4	—	—	2	2	—	—
Leguminosae	large	0	—	—	—	7	—	—	7
	small	11	11	—	—	0	—	—	—
other dicotyledons	large	0	—	—	—	22	—	1	21
	small	43	43	—	—	9	8	1	—
Gramineae	large	14	—	8	6	9	—	2	7
	small	8	8	—	—	2	1	—	1
other monocotyledons	large	1	—	1	—	3	—	—	3
	small	6	5	1	—	3	2	—	1

hibit feeding, usually called feeding deterrents or antifeedants, are particularly important.

A wide range of chemicals known to occur in plants has been tested to evaluate their effects on short-term food intake. These tests have been made with *Locusta* and *Schistocerca* (DADD, 1960; COOK, in press; BERNAYS and CHAPMAN, 1977) and with *Melanoplus bivittatus* (Say) (HARLEY and THORSTEINSON, 1967). In all cases the chemicals have been evaluated over a range of concentrations approximating to that in which they occur in plants. All the major chemical classes of plant constituents have been tested.

Sugars are the principal phagostimulants for *Locusta* (Table 2). A few amino acids are also stimulatory, but their effect is small compared with the sugars (Fig. 1) (COOK, in press). The high molecular weight carboxylic acids also stimulate feeding; they are frequent components of leaf-surface waxes. Sucrose and fructose, both principal leaf sugars, increased the intake of pith discs by over 40 times at typical leaf concentrations whereas maximum stimulation by the amino acids and carboxylic acids was much less even at high concentrations (Fig. 1).

The sugars are also stimulating over a wide range of concentrations (Figs. 2, 3) which correspond with the ranges occurring in host plants and non-host plants. From these data it seems likely that most plants will possess qualities capable of inducing feeding by *Locusta* and the distribution of phagostimulants cannot generally be regarded as limiting the range of plants ingested by tbis species. On the other hand, feeding is deterred by a range of chemicals helonging to many different chemical classes (Table 2). Many of these deterrent chemicals may occur in one plant.

To relate these data on individual chemicals to host-plant selection it is important to understand the manner in which they interact with each other. Complex mixtures have not yet been investigated, but experiments with pairs of phagostimulants or pairs of deterrents indicate that they act additively; there is no evidence from the work on *Locusta* and *Schistocerca* of synergism (Table 3). THORSTEINSON (1960), however, reports that synergism does occur between some amino acids presented in a mixture to *Camnula pellucida* (Scudder), another grass-feeding species. He also observed enhancement of

TABLE 2 - *Effects of different chemicals at naturally occurring concentrations on the consumption of sucrose impregnated discs by* Locusta *and* Schistocerca. *Chemicals are categorised as inhibiting, having no effect, or promoting feeding. The numbers show the number of chemicals in each class which produce the effect.*

Chemical Group	*Locusta*				*Schistocerca*			
	Number of chemicals				Number of chemicals			
	total tested	inhibiting	having no effect	promoting	total tested	inhibiting	having no effect	promoting
Inorganic salts	7		6	1	6		5	1
Sugars								
hexoses	6		1	5+	5		1	4*
pentoses	4	1	2	1+	1		1	*
disaccharides	6		1	5+	3			3*
polysaccharides	2			2+	1			1*
sugar alcohols	3			3+	—			
Amino acids								
protein	19		14	5+	6		4	2
non-protein	18	7	9	2	18		17	1
Carboxylic acids								
low mol. wt.	4	1	3		3			3
high mol wt.	5	1		4	—			
Triglycerides				×				×
Phospholipids	5		5	+	—			×
Alkaloids etc.	16	11	5		13	4	5	4
Sulphur compounds	5	2	3		4		4	
Glycosides	4	3	1		3		2	1
Phenolic compounds								
phenols and flavonoids	14	6	8		12	3	9	
quinones	3		3		3		1	2
phenolic glycosides	5	4	1		5			5
tannins	3	3			3	3		
Terpenoids								
monoterpenes	9	9			9	8		1
sesquirerpenes	11	6+(2)	3		11		11	
diterpenoid	1		1		—			
triterpenes	11	4+(5)	2		1	1		
Steroids	4	1	3		4		2	2
Vitamins	7		7	+	—			
Purines and related compounds	3		3		2		1	1

× indicates that the group of chemicals was found to promote feeding by MEHROTRA and RAO (1972)
\+ data from COOK (in press), method of assessment not revealing possible inhibitory effects
* data from DADD (1960)
figures in brackets indicate inhibitory only at the higher concentrations occurring in plants.

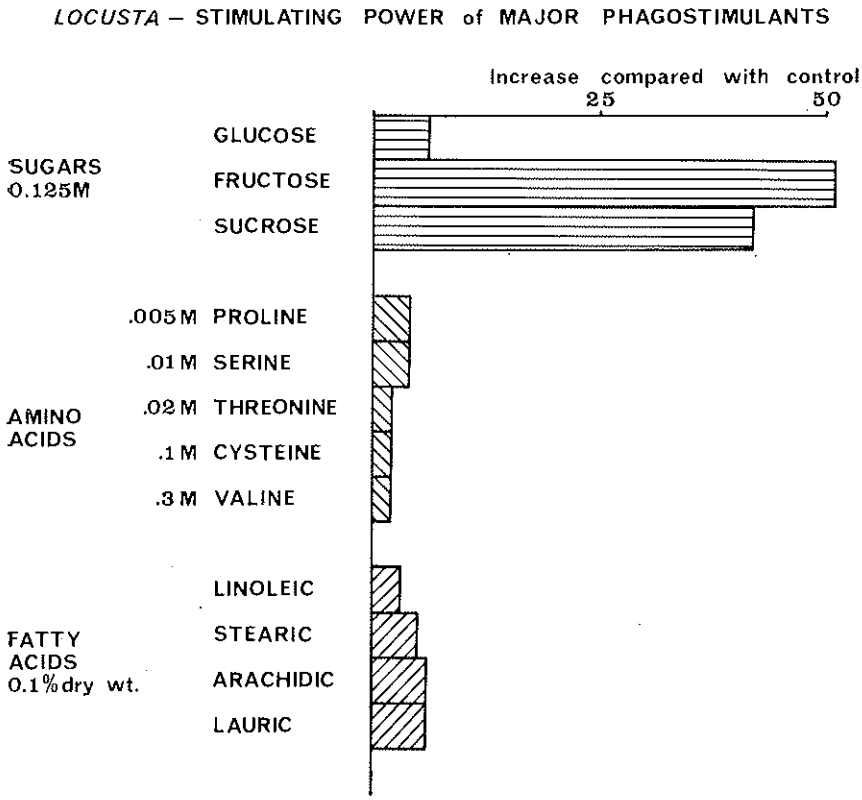

FIG. 1 - Stimulating power of various phagostimulants expressed as the increase in amount of pith disc eaten compared with untreated discs in 18 h experiments with fifth instar *Locusta* nymphs. The concentrations of sugars are similar to those occurring in leaves; the amino acids and fatty acids are generally only found at lower concentrations (data from COOK, in press).

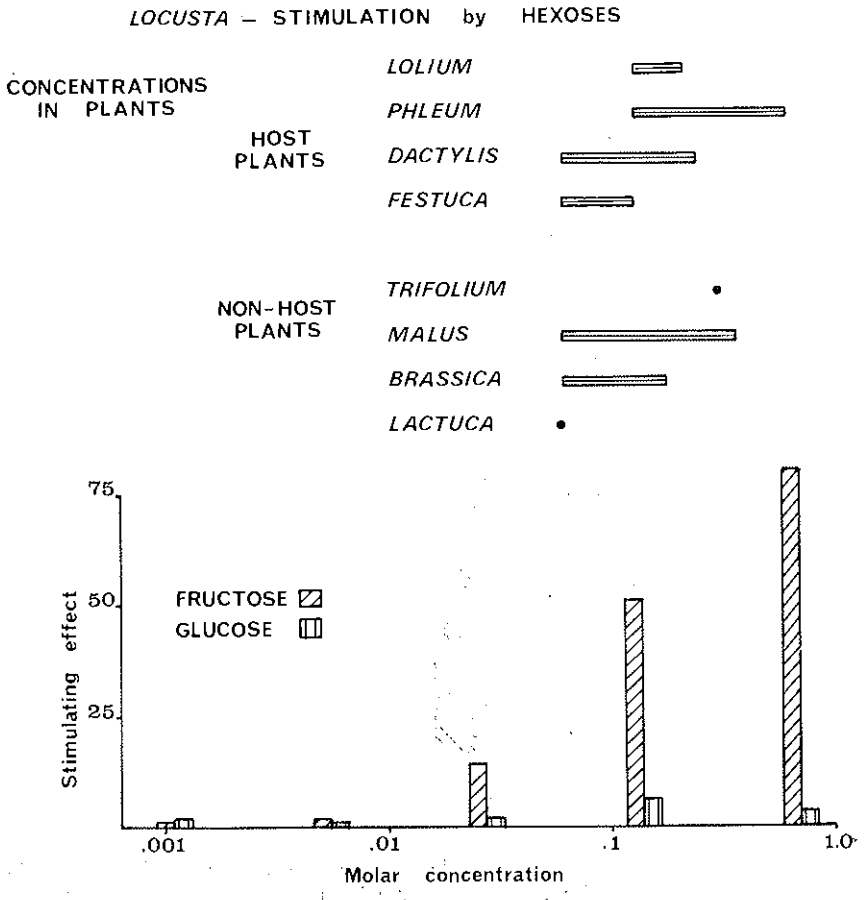

FIG. 2 - Phagostimulatory effect of fructose and glucose for *Locusta* compared with the concentrations of hexose sugars occurring in host and non-host plants. (data partly from COOK, in press).

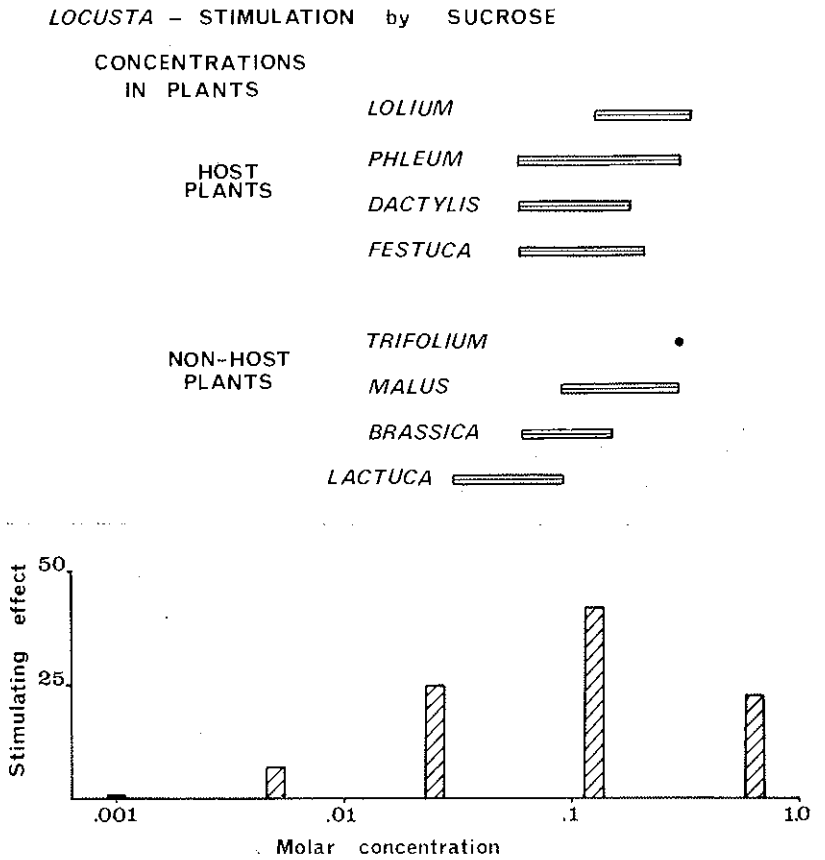

FIG. 3 - Phagostimulatory effect of sucrose for *Locusta* compared with the concentrations occurring in host and non-host plants (data partly from COOK, in press).

TABLE 3 - *Effects of mixtures of chemicals on food intake by* Locusta *nymphs compared with levels expected if the chemicals were additive in their effects. Data on phagostimulants from* COOK (*in press*).

	(% change compared with control)	
Phagostimulants	*expected*	*observed*
0.025M sucrose + 0.0125M serine	+ 68	+ 30
0.025M sucrose + 0.0125M methionine	+ 44	+ 54
0.025M sucrose + 0.0125M ascorbic acid	+ 47	+ 63
0.025M sucrose + 0.1% lecithin	+ 46	+ 57
0.025M sucrose + 0.025M fructose	+ 67	+ 73
0.025M sucrose + 0.02% KH_2PO_4	+ 43	+ 30
Deterrents *		
5.0mM albizzine + 10mM pipecolic acid	— 38	— 43
0.7mM azadirachtin + 10mM pipecolic acid	— 22	— 25
0.7mM azadirachtin + 0.25mM tomatine	— 33	— 21
0.25mM sinigrin + 0.25mM tomatine	— 42	— 42

* All tested on glass fibre discs with 0.1M sucrose.
+ indicates an increase in feeding; — a decrease compared with controls.

sucrose intake with KH_2PO_4 added to the sugar. This effect was not observed by COOK (in press) using a higher salt concentration.

The interaction between phagostimulants and deterrents varies with the relative concentrations of the materials. The addition of 0.1% dry weight of tomatine to wheat flour wafers almost completely inhibits feeding (Fig. 4), but with the increasing concentrations of sucrose the amount eaten is increased. With 0.5M sucrose the tomatine has no effect at all.

Plant chemistry is too complex and our knowledge of its quantitative aspects too scanty to allow us to build up a comprehensive picture of the interactions of the plant chemicals with the insect, but the evidence presented provides reasonable support for the hypothesis that host-plant selection by *Locusta* is governed by the distribution of chemicals which inhibit feeding. We cannot rule out the

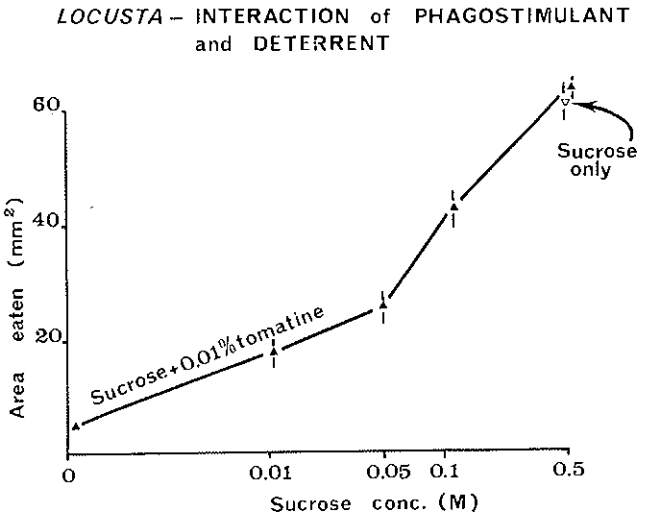

FIG. 4 - Amount of wheat flour wafer eaten by nymphs of *Locusta* in the presence of tomatine. The effect of tomatine was reduced by the addition of sucrose (from BERNAYS and CHAPMAN, in press).

possibility that intake is affected by the *balance* of nutrients, as VAN EMDEN (1972) records in aphids, but we have no evidence of this.

A much wider range of chemicals acts as phagostimulants for *Schistocerca* than is true for *Locusta* (Table 2). Many of the chemicals which deter feeding in *Locusta* are phagostimulants for *Schistocerca* at comparable concentrations. These include secondary plant compounds such as alkaloids and phenolic glycosides. A relatively smaller number of the chemicals tested inhibit feeding by *Schistocerca*, generally at higher concentration than those needed to deter feeding by *Locusta* (Table 4). Azadirachtin is quite exceptional, *Schistocerca* being 1000 times more sensitive than *Locusta*. Both species are very sensitive to aristolochic acid.

The available evidence suggests that host-plant selection by *Schistocerca* is also dependent on the balance between deterrent (antifeedant) chemicals and phagostimulants. The wider range of phagostimulants coupled with reduced sensitivity to antifeedants will account for the much wider range of acceptable host plants.

TABLE 4 - *Dry weight concentrations (% dry weight) of chemicals inducing a 50% reduction in the intake of flour wafers by* Locusta *and* Schistocerca.

Concentrations inhibiting *Schistocerca*	Concentrations inhibiting *Locusta*							
	.000001	.00001	.0001	.001	0.1	.1	1.0	10.0
.000001	.	.	.	.	.	.	.	.
.00001	1*	.	.	.	1+	.	.	.
.0001	.	.	.	.	.	.	.	.
.001	.	.	.	1	.	.	.	.
.01	.	.	.	2	2	.	.	.
.1	.	.	.	3	3	1	.	.
1.0	.	.	.	1	4	3	2	.
10.0	.	.	.	.	.	3	10	16
no effect or stimulating	.	.	.	1	.	6	12	11

* Aristolochic acid.
\+ Azadirachtin.

Melanoplus bivittatus also feeds on a wide range of plants, including grasses and broad-leaved plants (MULKERN *et al.*, 1962, 1964). HARLEY and THORSTEINSON (1967) observed the effects of different secondary plant compounds on the drinking response of the insect. Several of the compounds, including alkaloids, a glucoside and a saponin, inhibited drinking and the same compounds reduced the intake of artificial diet by the insects over 3-day periods. These experiments again indicated that deterrent chemicals are likely to be important in food selection, but there are insufficient data to permit any general conclusion about their role.

No studies have been carried out on the control of feeding in a grasshopper exhibiting a high degree of specificity. It is conceivable that in species with a very restricted host-plant range, feeding is largely governed by the presence of a specific chemical which promotes feeding. Examples of this in other groups of insects are hypericin which stimulates feeding by *Chrysolina brunsvicensis* Gr. on

Hypericum hirsutum (REES, 1969), and the mustard oil glucosides which promote feeding by *Pieris brassicae* (L). and regulate its normal host-plant range (MA, 1972; SCHOONHOVEN, 1973).

Nutrition and antibiosis

Knowledge of the long term chemical interactions of the insect with its host-plant is important for the understanding of development and survival. Many plants do not support survival even when the insects are forced to feed on them by the lack of alternatives, but in no case have sufficiently detailed studies on acridids been made to separate the metabolic effects of chemicals in the plants from slow starvation due to antifeedant chemicals.

This distinction is made, however, in the work of MULKERN and TOCZEK (1970, 1972) on *Melanoplus* spp. using whole plant extracts. They offered the grasshoppers pellets of a basic diet impregnated with the aqueous extracts of many different plants. Survival and development on the various plant extracts was very variable and with the extracts of some species total mortality occurred although the consumption rates with different extracts did not differ markedly. Clearly some of the plants yielded extracts which were metabolically inadequate or toxic.

Morphological differences may result from feeding on different plants, the most obvious being in wing development. It has been observed that a relatively high proportion of brachypterous adults may be produced in adult *Schistocerca* if the nymphs are fed on lucerne (TELENGA, 1930; WARDHAUGH *et al.*, 1969), spinach (TOYE, 1973) or cabbage (MUKERJI and BATRA, 1938). In other cases wing deformities may occur (BERNAYS and CHAPMAN, 1973).

Fecundity may also be affected by food quality. PFADT (1949) observed low egg production by *Melanoplus mexicanus* (Sauss.) on *Poa* compared with *Taraxacum* and wheat, although the grass was freely eaten. Mortality and abnormal development could result from nutritional deficiencies of the food, or could arise from adverse biochemical effects of some dietary constituents. The question of the nutritional adequacy of different plants for acridids remains open. There is evidence that acridids adjust the amounts of artificial diets eaten to maintain an appropriate intake of nutrients. If the nutrients

are diluted with cellulose more diet is eaten (DADD, 1960; MCGINNIS and KASTING, 1967), but the extent to which acridids may modify food intake to make good specific nutritional deficiencies is not known.

There is some evidence for the antibiotic effects of some plants on acridids. Nymphs of *Zonocerus variegatus* (L.) survive less well on *Newbouldia* than if they have no food at all (BERNAYS *et al.*, 1975); a toxic effect of the plant is indicated. An extract of *Medicago* in pellets of artificial diet caused rapid mortality in first instar nymphs of *Melanoplus femur rubrum* (MULKERN and TOCZEK, 1970). HARLEY and THORSTEINSON (1967) showed that similar effects were induced by specific secondary plant chemicals added to an artificial diet for *M. bivittatus*. With nornicotine, solanine, tomatine, digitonin or saponin added to the diet in amounts equivalent to those in plants, the insects failed to survive altogether. Other chemicals permitted survival to the adult stage, but mortality was high.

The experiments of PRUESS (1970) with *Ageneotettix deorum* (Scudder) illustrate another aspect of antibiosis. When the powder of an unpalatable plant was added to a palatable plant, not only was intake reduced but the digestibility of the food was reduced out of proportion. This seems to indicate some interference with digestive or absorptive processes.

Relatively little is known of the effects of long term interactions of plant chemicals with acridids, but one unusual instance is worth noting. *M. bivittatus* survived better on artificial diet pellets if the saponins tigogenin or hegogenin were added (HARLEY and THORSTEINSON, 1967). The chemicals are either digested and increase the availability of some dietary constituent, or they facilitate digestion or absorption of the normal dietary constituents.

Changes in host-plant selection due to changes in the insect

The range of host-plants eaten by acridids increases as the period for which the insects are without food lengthens. After only 1h without food *Chortoiceter terminifera* (Walker) rejected 20 plants without feeding on them at all. After 8h without food 35% of these plants were eaten and the percentage increased to 50% by 32h (Fig. 5). Meal sizes on these plants remained small, but on 5 of the

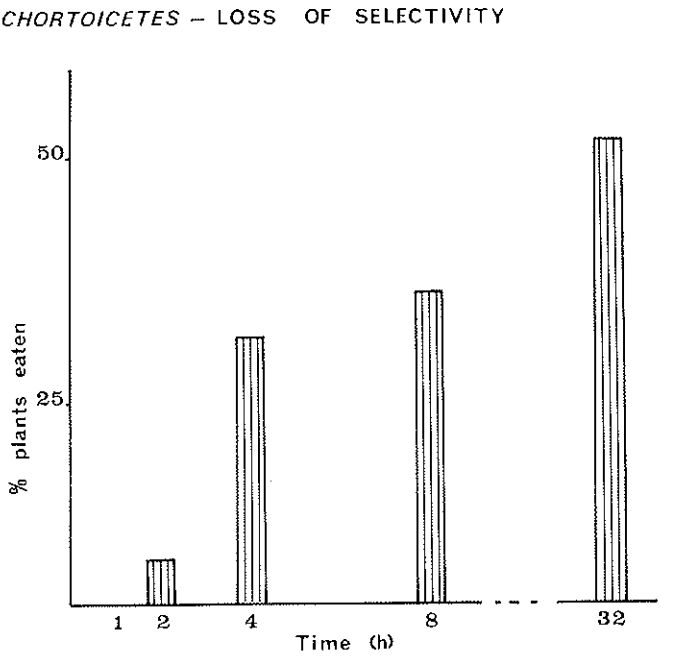

FIG. 5 - The percentage of 20 non-host plant species which were eaten by nymphs of *Chortoicetes* after different periods without food. All twenty plant species were rejected after 1 h.

plants enough was eaten to permit survival for at least 26 days. Similar data are recorded by VUILLAUME (1954) for *Zonocerus* and by BERNAYS and CHAPMAN (1970b) for *Chorthippus*. Some of the plants which KHOZANCHIKOV (1950) showed were adequate for the survival of *Locusta* are rejected by the insects if they are not deprived of food for an extended period (BERNAYS and CHAPMAN, 1977). These observations show that the deterrent effects of the plant chemicals vary with the degree of deprivation to which the insects are subjected.

Individual insects also differ in their acceptance of food even when they are standardised with respect to age and degree of food deprivation. For instance, in experiments with 3-day-old fifth instar nymphs of *Locusta* which had been without food for five hours, 18

plants were rejected by at least half of the nymphs to which they were offered. Most of the other insects ate small meals of less than 20mg, but a few ate much larger amounts (Fig. 6). Presumably these insects were less sensitive to the deterrent chemicals which prevented feeding by a majority of the insects.

The psysiological status of the insects may also influence selectivity. HILL *et al.* (1968) and MORDUE and HILL (1970) found that female *Schistocerca* fed a mixed diet of bran and lettuce selected

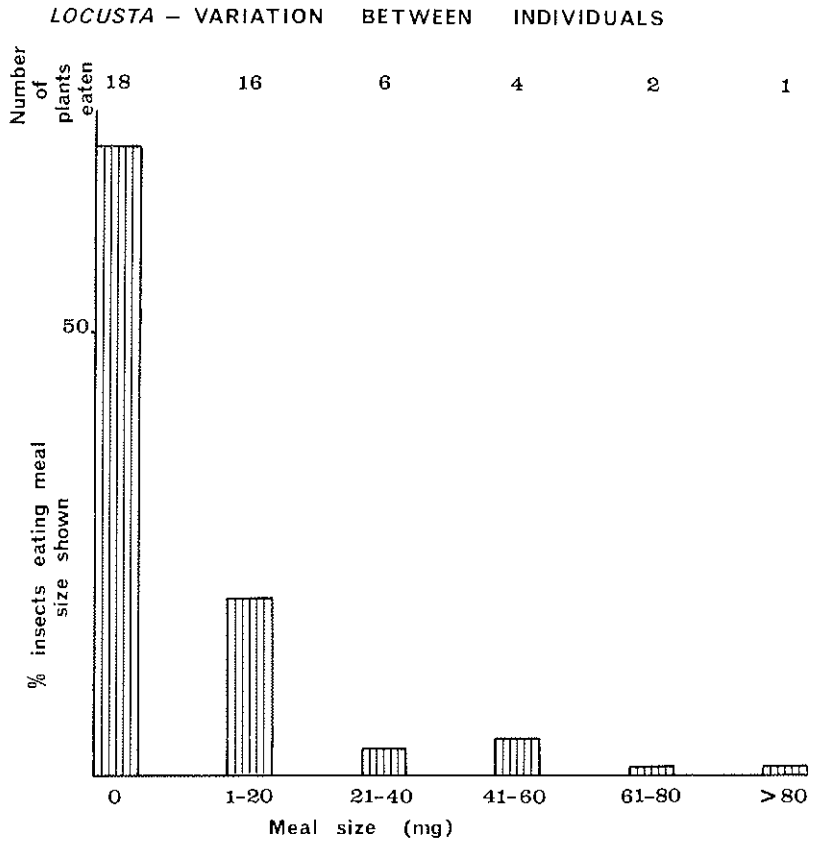

FIG. 6 - Variation in amounts of non-host plants eaten by different individuals of *Locusta*. Most insects rejected the plants without feeding at all, but a few ate relatively large meals.

much more bran during the period of somatic growth than at other times. This could reflect some nutritional regulation of food intake, but it may be more closely related to the maintenance of the insects' water balance. It is known that insects fed on dry grass eat larger meals of lush grass and smaller meals of dry grass than insects fed continuously on lush grass with a high water content (BERNAYS, unpublished).

Conditioning to a particular diet can occur. Wheat seedlings and mature *Agropyron* and *Poa* are all accepted by *Locusta* though wheat seedlings are eaten only in small amounts (BERNAYS and CHAPMAN, 1972). If insects are reared from hatching to the fifth instar on wheat seedlings they eat bigger meals on wheat than other insects reared on *Agropyron* or *Poa*. PFADT (1949) observed some changes in preference after rearing on specific food plants, but concluded that in general there was little evidence for conditioning in *Melanoplus mexicanus* (Saussure). FREELAND (1975) suggests that the amounts of different foods eaten by *Valanga irregularis* (Walk.) depend on previous experience of those foods. No information is available on the effects of feeding on a normally unacceptable food, though HASKELL *et al.* (1962) report that *Schistocerca* reared on an artificial diet subsequently preferred this to grass. It is possible that in this case the insects were conditioned to the texture of the diet rather than its chemical properties. Much more marked examples of conditioning are known from the Lepidoptera. *Papilio aegeus* Don., for example, normally feeds on *Citrus* and larvae reared on *Citrus* will eat very little *Cinnamomum camphora* if transferred to this plant. The converse is true of larvae which are reared in *Cinnamomum* (STRIDE and STRAATMAN, 1962).

There are no known instances of acridids developing distinct biotypes in relation to the availability of different foods, but there is good evidence of this occurring in a number of crop pests (PATHAK, 1975). As a result, plants which were once highly resistant to attack have become susceptible.

Changes in host-plant selection due to changes in the plant

The susceptibility of a plant may vary with its age. Seedling grasses are much less susceptible to attack by acridids than mature

plants of the same species (BERNAYS *et al.*, 1974). For instance, the meal size of *Locusta* nymphs on seedling *Zea* is 7±2mg. Many insects initially reject the grass without feeding at all and if forced to feed only on seedling *Zea* all the insects die (Fig. 7). On mature *Zea* the average meal size is 62±2mg and the insects survive normally. Similar results have been obtained with other acridids on a range of other grasses.

The resistance of the seedling has a chemical basis since if seedling *Zea* is soaked in chloroform and dried to remove the solvent before being offered as food the meal size is increased to 48±4mg. The precise nature of the resistance is unknown, although it is known that reductions in some secondary plant chemicals occur during the seedling period. Hordenine is absent from plants of *Hordeum vulgare* after 4 weeks (MANN *et al.*, 1963) and halostachine in *Lolium perenne* is reduced to 20% of its original value in 5 weeks (AASEN *et al.*, 1969). In *Sorghum* the concentration

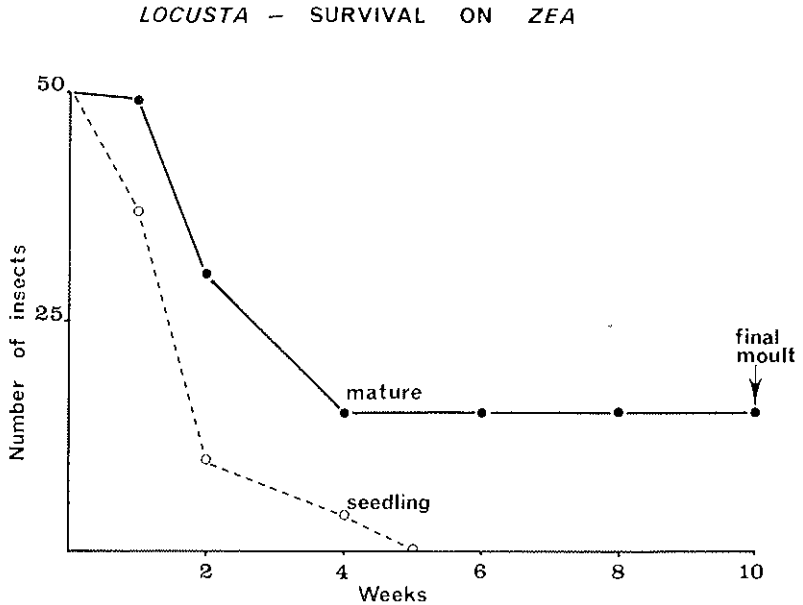

FIG. 7 - Survival of *Locusta* on seedling and mature maize (from BERNAYS *et al.*, 1974).

of the cyanogenic glycoside, dhurrin, falls throughout the seedling period (JONES, 1972) and in other plants phenolics are known to decrease (HARRIS and HARTLEY, 1976). The alkaloid halostachine and the phenolics, cinnamic acid, ferulic acid and dimethoxybenzoxazolinone (DIMBOA), are known antifeedants for *Locusta*. The inverse correlation between the concentration of halostachine in *Lolium* and the amount eaten by *Locusta* (Fig. 8) suggests that

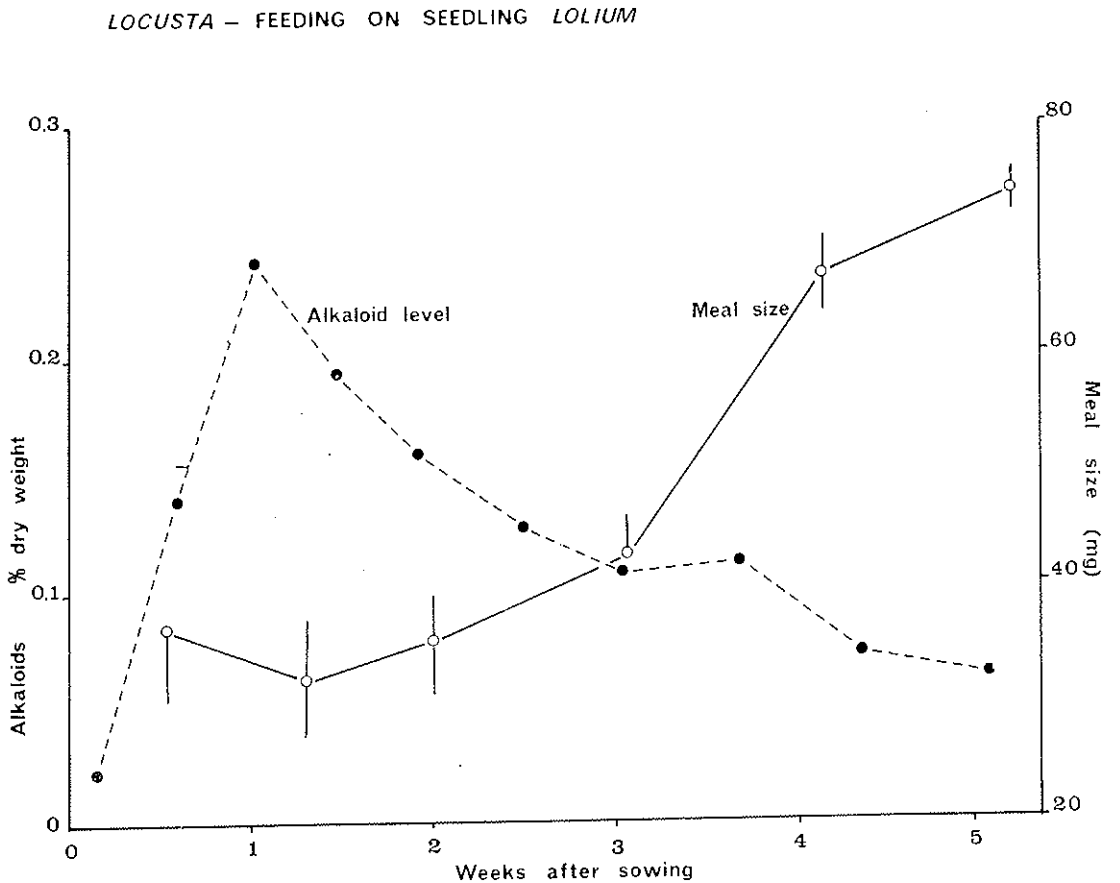

FIG. 8 - Meal size eaten by fifth instar nymphs of *Locusta* on Lolium of different ages, and the concentration of alkaloids present in the leaves (partly after AASEN *et al.*, 1969).

changes in the amount of alkaloid are important in regulating this change in the acceptability of the plant.

Various parts of a plant may differ in their resistance to insect attack. *Eupatorium odoratum* leaves do not permit good survival of *Zonocerus variegatus*, but the flowers appear to be important for the insect under some conditions. Field observations showed that of 175 insects feeding on *Eupatorium* 89% were feeding on the flowers although there was an abundance of leaves. Vacation of the *Eupatorium* clumps by the insects seemed to be associated with lack of flowers due to their consumption or death. A preference for flowers has been reported in the literature (*e.g.* PFADT, 1949), but in many cases this probably reflects only a preference for the highest parts of the plants.

In cages it was often observed that *Zonocerus* fed on the stems of *Eupatorium* rather than the leaves. This has also been observed with other acridids feeding on distasteful plants, both in the laboratory and the field. SHPAN-GABRIELITH (1965) records that adult *Schistocerca* ate the stalks of unpalatable plants in laboratory experiments, and in field cages WHITE and WATSON (1972) found that *Brachaspis nivalis* (Hutton) and *Sigaus australis* (Hutton) preferred the pedicels of *Gentiana* to the leaves. *Gentiana* was not a favoured food. In the field *Locusta* has been recorded as feeding on petioles of cotton leaves (COLENO, 1932; BEDFORD, 1933) and cutting the stems of potato (BLUNT *et al.*, 1931). It is known that the stems of pasture legumes contain higher concentrations of sugars than the leaves so that the relative importance of deterrent chemicals is reduced even if they are present at similar concentrations in leaf and stem.

In other insect groups it is known that the nutritional status of plants may affect the insects. For example, fertilisation of the balsam fir with urea leads to an increase in the nitrogen content of the foliage. The amounts of fats and sugars are also increased. Correlated with these increases it has heen shown that there is better survival of the larvae of *Choristoneura fumiferana* (Clem.) and that pupal weights are higher (SHAW and LITTLE, 1972). WHITE (1976) has suggested that survival of acridids may be enhanced by the nitrogen status of the vegetation, and this has been demonstrated in *M. mexicanus* by SMITH and NORTHCOTT (1951).

The influence of ecology and behaviour on host-plant selection

In species which are not highly selective, the food eaten depends to a large extent on the abundance of different food-plants in the habitat. For instance, *Chorthippus parallelus* eats most grasses in its habitat in relation to their abundance, and in different localities different grasses may be dominant in the food simply as a reflection of differences in the abundance. In one locality where *Molinea* was the commonest grass it formed the principal component of the food; elsewhere *Agrostis* was commoner and was eaten more by the insects (BERNAYS and CHAPMAN, 1970a). Similar opportunism has been shown to occur in North American grasshoppers by, for instance, MULKERN *et al.* (1962, 1964), UECKERT *et al.* (1972), PRUESS (1969), CAMPBELL *et al.* (1974) and CHU and KNUTSON (1975).

A lack of available alternative food plants may lead to the ingestion of relatively unpalatable foods. The later instars of *Zonocerus variegatus* commonly reject cassava on first contact when they are deprived of food for 24h, and if the adults are bagged individually on growing cassava they lose weight and die. Yet in the field these stages are responsible for extensive defoliation of cassava. In southern Nigeria this damage occurs at the height of the dry season. Before this the insects feed on herbs, but as the dry season progresses herbs become scarcer and the insect is apparently forced to the cassava by a lack of alternative foods. This suggestion is supported by the fact that in a season with less rain in December, damage occurred earlier in the dry season than when the December rainfall was higher (Fig. 9) (BERNAYS *et al.*, in press).

The literature on the damage inflicted by swarms of *Locusta* suggests that it is not unusual for acridids to be forced to feed on plants which are normally unacceptable. *Locusta* is graminivorous and when it is well-fed it rejects plants belonging to almost all plant families other than Gramineae. But out of 698 records of damage which have been analysed 33% indicate feeding on non-grasses. Even allowing for some exaggeration of reports it is obvious that feeding on "non-hosts" must have occurred relatively frequently. There are also reports of those same "non-hosts" remaining undamaged on other occasions and examination of the more detailed

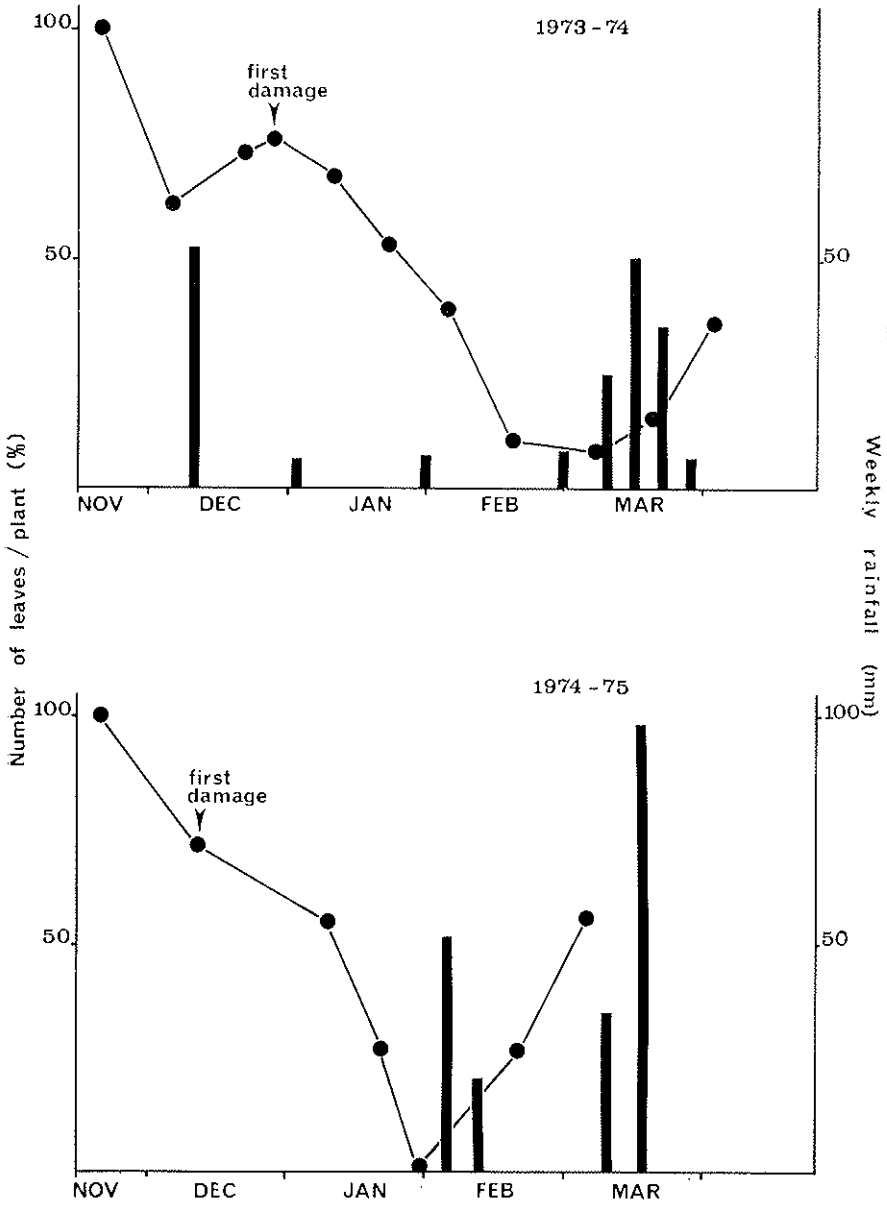

FIG. 9 - Defoliation of cassava plants in Nigeria by *Zonocerus variegatus* (from BERNAYS *et al.*, in press).

records reveals that attacks on non-grasses occurred under exceptionally dry conditions or if, for some reason, no grasses were present in the habitat (BERNAYS *et al.*, 1976).

Food selection is also influenced by aspects of behaviour not directly connected with feeding. *Nomadacris septemfasciata* (Serville) roosts in tall vegetation at night and moves down into shorter vegetation during the day. As a result, the insect eats the tall grasses in the morning and evening and short grasses during the day (CHAPMAN, 1957). *Melanoplus differentialis* (Thomas) is also well-adapted to climhing and KAUFMANN (1968) showed that tall *Dactylis* was eaten before short *Taraxacum* despite a preference for the latter if the species were presented in a comparable manner. Conversely *Encoptolophus sordidus costalis* (Scudder) is geophilous, and BAILEY and RIEGERT (1971) suggest that this habit influences its preference for *Carex eleocharis* which is eaten more commonly than would be expected from its abundance in the habitat.

KAUFMANN (1968) has also shown that selection by *M. differentialis* may be affected by the positions of plants in relation to sunlight and shade. In a uniform environment *Taraxacum* is preferred to *Poa,* but young nymphs will eat *Poa* rather than *Taraxacum* if the former is in the sun and the latter in the shade. If *Taraxacum* is in the sun, the *Poa* is uneaten. The effect is less obvious in older insects because of their greater mobility.

The biology of varietal resistance

Up to the present time the development of plant varieties which are resistant to insect attack has been largely empirical. Resistant varieties have been developed with little or no knowledge of the mechanisms involved (MAXWELL, 1972; PATHAK, 1975) and even now very little consideration has been given to the best ecological contexts in which to use varietal resistance for pest control.

The present-day relationship of an insect to its host plant is the result of a continuous evolutionary struggle between the two (FEENY, 1975). This is a dynamic relationship and, as BECK (1974) has pointed out, in developing varietal resistance we are directing and accelerating adaptations of the plant which favour it in encounters with pest insects. Varietal resistance does not, then, differ

fundamentally from the resistance of any plant to insect attack and from a study of the insect/host plant relations in normal ecosystems we may learn a great deal which is relevant to the development and use of resistant varieties in agriculture.

In this paper we have restricted ourselves to a consideration of the chemical interactions between acridids and their host-plants, but we believe some general conclusions can be drawn which are applicable to other situations.

All plants contain a very large pool of resources which could be utilised in producing resistant varieties. A particlar insect species may be deterred from feeding by, or suffer adverse metabolic effects from, a wide range of chemicals belonging to many different chemical classes. Even the most favoured host-plants may be suboptimal because of the deleterious chemicals they contain which require metabolic degradation (BECK, 1974).

Resistance to attack may depend on chemicals either with antifeedant or with antibiotic effects. The distinction is important in determining the manner in which resistance is utilized. Antifeeding is of value in preventing damage by pests invading a crop from outside and especially against vectors of disease; it may break down unless alternative host-plants are available. Antibiosis is most relevant with chronic pests, producing population reductions over successive generations. To some extent antibiosis and antifeeding are mutually exclusive since the former can only take effect if the insects feed. Antifeedants may be ineffective at high population pressures. They are not, therefore, appropriate to situations where the insects have no alternative food plants and such considerations should influence agricultural practice. Ideally an antifeedant variety of a critical crop might be grown with another which was tolerant of insect attack and which, though attacked, possessed antibiotic properties. The second variety or species would act as a trap crop.

In developing a resistant variety it is important to understand the basis of resistance and to take account of the effects on other possible pests. Different insects vary in their responses to chemicals, and a chemical which is deterrent to one may be a phagostimulant for another. Breeding for features such as high yield may involve developing characters, such as high nitrogen content, which favour

pests. In an uncoordinated breeding programme the anomalous situation could arise in which factors tending to favour and to suppress a pest are developed simultaneously.

Polyphagous insects are less sensitive to antifeedant chemicals than oligophagous species as JERMY (1966) has previously observed. So it is unlikely that resistance which depends on antifeeding will commonly be an effective means of protecting crops against polyphagous pests. On the other hand, because of this lack of sensitivity, polyphagous species will ingest relatively large amounts of potential antibiotic chemicals and so may be vulnerable to this method of attack. Control of polyphagous species must involve the synchronous development and use of resistance in a number of potential hosts.

Host-plant selection may be modified by aspects of the physiology, behaviour and ecology of the insect which are not directly concerned with feeding. These general features of pest biology may affect the successful use of resistant varieties and need to be considered in developing a rational control programme.

The variability and adaptability of food selection by phytophagous insects emphasises that the development of a resistant variety can never be regarded as an absolute solution to a pest problem. By artificially favouring the plant in the plant/insect struggle we increase the pressure on the insect to adapt to the new situation and it should be no surprise if resistance "breaks down". One solution is to reduce the single pressure on the insect to adapt by using a number of varieties involving different mechanisms of resistance (BECK, 1974), but, like any other method of pest control, varietal resistance will require sustained inputs by biologists to meet the changing pest pressures imposed on the crops.

These general conclusions are not new in themselves, but we believe that insufficient attention has been paid to them in developing and using varietal resistance. This method probably has greater potential than any other single technique of pest control, but the interrelationships between insect and host-plant are complex and its success depends on using the right variety in the right environment. While it is employed in a largely empirical way frequent failures must be expected. These failures must be seen as the result of ignorance, not an inadequacy of the method. It is unrealistic to think that we can have a total understanding of the

complexities of every crop situation, but by studying insect/plant relationships in naturally occurring ecosystems we can gain an understanding of the principles involved in the relatively stable coexistence of plants and insects. Only by applying these principles to agricultural ecosystems and by developing our knowledge of the details of the specific insect/host-plant relationships involved shall we ever realise the enormous potential of varietal resistance in pest control.

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DISCUSSION

SHOREY

Professor Chapman, I spoke to you briefly after your very interesting discussion yesterday but I would like to repeat here a few of my comments. I agree with your closing comments, during your discussion, in which you (perhaps expressing your personal bias) expressed the feeling that this area of research directed towards the obtaining of a detailed chemical knowledge concerning host-plant resistance to insects may be the most promising of the various areas which we have been discussing, as far as potential advances for protection of agriculture are concerned.

I agree with this sentiment. I do not think that I am doing disservice to my own field of pheromone research or to other people's specific research fields by so saying. It would seem that the scientific approach to host plant resistance mechanisms is still in its infancy, when compared with some of these other areas. Until the present date, most of the varieties which have been produced and which are relatively resistant to insect or pathogen attack have been developed through research by plant breeding specialists. They walked into fields of lettuce (as an example) and found the one lettuce plant that did not have aphids crawling all over it. They then selected that lettuce plant and bred it, and eventually worked out a resistant variety without really knowing why it was resistant. It would seem there is such an important interaction occurring as you have recognized at the interface between the plant and the insect which is attacking it. The interaction is absolutely critical to the survival of the insect. If I were to advise a young natural product chemist or a young biologist who was just entering the science of plant protection in which we are involved, I would advise him to specialize in your research area. I think that some of the most exciting new advances to be made and new discoveries to be made are present in this area. I have a couple of specific questions. You never used the term "secondary plant chemicals", although this term is

used by some authors. I presume that this is the same group of chemicals to which you refer. Also some authors make the statement, mainly hypothetically, that this whole group of chemicals would appear to have little function as far as the physiology of the plant is concerned, and that it would appear that the main pressure which has forced the plants to develop these chemicals is a defensive pressure — as a mechanism to resist attack by insects. Now what is your feeling on this matter?

CHAPMAN

One of the reasons that we tend not to use it is because we feel the term is often misused. Now, if by " secondary plant chemicals " you simply mean, as I believe the botanists in general do, chemicals which are not in the main metabolic pathways of the plants, then we are talking about " secondary plant chemicals ". But it seems to me that it really does not matter whether we are talking about secondary plant chemicals or not because in our view all the chemicals are important and it does not matter whether they are phytoalexines or secondary plant chemicals they are all going to have an influence on the insect one way or another. So we have tended to think in terms of the chemicals rather than trying to classify them in that way. As far as the uses of these chemicals are concerned, I think that there are some instances where it is now being recognized that the plant can make use of the so-called secondary plant substances but I think that does not mean to say that they are not used as well in plant defense. I think we should not think of things in water-tight compartments where the chemical is doing one thing or another because in my view they are all doing everything. If the plant has these toxic chemicals, it has also often got the problem of de-toxifying them itself because they may have a harmful effect. If you have a cyanogenic plant and it is damaged and it produces cyanide, the plant itself has got to de-toxify that cyanide. And this is costing it effort. So of course it does not have it there for nothing and I would think in many instances the chemical is produced for defense. It may not be defense against insects of course. One has got to consider the herbivores in a broad sense and I think the insects may have been important in some roles but other bigger herbivores have been important in other instances too. I do agree with your general comments of course. One of the problems is that this is a fringe area.

in direct agriculture application and there is pressure on the places which can do this work to undertake studies which are more immediately useful and therefore there is the tendency to think that all these other things are not necessary.

SHOREY

I have a follow-up question: I am trying to understand the differences between the various insect species, and how they have developed mechanisms for de-toxifying these chemicals. And I cannot really understand how any of the chemicals continue to work. We frequently say — almost as a truism — that the pest organism can continuously develop resistance mechanisms to factors that we or the plants use against them, whereupon either we or the plants develop new defensive measures and thus keep the battle going. It is a continuous struggle. Over a long period of time you find some species which become much more limited in their plant range, because they avoid or are killed by defensive chemicals present in most plants. But if insects in general have this inherent ability to de-toxify plant-produced chemicals, I cannot understand why many species have not responded to the pressure by de-toxifying a much larger array of these chemicals, and why over the processes of a few bundred generations, most species do not detoxify each additional substance that the plant has to throw at them.

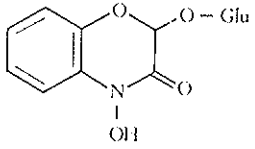
CHAPMAN

Yes, that is a rather difficult one to answer. They have developed means of getting round the problem although I am not sure about de-toxifying. The acridids have gone past the stage of de-toxification because the insect never ingests them. And this is where the acridids — the grasshoppers and locusts — are extremely advanced. They are really rather better than the Lepidoptera in this respect. We started working on non-protein amino acids with Professor Bell primarily because we were interested in antibiotic effects that people have produced with Lepidoptera larvae. But our insects will not eat them at all, even in very, very low concentrations. They have got to the stage wbere they are selecting at a sensory level. So that this is a central nervous phenomenon that the insect is really coming into contact with these chemicals and saying

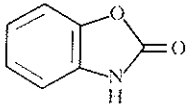
" No, this won't do ". So actually it has got beyond the stage of drinking out of the bottle, it is reading the label on the bottle. This is a much more advanced stage of development and of course the plants are at the same time countering this because it does not pay a plant to be bitten and damaged, in order for the insect to decide it does not like it, since if you damage a plant, you open the way for subsequent infections and the plant has got to repair itself. So the plant in its turn is developing mechanisms which provide a label on the outside and say: " Do not eat me, I am poisonous ". I am sorry that does not really answer your question. I do not think I can answer your question.

NAKANISHI

I would like to show two slides: This slide shows two feeding stimulants — namely sucrose and adenosine — for the army worm as detected by electrophysiology and characterized spectroscopically (by Drs. I. Kubo and W. Ma, at ICIPE, 1975). These are natural phagostimulants contained in the corn. I think it would be interesting if you checked the nucleic acid bases. Especially it is interesting that for the Japanese at least, inosinic acid is a phagostimulant but adenosine is not. Now the second slide shows:



1



2

1 is quite interesting and has appeared in a 1959 paper, so it may have to be checked again. It is a corn constituent which the Authors mentioned is contained in corn leaves, and it is a stimulant. When corn leaf is collected, compound *2* starts to build up — the latter is an antifeedant (Dr. I. Kubo, ICIPE). It is clearly derived from *1* by loss of glucose and one carbon atom.

BELL

I would like to follow up Prof. Chapman's comment on cyanogenetic

glycosides. He said that the plant has to de-toxify these. I think this supposition underlies a common fallacy that we all subscribe to as human beings. We see the whole world from the point of view of human beings rather than the point of view of plants. I say this because although the cyanogenetic glycosides clearly protect the plant against insect attack, experiments which we made some years ago showed that hydrogen cyanide could be metabolised by plants and incorporated into asparagine. In fact its role was more that of a metabolic intermediate in the plant than a toxin.

CHAPMAN

Thank you Professor Bell. Thank you Professor Nakanishi. I cannot really comment on that. It is an extremely interesting point. I do not know whether it actually occurs in other situations but certainly that would be nice to follow up.

Could I just say something about the point Professor Bell has made? Actually, most people think of the glycosides themselves as being distasteful or harmful to the animal and very often this is not true. And there are of course varieties of clover and ferns, for instance, which do have the glycoside but which lack the enzyme. These varieties are readily eaten by insects and other animals. But the plants have to have both the glycoside and the enzyme so that when the plant is damaged, the cyanide is produced. So that coming back to the plant chemistry, I think one has to be rather careful in saying that you have isolated this or that compound because things are not always what they seem, the compound you isolate may not be the compound that is important as far as the insect is concerned.

BOWERS

I was very pleased to see this very deliberate and reasonable approach to developing a basis of understanding of plant resistance because I see that it has two great major effects — one of which will hopefully allow faster development of resistant varieties on a very rational basis, and knowing how it was developed one would prevent the development of toxic plants. Our interests do run parallel since my interest is in finding compounds in plants which possess insect growth regulant activity.

Years ago I spoke to several plant breeders and asked them about their resistant varieties, and what was the chemical basis of resistance to insects. Without exception they said they did not know and did not care. It really was not important to them as long as they achieved resistant varieties. Now the U.S. government is insisting upon testing some of these new varieties to make sure they are safe for human consumption. So I think your work is very timely, especially in view of these new regulations and the fact that such an approach will certainly allow much more rapid development of pest-resistant varieties.

CANONICA

I am an organic chemist and so probably I say incorrect things on this topic but in my opinion the problem is complicated by different factors. If I consider a man, this man can refuse to eat in a case because I offer him a meal which has an unpleasant flavour. But this man can refuse also to eat because he has already eaten enough. The sensation of having eaten enough is due not only to the quantity of ingested food but also to the kind of food. It is as if organisms possess a kind of quickly acting analytical device. This is a fascinating but up till now obscure problem of physiology. So, an insect can feel full after having eaten 100 mgr. of a leaf, whereas 200 mgr. of another leaf are probably required to produce the same effect.

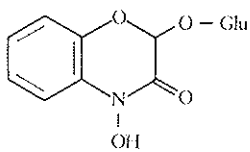
CHAPMAN

This is obviously a very knotty problem. It is quite difficult to answer because there are various ways of looking at it, but we do know that meal size — any one meal size — is generally determined mechanically and not chemically, provided the insect is on a favourable food plant. In our insects, that is, the acridids, the insect always distends its gut until the stretch receptors just inside the mouth reach a certain level of stimulation. But nevertheless the insect is able to adjust the amount of ingestion by the rate with which food passes back through the gut and therefore the rate with which it takes meals. We do know that if you use an artificial diet, the insect eats more if this diet is deficient in nutrients; that is to say if you have a certain body of amino acids and carbohydrates and all things mixed together and you dilute

these with cellulose, which is not digested, the more cellulose you add the more the insect will eat. In other words, it is making up for the nutritional deficiency of the diet. What we do not know is whether the insect is capable of making any more detailed selection than this. There are suggestions — indeed there are statements in the literature — that if the insect is deficient in, say, protein, it will eat a protein diet. Now this is certainly true I think in the Diptera. In the acridids I think the evidence is very slender indeed. I think that probably the acridids are not able to make a selection between say a high protein diet and a low protein diet, but this is something which we are really just going to start looking at. If you think of it in terms of how the insect would make this choice, it is clear that selection of this type is very difficult because you are never going to get a leaf which has no protein or no carbohydrate. So if the insect is protein deficient and it comes to a leaf which contains only relatively little protein, it has somehow got to make a decision: I will not eat this leaf in case subsequently I find a better leaf with more protein. And I think that is rather a hard decision for the insect to make. So I am not sure; think that the acridids may not have this capacity but I really don't know.

NAKANISHI

When you study your phagostimulants do you consider factors like swallowing and biting factors? The reason is that the feeding stimulant:



could be the biting factor. This substance was isolated from the corn fraction which induces army worms to bite. For example you can put it on Styrofoam and induce the insect to bite; however it does not swallow. We are trying to identify what compound this is and we have the feeling that the feeding stimulant should be defined. I was wondering how in your bioassays you differentiate between swallowing, biting and other such factors.

CHAPMAN

This is an interesting one because it is a thing we are always arguing about ourselves. I think that the differentiation is not very easy or perhaps not possible in the case of the grasshoppers but Dr. Bernays certainly would not agree with me. If we are pushed into a corner and we have to say what are biting factors and what are not, then I think we could probably give you some — for instance, it seems to us that the long chain carboxylic acids which I mentioned as phagostimulants yesterday and which occur naturally on the outside of the leaf, could probably classify as biting factors. But it does seem to me that within the acridids any of the phagostimulants is basically a biting factor. And whether it necessarily follows that it also leads to swallowing we really do not know; we have not looked at that angle, so I cannot answer — I think probably we could make the differentiation — I am not sure that it is valid.

NAKANISHI

The swallowing factor of *Bombyx mori* has been identified as β-sitosterol.

CHAPMAN

Yes, that is true, but Hsiao disagrees with that finding.

WAIN

I was talking to Dr. Bernays the other day and she informed me that the wilted plant is more acceptable to the insect than the turgid plant. This, of course, ties very clearly into what I was saying yesterday about the hormone inhibitor status of plants under physiological stress. You remember that I told you that a plant which is showing the wilting symptoms can have up to 50 times more abscisic acid in its wilting leaves than the normal plant and this abscisic acid is very active physiologically in plants. If the insect does prefer wilting tissues then he will take up more abscisic acid. This raises the question whether abscisic acid might be a feeding stimulant or whether the increased load of abscisic acid taken from the wilted tissue exerts physiological effects within the insect.

I am very pleased that Dr. Bernays and Dr. Chapman are going to look at this problem in their laboratory and we shall be sending them some abscisic acid for this purpose.

CHAPMAN

Thank you, Professor Wain. Yes that is a very interesting point — actually I should emphasize that we would not generalize on this point — we are not saying necessarily that it applies to all wilting plants, but that does not affect the argument. The specific one that I think we probably mentioned was cassava, where, as I mentioned yesterday, on the standing plant the insect really will not eat at all. And yet if you bring cut leaves into the laboratory, they develop into the best food you could possibly give it. And this seems to be a simple reflection of turgidity and the rate of production of HCN from the glycoside, that on the wilted plant the HCN production is slow and the insect can somehow cope with it or the HCN moves away fast enough for the insect not to be affected, whereas on the turgid plant as soon as the insect bites, we presume it gets a puff of cyanide, and you can see it leap away from the leaf — a very positive repellent action.

MARINI-BETTÒLO

I should now ask Professor Ballio to review our present knowledge on phytotoxins. This group of substances is most important for the scope of our meeting. They represent in effect the chemical agents produced by microorganisms which may account for their mechanism of action.

PHYTOTOXINS: CHEMICAL STRUCTURE AND BIOLOGICAL ACTIVITIES

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Phytotoxins are metabolites of microbial plant parasites which interfere with, or alter the metabolism of plant cells and so injure the plant symplast. They are produced either in the host, or on its surface, or even outside the host, for example in the soil [1]. It is now recognized that a number of phytotoxins play a definite role in plant diseases; in consequence the study of these metabolites is of interest for the understanding of the proper basis of host-parasite interactions. Knowledge of the structure, biosynthesis and mode of action of phytotoxins can suggest rational approaches to the development of new protecting agents for plants, as for instance:

a) non-toxic analogues effective in competing with the toxic metabolite at the level of receptors of the target cells;

b) specific inhibitors of toxin biosynthesis;

c) compounds capable of modifying, and thus inactivating, the toxin [2].

In particular, host-specific phytotoxins [3], namely those metabolites which are toxic only to the host of the pathogen producing them, have already found practical use in plant breeding programs as tools for a rapid mass screening of seedlings resistant to the pathogen [4].

Lycomarasmin (I) was the first phytotoxin obtained as a pure compound. It was isolated in 1944 hy GÄUMANN and associates [5]

from culture filtrates of *Fusarium oxysporum* f. sp. *lycopersici*, the agent of fusarial wilt of tomato. The significance of lycomarasmin in this disease is rather problematic, but in spite of this it must be recognized that its discovery has opened a new field of investigation in plant pathology and has long served as a model for physiological studies on phytotoxins.

Since the discovery of lycomarasmin several dozen phytotoxic metabolites have been isolated from plant pathogenic microorganisms and their structures have been elucidated. In general they are low molecular weight compounds belonging to very different chemical groups. Their role in pathogenesis has not always been ascertained and also their biosynthesis and mechanism of action are often unexplored.

As a number of excellent reviews on the chemistry and the biology of phytotoxins have appeared during the last ten years [3, 6-12], I felt it preferable to concentrate on a "case history" in this area taken from my own expetience, rather than recollect data which have already been critically evaluated. Just to illustrate the variety of structures found among phytotoxins, I have listed a few of them in Tables I and II; examples are limited to phytotoxic metabolites of pathogens belonging to three genera of fungi, and to those of three phytopathogenic bacteria.

In the following I will deal with the study of a plant disease caused hy a toxin-producing microorganism, which started in the early 60's and developed through an interdisciplinary joint effort of plant pathologists, plant physiologists and chemists, in which I had the fortune to participate.

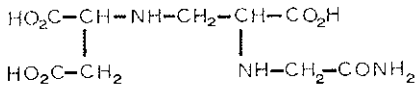
In the Mediterranean area, as well as in other parts of the world, almond and peach are frequently attacked by a pathogenic fungus called *Fusicoccum amygdali* Del. This organism infects twigs and leaves causing necrotic spots and later, as a result of a defence mechanism of the plant, formation of cankers around buds and nodes [29]. Foliar necrosis is also observed a few days after isolates of *F. amygdali* are inoculated in almond [30], or other species of *Prunus* [31], and it involves even leaves located well above the infection wound and not yet invaded by the fungus. For this reason it was postulated [30, 32] that the fungus can

TABLE I — *Phytotoxins produced by fungi of the genera* Fusarium, Alternaria *and* Helminthosporium, *and by three bacteria.*

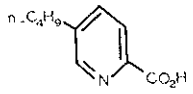
Organism	*Name of the toxin*	*Disease caused by the organism*	*References*
Fusarium oxysporum f. sp. *lycopersici*	Lycomarasmin (I) and Fusaric acid (II)	Fusarial wilt of tomato and other plants	5, 13, 14 13, 14
Fusarium oxysporum f. sp. *carthami*	Diacetoxyscirpenol (III)	Fusarial wilt of safflower	15-17
Fusarium Martii var. *pisi*	Naphtazarins (IV-VIII)	Root and stem rot of pea	13
Alternaria solani	Alternaric acid (IX)	Blight of tomato and potato	10
Alternaria zinniae	Zinniol (X)	Leaf spot and seedling blight of zinnia and other plants	10
Alternaria tenuis	Alternariol (XI), Alternariol methyl ether (XII), Altenuene (XIII), Tentoxin (XIV)	Seedling chlorosis of cotton, citrus and other plants	10, 18-20 21
Alternaria mali	Alternariolide (XV) and related compounds (XVI and XVII)	Leaf spot of apple	22, 23
Helminthosporium sacchari	Helminthosporoside (XVIII)	Eyespot of sugarcane	9
Helminthosporium sativum	Helminthosporal (XIX)	Blight, root rot, leaf spot of wheat and barley	9, 17
Helminthosporium oryzae	Ophiobolins (XX and XXI)	Brown spot of rice seedlings	9, 17
Helminthosporium victoriae	Victorine (*)	Victoria blight of oats	9
Rhizobium japonicum	Rhizobitoxin (XXIII)	Chlorosis of soybean	25, 26
Pseudomonas tabaci and *Pseudomonas coronafaciens*	Tabtoxin (XXIV) and its serine analogue	Wildfire of tobacco Halo-blight of oats	27, 28

(*) The total structure of this toxin is still unknown; one component of the molecule is victoxinine (XXII) [24].

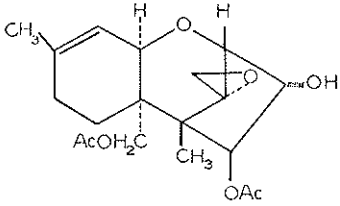
TABLE II — *Structure of the toxins listed in Table I.*



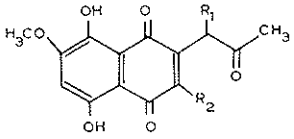
(I)



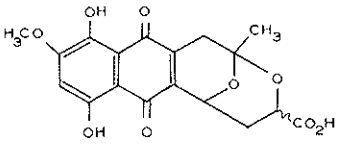
(II)



(III)



(IV-VII)



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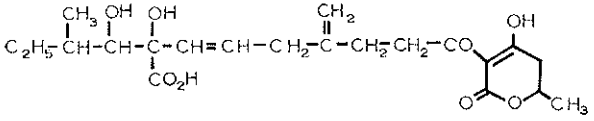

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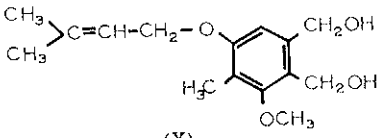
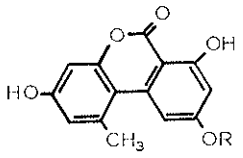
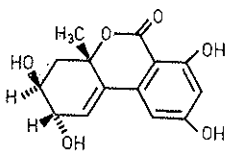


(X)



(XI-XII)



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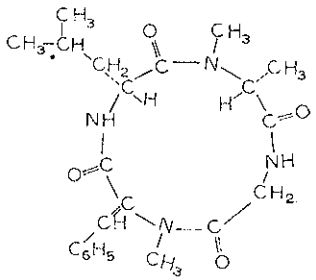

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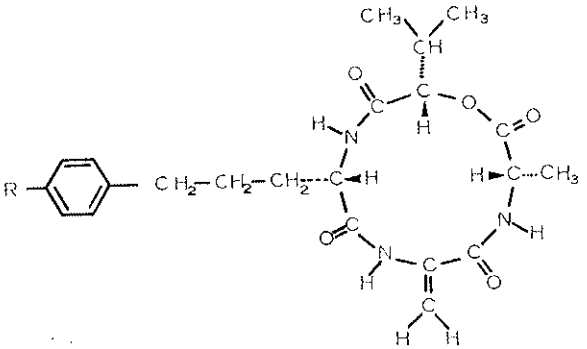
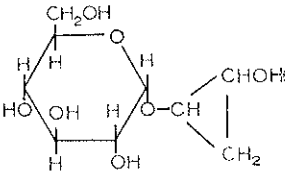


(XV-XVII)



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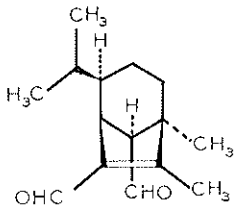

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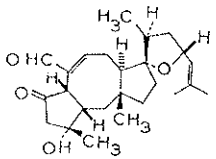
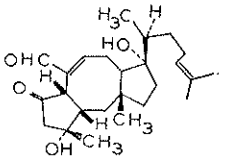
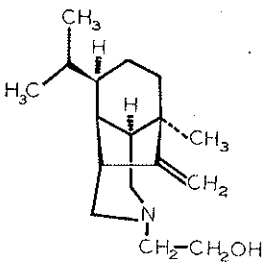


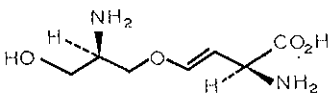
(XX)



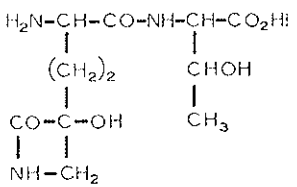
(XXI)



(XXII)



(XXIII)



(XXIV)

Lycomarasmin (I), fusaric acid (II), diacetoxyscirpenol (III), norjavanicin IV: R_1 and R_2 = H), novarubin (V: R_1 = $-CH_2OH$, R_2 = H), javanicin (VI: R_1 = H, R_2 = $-CH_3$), fusarubin (VII: R_1 = H, R_2 = CH_2OH), marticin and isomarticin (VIII), alternaric acid (IX), zinniol (X), alternariol (XI: R = H), alternariol methyl ether (XII: R = $-CH_3$), altenuene (XIII), tentoxin (XIV), alternariolide (XV: R = = $-OCH_3$), AM-toxin II (XVI: R = H), AM-toxin III (XVII: R = $-OH$), helminthosporoside (XVIII), helminthosporal (XIX), ophiobolin A (XX), ophiobolin B (XXI), victoxinine (XXII: part of the victorine molecule), rhizobitoxin (XXIII), tabtoxin (XXIV).

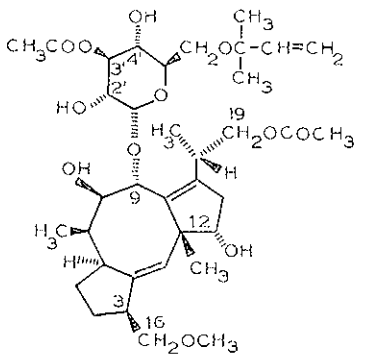
produce one or more translocatable toxins which are spread systemically to cause generalized symptoms. This hypothesis received strong support by the fact that leaf necrotic injuries, similar to those caused by the fungus, are observed when almond shoots or twigs take up small amounts of *F. amygdali* culture filtrate, either through their cut end or through scars or wounds [30, 32]. This observation, together with the successful development of a medium suitable for growing the fungus in submerged culture and of a bioassay to test the phytotoxicity [33], prompted work toward the isolation and characterization of the toxin(s). Pilot plant fermentations [34] gave material sufficient for extraction and purification of the active principle. A crystalline phytotoxic compound, named fusicoccin A (1), was isolated through a rather tedious procedure involving solvent extraction and column chromatography [35]. This procedure was replaced by a much simpler method, consisting in charcoal absorption followed by acetone desorption [34], when fermentation broths with a ten-fold higher titer became available using new strains derived from the original isolate by u. v. treatment and selection. The availability of pure fusicoccin immediately promoted extensive work along two lines: the assessment of the role of fusicoccin as a causative agent of plant disease, and the elucidation of its structure.

Almond shoots or twigs which have taken up fusicoccin solutions through their partially cut leaves or leaf scars develop foliar symptoms similar to those which follow infection with the pathogen under natural conditions [36]. Fusicoccin causes necrosis and wilt in a number of green plants at 0.1-0.2 ppm and therefore is a non-specific but highly active phytotoxin [36]; it is devoid of toxicity towards microorganisms [36, 37] and mammals (unpublished results). Its minimal dose was determined on tomato cuttings, the plant routinely used for the bioassay, and, according to the laboratory conditions used, values between 0.1 and 0.6 mg/kg fresh weight were obtained [36, 38]. Compared to other known wilt toxins these figures represent a high toxicity. Recently fusicoccin has been isolated from unripe peaches artificially infected with *F. amygdali* and from cankers formed on naturally infected almond shoots in

(1) In what follows this will be called fusicoccin.

concentrations high enough to account for toxicity symptoms on leaves [39], thus providing conclusive evidence that it is a chemical determinant of pathogenesis, *i. e.* a "vivotoxin" sensu DIMOND and WAGGONER [40].

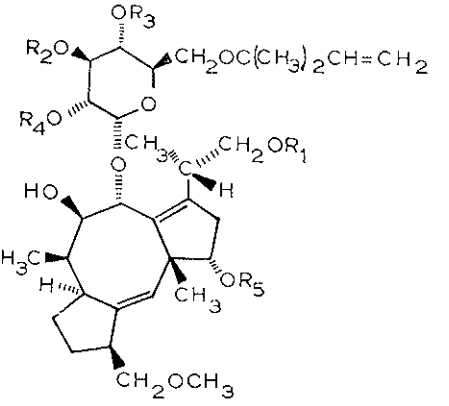
A preliminary chemical characterization of fusicoccin indicated that it was a $C_{38}H_{58}O_{13}$ glucoside containing one *O*-methyl and two *O*-acetyl groups [35]. Further investigations, implying a very restricted use of conventional organic chemistry operations, an extensive application of n.m.r. and mass spectrometry, and finally a three-dimensional X-ray analysis of a p-iodobenzenesulphonyl derivative,



(XXV)

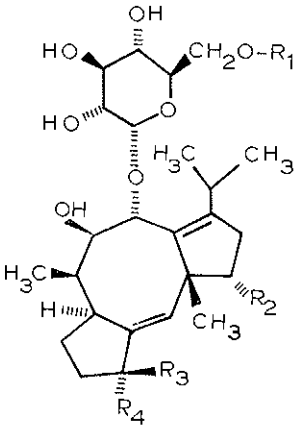
led to the amended molecular formula $C_{36}H_{56}O_{12}$ and to the complete determination of the structure (XXV), absolute configuration included [41]. This structure, soon confirmed by an independent investigation carried out at Imperial College, London [42-44], contains the same carbotricyclic system found in the ophiobolins (XX and XXI), toxic sesterterpenoids also produced by a phytopathogenic fungus [9, 17, 45].

A careful investigation of culture filtrates of *F. amygdali* led also to the isolation of a number of minor metabolites chemically related to fusicoccin. Ten of them (XXVI-XXXV) differ from the major phytotoxin only for the number and/or the position of the *O*-acetyl groups [34, 46-49], whereas four (XXXVI-XXXIX) are

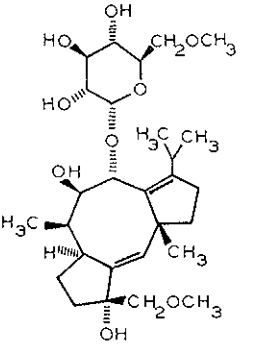


(XXVI-XXXV)

Dideacetylfusicoccin (XXVI: $R_1 - R_5 = H$). Monoacetates (XXVII: $R_1 = -COCH_3$, $R_2 - R_5 = H$; XXVIII: $R_2 = -COCH_3$, $R_1, R_3, R_4, R_5 = H$; XXIX: $R_3 = -COCH_3$, $R_1, R_2, R_4, R_5 = H$; XXX: $R_4 = -COCH_3$, $R_1, R_2, R_3, R_5 = H$; XXXI: $R_5 = -COCH_3$, $R_1, R_2, R_3, R_4 = H$). Diacetates (XXXII: $R_1, R_3 = -COCH_3$, $R_2, R_4, R_5 = H$; XXXIII: $R_1, R_4 = -COCH_3$, $R_2, R_3, R_5 = H$). Triacetates (XXXIV: $R_1, R_2, R_5 = -COCH_3$, $R_3, R_4 = H$; XXXV: $R_1, R_3, R_5 = -COCH_3$, $R_2, R_4 = H$).



(XXXVI-XXXIX)

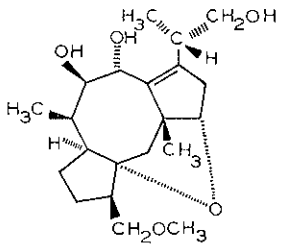


(XL)

XXXVI: $R_1 = -C(CH_3)_2CH=CH_2$, $R_2 = -OH$, $R_3 = -CH_2OCH_3$, $R_4 = H$.
XXXVII: $R_1 = -C(CH_3)_2CH=CH_2$, $R_2, R_4 = -OH$, $R_3 = -CH_2OCH_3$.
XXXVIII: $R_1 = -C(CH_3)_2CH=CH_2$, $R_2 = -OH$, $R_3 = H$, $R_4 = -CH_2OH$.
XXXIX: $R_1, R_2, R_4 = H$, $R_3 = -CH_2OH$.

derivatives of deacetylfusicoccin having as a common feature the absence of an oxygen atom at C-19 [48, 50-55]. The presence of an extra oxygen atom on C-3 in compound XXXVII is of some interest, as this also occurs in a group of fungal metabolites recently studied by SASSA in Japan, the cotylenins (2) [56-64], which exhibit close chemical and biological similarities to fusicoccin.

A number of other structurally related analogues were prepared by chemical modification of fusicoccin and of some minor metabolites of *F. amygdali*; these were in part obtained in the course of the structural investigations (*e. g.* XLI [65]), and in part (un-



(XLI)

published results) prepared either for the purpose of determining the structural features essential to biological activity, or for establishing the sites of incorporation into fusicoccin of labeled atoms contained in radioactive precursors fed to growing cultures of *F. amygdali* (*e. g.* fusicoccins with an inverted configuration at C-9).

Experiments with labeled precursors, aimed at the elucidation of the biosynthetic path leading from mevalonate to fusicoccin, are complicated by the low yields of toxin produced by the mould, by the poor incorporation of tagged mevalonate, and by the peculiar bebaviour of fusicoccin in several chemical reactions. From work so far performed (all unpuhlished) it is apparent that the cyclization mechanism of an activated C_{20} precursor, derived from four mevalonate units, is different from that operative in the case of the

(2) Formula XL represents the simplest member, namely cotylenin E.

ophiobolins [66]. Results obtained on feeding the mould with tritiated minor metabolites suggest that XXXVIII is a shunt product, whereas XXXIX, XXXVI, and XXVI lie in sequence on the main pathway to fusicoccin ([3]).

Several potential inhibitors of the biosynthetic pathway have been tested with the hope of blocking it at specific steps with consequent accumulation of intermediates. So far inhibitors of mixed function oxydases and methyl transferases have yielded "all or none" results (unpublished results). Some of these inhibitors might perhaps prevent the production of the toxin also in naturally infected plants and thus achieve chemical control of the disease without affecting the pathogen directly.

The study of the mechanism through which fusicoccin affects plant tissues took at first into consideration the loss of turgor and the wilting observed in tomato cuttings [29]. It was soon found that the stomatal transpiration was strongly increased by fusicoccin [68] and that stomata in tomato and a number of other species opened, both in the light and in the dark, either on absorption of the toxin through the petiole or on painting it on to the leaf [69-71]. Leaf water potentials were decreased after stomatal opening and this probably represents the major cause of the wilting observed in diseased tissue. A remarkable stimulation of water uptake by tomato cuttings took place during the first hours of treatment, a fact previously noted in tomato leaf fragments [72] and then in etiolated pea tissue [73]. Isolated internode segments of the latter tissue showed a large increase in their fresh weight, elongation and cell wall plasticity on addition of fusicoccin [73]. The growth promoting activity was strongly reminiscent of an auxin effect and encouraged further physiological and biochemical studies which soon established that fusicoccin produces effects similar to, but much larger than, those of indoleacetic acid [74-81]. This "amplification" turned out of great help in demonstrating that auxin induces proton extrusion from plant tissues sensitive to it [82-84], acidification being responsible for the cell wall stretching effect [85].

([3]) Results of biosynthetic studies have been reported by the Imperial College group [50, 55, 67].

Fusicoccin-induced H^+ extrusion is tissue aspecific [75, 86], in contrast to the effect of indoleacetic acid, thus suggesting different primary sites of action of the two substances.

Another interesting effect of fusicoccin is the promotion of seed germination by breaking of dormancy [87]; here again the effect is accompanied by proton extrusion [88].

Acidification by fusicoccin is consistently accompanied by stimulation of K^+ uptake [77-79, 81, 89, 90] and increase of the negative transmembrane potential [78, 79, 91, 92]. Also stomatal opening induced by fusicoccin involves a very marked stimulation of active K^+ uptake in the guard cells [93] and acidification of the medium [94].

Evidence has been obtained that fusicoccin specifically binds at the plasma membrane [95], to a site different from that for auxin and that the fusicoccin receptor is most probably the plasmalemma-bound K^+, Mg^{2+}-dependent ATPase, which in fact is significantly stimulated by fusicoccin "in vitro" [96]. It has been speculated that the common mechanism responsible for all physiological effects of fusicoccin might consist in the activation of this H^+-pumping ATPase, which would cause the hyperpolarization of the transmembrane electric potential, only partially neutralized by simultaneous K^+ uptake [96]. It has also been proposed that such a mechanism might be operative in all higher plant tissues, and that a number of specific physiological activities of indoleacetic acid and other plant hormones might depend upon interaction, in a less direct and more sophisticated way than fusicoccin, with the H^+/K^+ exchange system [96].

The availability of several dozen fusicoccin derivatives and analogues have afforded a large amount of material for structure-activity studies. The phytotoxicity of a number of compounds towards tomato cuttings has been determined and it has been found that, with the exception of dihydrofusicoccin (where the *t*-pentenyl group in the glucosyl moiety is hydrogenated), all modifications of the molecule result in a very marked drop in the activity [97, 98]. It is of interest that the phytotoxicity of cotylenin A, which was discovered as a consequence of its growth promoting properties [56-58, 63], is nearly as high as that of fusicoccin (unpublished results).

Recently a number of fusicoccin derivatives and analogues, as well as some cotylenins and their aglycone (cotylenol), have been compared with the toxin for their activity in several biological tests (opening of stomata in broad beans, cell enlargement and proton extrusion in pea stem segments and in squash cotyledons, germination of dormant lettuce and radish seeds) and in their ability to compete with labeled dihydrofusicoccin in the binding to plasmalemma-enriched membrane preparations from maize coleoptiles. Results so far obtained [73, 99-101] (also unpublished results) indicate that:

a) structural requirements for activity are much less stringent in these tests than in the phytotoxicity test;

b) overall polarity of the molecule is important;

c) correct configuration at C-9 is essential.

Up to now data of binding to plasma membranes correlate quite well with proton extrusion in pea stem segments, while in other tests some divergence is noted. It is likely that the divergence depends on the limited availability of the binding site, rather than on the existence of independent sites in the different tissues examined.

The information so far obtained from structure-activity studies has been particularly useful in making the proper choice of fusicoccin derivatives suitable for linking to macromolecules; both, stationary phases for affinity chromatography and conjugates to bovine serum albumin for immunological studies have been prepared (unpublished results). Furthermore, the finding that a number of non-phytotoxic derivatives are still active in several physiological tests prompts an investigation of their potential "in vivo" applications.

The investigations which have led to the discovery of fusicoccin and its remarkable biological effects were started in connection with a phytopathological problem, but eventually showed that the phytotoxin is a very active plant growth regulator. Such a finding is not astonishing, as it is ascertained that symptoms of some plant diseases can be caused by an excess of growth-regulating substances (*e. g.* indoleacetic acid, gibberellins, etc.), either produced by the parasite or accumulated in consequence of changes of the host metabolism induced by the pathogen [102, 103]. The most famous case is that of gibberellic acid, discovered in connection with studies on the *bakanae* disease

of rice caused by *Gibberella fujikuroi*. In contrast with the plant hormones, fusicoccin is neither a normal constituent of plants, nor synthesized and degraded under the metabolic control of the plant. The stability in various tissues (unpublished results) and the high affinity of fusicoccin for its receptor [95] are probably responsible for the development of the physiological effects towards a pathological situation.

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NEW TRENDS IN THE USE OF PHYTOTOXINS

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In plant pathology phytotoxins are properly referred to as products of the pathogen which are harmful to the host plant (GÄUMANN, 1954; GRANITI, 1972). This is a heterogeneous class of natural substances ranging from simple aliphatic or aromatic molecules to high-molecular-weight compounds.

A substance can be classified as a phytotoxin when:

1) it is produced by a microorganism, i. e., it is a microbial metabolite;

2) it acts at very low concentrations;

3) it interferes with or alters the plant cell metabolism or structure, thus resulting in injuries to protoplasts.

Compounds satisfying the requirements reported above are often found in culture media of many plant pathogenic — and even non pathogenic — bacteria and fungi. Phytotoxins found in the diseased plant ("vivotoxins") and proved to have a function in pathogenesis and in symptom expression ("pathotoxins") are however relatively few. These can be further classified as "selective" and "non-selective" phytotoxins according to their ability to act selectively when applied to plants susceptible or resistant to the disease involved (SCHEFFER and YODER, 1972; WHEELER, 1976).

Sometimes, ability to produce phytotoxin(s) has been shown to confer virulence to a parasite. Since toxin production is consequently required for infection, "non producer" strains are non

pathogenic. In some cases (*Alternaria kikuchiana* Tan., a parasite of Japanese pear, for example) conidia from avirulent strains can infect host tissues only if they are inoculated along with traces of the specific AK-toxin (NISHIMURA *et al.*, 1974); in other cases, for example in some wilt diseases, toxins are required during pathogenesis, i.e., they are not essential for infection and first colonization of the host.

That a parasite could damage its host by poisoning it with its *excreta* was hypothesized since the early days of plant pathology. For a long time, however, findings did not encourage a systematic search for a toxic origin of symptoms of certain plant diseases. Only after the 2nd world war E. GÄUMANN emphasized the importance of toxigenic diseases and called the attention of plant pathologists to the effects of phytotoxins on plants. But his generalization (1954) that "micro-organisms are pathogenic only if they are toxigenic" was received with much scepticism. However, the researches carried out at Zurich and those which developed in various countries afterwards brought the number of phytotoxins from the few fusarial toxins studied at the beginning to the many bacterial and fungal compounds which are known nowadays. At the same time, the studies on the mode of action of phytotoxins on plants progressed considerably. It was soon ascertained that phytotoxins represent a key factor for the pathogenicity of certain parasites.

New approaches in biological research often result in an expected progress of other fields of study. Phytotoxins are not an exception. As a by-product of investigations on phytotoxins and other chemical factors involved in plant diseases, a better understanding of how the healthy plant functions has resulted. For example, both gibberellins and fusicoccins, which affect plant growth and therefore are studied by plant physiologists, were first discovered as metabolites of plant pathogenic fungi.

Three possible applications of recent studies in the use of phytotoxins will be briefly considered here, to exemplify what is said above:

1. Remission of toxicity in diseases
2. Resistance of plants to diseases
3. Beneficial applications in agriculture.

REMISSION OF TOXICITY IN DISEASES

Three different cases will be considered (see also: GROSSMANN, 1972).

1. *Removal of metabolic blocks.*

Certain phytotoxins acting as antimetabolites cause enzyme inhibition and blocks in the metabolism of plant cells. If these metabolic blocks are removed by artificial application of the specific metabolite, symptoms are consequently repressed.

Tabtoxin, a β-lactam-threonine produced by the bacterium of tobacco wildfire, *Pseudomonas tabaci* (Wolf et Fost.) Stev., was thought to inhibit glutamine synthetase in tobacco leaves; this should lead to the accumulation of toxic amounts of ammonia and to the appearance of chlorotic spots (SINDEN and DURBIN, 1968). Although this way of action was not confirmed when pure preparations of tabtoxin were used, yet tobacco leaves infiltrated with glutamine were protected by the toxic effects of the wildfire toxins.

Phaseotoxin, a toxic peptide produced by *P. phaseolicola* (Burkh.) Dows., has been reported to inhibit the enzyme ornithine carbamyl transferase, which catalyzes the carbamylation of ornithine to citrulline. This causes a citrulline deficiency and hinders the formation of chlorophyll synthetizing enzymes. As a consequence, chlorotic haloes are formed on infected leaves of bean (*Phaseolus vulgaris* L.). These show no symptoms when treated with L-citrulline prior to application of phaseotoxin (PATIL, 1974; RUDOLPH, 1976).

2. *Toxin deactivation.*

Application of chemicals to plants infected by toxigenic pathogens may result in a modification of the toxin molecule that makes it ineffective. These chemicals act as chemotherapeutants.

Lycomarasmin was the first fungal metabolite studied as a phytotoxin. It was isolated from *Fusarium oxysporum* (Schl.) Snyd. et Hans. f. sp. *lycopersici* and other *formae speciales*, but its

role in tomato wilt or in other diseases caused by species of *Fusarium* remains uncertain.

Aspergillomarasmin B (lycomarasmic acid), a more toxic derivative of lycomarasmin, is produced by populations of the anthracnose fungus *Glomerella cingulata* (Ston.) Spauld et v. Schr. attacking fruits and branches of olive trees (BALLIO *et al.*, 1969).

It was postulated that most of the foliar symptoms shown by the affected trees could be induced by translocation of the toxin from infected bark to the leaves. Like lycomarasmin, aspergillomarasmin B forms complexes with metal ions. Iron complexes were shown to be 2-3 times more toxic to test plants, while no other tested cations enhanced toxicity. It was decreased from about 50% up to 97% respectively, by the addition of zinc, copper, aluminum, cobalt; nickel gave a completely detoxified complex (BOTTALICO, 1973).

The ability of lycomarasmin and derivatives to form complexes with heavy metals could be utilized to reduce the severity of the disease they were associated with, when no other means of control was available. Theoretically, absorption of copper or other metal cations by infected plants should have a detoxifying effect which would help to keep the disease under control. As a matter of fact, bordeaux mixture or copper oxychloride are by far the most efficient fungicides for controlling olive anthracnose.

Fusaric acid, another phytotoxin from pathogenic populations of *Fusarium oxysporum*, is able to form chelates with iron, cobalt, copper, and other cations. They differ in their toxicity towards plants (BÄR, 1963; MALINI, 1966).

Toxicity of naphtazarin derivatives (marticins etc.) produced by *Fusarium solani* (Mart.) Snyd. et Hans. is also counteracted by heavy metal ions, especially by copper (KERN and NAEF-ROTH, 1966).

Ferric ions detoxify ascochitine, a phytotoxic metabolite of *Ascochyta fabae* Speg., a parasite of broad bean (OKU and NAKANISHI, 1963).

Organic substances like chlorogenic or ferulic acid were reported to detoxyfy piricularin, the toxin of *Piricularia oryzae* Cav. Application of these phenolics to rice plants resulted in an increased

resistance to rice blast (TAMARI *et al.*, 1963), although no evidence is available that this is due to toxin deactivation, and if so to what extent.

3. *Suppression of biosynthesis.*

Synthesis of phytotoxins by plant parasites is genetically determined, but may require a suitable environment, presence of precursors or a proper substrate to be accomplished. An adequate supply of nutrients and other favourable conditions of the medium are often requested. These include both macro- and micro-elements, for example type of nitrogen and presence of zinc for production of fusaric acid and otber *Fusarium* toxins. The nutritional status of the host is known to affect the severity of toxigenic diseases, but little information is available on how this is related to suppression or reduction of toxin synthesis. More promising seems the use of certain chemicals which inhibit the enzymes involved in toxin biosynthesis or repress the genetic information (GROSSMANN, 1972; WHEELER, 1975); but more work is needed in this respect.

RESISTANCE OF PLANTS TO DISEASES

Resistance of plants to toxigenic diseases may be linked to tolerance or insensitiveness to the toxins produced by the parasites involved. Barriers which prevent toxin diffusion within plant tissues, deactivation or degradation mechanisms, lack of proper toxin receptors at the cellular level, and other conditions may explain why certain plant species, varieties or clones are immune or tolerate concentrations of toxins which in other cases are fully effective.

Consequently, use of phytotoxins as a means for assaying resistance of plants to diseases and for screening the progenies of crosses may be useful in breeding for resistance. In fact, a group of phytotoxins has been used by plant breeders to detect plants in which tolerance to each toxin is correlated with resistance to the pathogen, and to select for disease resistance on the basis of the reaction of the host to the toxin.

The best known example is victorin, a phytotoxin produced by the fungus *Cochliobolus victoriae* Nels. (imperfect stage: *Helminthosporium victoriae* Mee. et Mur.) (LUKE and GRACEN, 1972; WHEELER, 1975; SCHEFFER, 1976). This is a specialized pathogen which infects only "Victoria" oats and derived cultivars, causing a seedling disease known as "Victoria blight". Toxin production by different isolates of *C. victoriae* was shown to be correlated with pathogenicity. If applied at very low doses (2×10^{-4} µg/ml) to oats susceptible to the pathogen, victorin-induced symptoms mimic the disease caused by the inoculation of the fungus. The same dose of victorin, however, fails to cause damage if applied to cultivars resistant to the disease which apparently are able to inactivate or to bind the toxin. Seedlings of resistant cultivars tolerate more than 400,000 times higher concentrations of victorin. Since oat sensitivity to victorin parallels susceptibility to the pathogen, the toxin has been successfully used as a substitute for the pathogen in mass screening for disease-resistant mutants in oat populations (WHEELER and LUKE, 1955; SCHEFFER and YODER, 1972).

Another species of *Cochliobolus, C. carbonum* Nels. (imperfect stage: *H. carbonum* Ullstr.), race 1, the causal agent of a leaf spot disease of inbred maize, produces a toxic polypeptide, called HC-toxin (PRINGLE and SCHEFFER, 1964), which is about 20 times more active on susceptible than on resistant maize cultivars. HC-toxin selectively affects the genotypes of maize that host the fungus. They possess a homozygous recessive factor at the locus conditioning disease reaction.

In both *C. victoriae* and *C. carbonum* a single gene pair appears to control both pathogenicity and toxin production. Crosses between the two species gave a segregation rate of 1:1:1:1 for pathogenicity to oats or to maize, to both or to neither, which parallels the ability of the progeny to produce victorin, HC-toxin, both or neither (SCHEFFER *et al.*, 1967).

The widespread use of inbred lines or hybrids of maize with "Texas male sterile" (Tms) cytoplasm in the U.S.A. resulted in a disastrous outbreak of the "southern corn leaf blight" that occurred in 1970. The disease is caused by *C. heterostrophus* Drechs. (imperfect stage: *H. maydis* Nis. et Miy.) race T, which attacks Tms plants only. The pathogen produces a toxin (T-toxin) with se-

lective toxicity for corn lines susceptible to the disease (HOOKER *et al.*, 1970). Crosses between race T and race O (with no specificity for corn cytoplasm) gave a segregation rate of 1:1 and showed that T-toxin production is required for race T-type pathogenicity (YODER and GRACEN, 1975). A rapid test based on inhibition of primary root elongation has been developed which can distinguish between susceptible corn lines with Tms cytoplasm, which are sensitive to low doses of T-toxin, and resistant lines with normal (N) cytoplasm, tolerating higher (up to 25 times) levels of toxin. A clear separation was obtained with this method when artificial mixtures or commercial blends of seeds of Tms and N hybrids were assayed (WHEELER *et al.*, 1971).

Similarly, the host-selective PC-toxin is utilized in breeding for resistance to *Periconia circinata* (Mang.) Sacc. in sorghum (SCHERTZ and TAI, 1969).

The toxin reported above and a few others, like PM-toxin produced hy *Mycosphaerella zeae-maydis* Muk. et Boothr. (imperfect stage: *Phyllosticta maydis* Arny et Nels.) (YODER, 1973) have been called "host specific" (PRINGLE and SCHEFFER, 1964) or "host selective" (WHEELER, 1976), since they affect only the plants which host the pathogen, and do not affect — or do so only at relatively high concentrations — plants resistant to the disease or non-hosts.

The nature of such a selectivity of toxin for plants is still little understood. It has been postulated that certain toxins possess receptor sites on sensitive cultivars and no receptors — or sites with lower affinity — in non sensitive cultivars.

Helminthosporoside, a cyclopropyl-galactopyranoside produced by *Helminthosporium sacchari* (Van Br.) Butl., the causal agent of the eye-spot disease of sugarcane, might be a case in point (STEINER and STROBEL, 1971). Injection of helminthosporoside at very low concentrations (5×10^{-12} M) into leaves of the majority of clones susceptible to the disease reproduces one symptom of the disease, the reddish stripes or "runners", but is ineffective on most clones resistant to eye-spot. A specific binding site for helminthosporoside has been claimed to occur in the plasma membrane of susceptible sugarcane clones or cultivars, but not of resistant ones. The latter possess a closely related protein which does not

bind the toxin (STROBEL, 1973, 1975). However, the evidence presented in support of the existence of a single host protein as a specific receptor site has been criticized (Daly, in: PATIL, 1976). Helminthosporoside has been used routinely to select sugarcane clones resistant to eye spot disease both at seedling or mature plant stage (BYTHER and STEINER, 1972).

Another example of the existence of a specific mechanism for virulence in a plant pathogen and a correspondent specific mechanism for susceptibility in host plants is given by tentoxin, a cyclic tetrapeptide produced by strains of *Alternaria tenuis* Auct. Tentoxin induces chlorosis by selective disruption of chloroplasts in sensitive species (lettuce, potato, cucumber, spinach), and has no effect, or a little one, in non-sensitive plants (radish, tobacco, corn). Sensitive chloroplasts were shown to possess a single receptor site for tentoxin ("coupling factor 1" or CF_1). When tentoxin is bound to CF_1, the CF_1ATPase is inactivated and cyclic photophosphorilation is inhibited (STEELE *et al.*, 1976).

In conclusion, the use of toxins may be an effective tool for selecting resistant plants through a simple assay of breeding material. Moreover, the use of a toxin in place of the pathogen would avoid the risk of introducing new strains of that pathogen, when screening is carried out in places or countries where the latter is not present.

BENEFICIAL APPLICATIONS OF TOXINS IN AGRICULTURE

A beneficial use of certain phytotoxins can be envisaged. As biologically reactive molecules, phytotoxins could be used in very low amounts to stop or modify undesired growth of plants, to cause fruit or leaf fall, to dry leaves, to kill noxious weeds, etc.

Since most phytotoxins affect only higher plants and some show a high degree of specificity, their use in practice would not cause much concern as far as environment, pollution, and residues in food are concerned.

Possible applications of phytotoxins will be exemplified by the work on fusicoccin.

Fusicoccum amygdali Del. may cause not only cankers around

infected buds and nodes of peach and almond trees, but also wilting of leaves of infected shoots. These foliar symptoms have been attributed to fusicoccin, a terpenoid glucoside elaborated by the pathogen at the site of infection and translocated via apoplast to leaves (GRANITI, 1962; BALLIO *et al.*, 1964, 1975). When applied to transpiring organs of a great number of higher plant species in concentrations as low as 2 μg/ml, fusicoccin produces flaccidity of leaves, necrosis, and eventually wilting of the whole plant (BOTTALICO, 1971, 1972).

It was first demonstrated that fusicoccin stimulates water uptake by plants as a consequence of cell enlargement. This effect is associated with an irreversible stretching of the cell walls and with changes in cell metabolism similar to those induced by the plant hormone indol-3-acetic acid (IAA). The greater amplitude of the effects induced by fusicoccin has made this toxin an appropriate tool for the study of the mechanism of action of auxins (MARRÉ *et al.*, 1971). On the other hand, fusicoccin and IAA show a different tissue specificity and a different primary site of action on cell structures.

Changes in cell wall are associated with an energy-dependent output of protons from cytoplasm into the cell wall. This causes a pH lowering which favours loosening of calcium bridges or other alterations that increase the cell wall plasticity (MARRÉ *et al.*, 1973, 1974).

It was also demonstrated that fusicoccin increases transpiration in a wide range of species, both mono- and dicotyledons, by causing the stomata to open more widely. When plants were allowed to take up dilute solutions of the toxin, the leaf resistance to transpiration decreased progressively and the leaf water potential declined until loss of turgor and wilting became manifest. In other experiments, painting or spraying leaves with fusicoccin opened the stomata both in the light and in the dark (TURNER and GRANITI, 1969; GRANITI and TURNER, 1970).

The mechanism through which fusicoccin opens stomata has been investigated. The results of this investigation showed that a potassium pump is rapidly activated by fusicoccin and K^+ is accumulated in the guard cells and produces stomatal opening (SQUIRE and MANSFIELD, 1972; TURNER, 1972, 1972a; DURBIN

and GRANITI, 1975). Although several chemicals are known to close stomata, only cytokinins were reported to have a limited effect on stomatal aperture. Thus, fusicoccin and related compounds are the only example of compounds that open stomata efficiently.

It is known that plants under water stress accumulate abscisic acid (ABA) which is able to reduce stomatal opening by lowering the osmotic pressure of guard cells. This is accomplished by inhibition of K^+ accumulation in guard cells. Experiments with many plants showed that the effect of ABA can be completely reversed by fusicoccin (TUCKER and MANSFIELD, 1971; SQUIRE and MANSFIELD, 1972).

Fusicoccin effects, like increased transpiration and higher flow of water from the soil through the plant, could be utilized for practical applications.

An enhanced transpiration may be requested to speed up drying of plants which are used as food, fodder or for industry. During hay drying, for example, a rapid loss of moisture from the green mass would be useful to reduce losses in dry matter and nutrients during prolonged field curing. Consequences of rains would be reduced as well. Experiments carried out in Connecticut showed that, by spraying alfalfa with a weak fusicoccin solution 3 hours before cutting, the time requested to reduce the humidity of the alfalfa up to 40% was halved. In the field, alfalfa hay could be safely stored after 3 days instead of 4 (TURNER, 1970).

Preliminary experiments performed in southern Italy (GRANITI, unpublished data) showed that fusicoccin can speed up drying of tobacco leaves and reduce the length of tobacco curing by farmers. However, more information is needed on the quality and commercial value of hay, tobacco or other treated products, before any practical application can be recommended.

Experiments with fusicoccin and some non-toxic derivatives are now in progress in our laboratories. The aim of this investigation is an increase of plant absorption of pesticides, weed-killers, and other water-soluble chemicals from soil.

Fusicoccin is also active in breaking the dormancy of many seeds; it replaces gibberellins and cytokinins or red light in stimulating germination of photosensitive seeds (lettuce, for example); finally, it can reverse the inhibitory effect of ABA on germination

of treated seeds (radish and cotton, for example) (LADO *et al.*, 1974; HALLOIN, 1976). These findings indicate that fusicoccin may prove useful for weed control. Early germination of dormant seeds in soil and subsequent use of herbicides before sowing crops would provide a tool to reduce the cost of weed control.

CONCLUSIONS

Although the meaning of the term toxin is commonly related to the idea of death as suggested by its Greek etymology, we do hope that the power of phytotoxins can be used in agriculture for man's welfare, as the examples reported above would indicate.

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DISCUSSION

KARLSON

I would like to ask how long does the effect of fusicoccin last in the plant if it is sprayed under normal conditions, field conditions.

GRANITI

What effect do you mean?

KARLSON

Dessicating effect.

GRANITI

Dessicating effect is an irreversible effect. Absorption by plants of a toxic concentration of fusicoccin results in a permanent wilting and drying of leaves. Lower doses or spraying leaves with non-toxic concentrations of fusicoccin cause a transitory effect on stomatal transpiration, which can last for days.

SIDDAL

I was intrigued by your statement that the structure of fusicoccin cannot be varied very much without loss of biological activity. I wondered if any of the analogs which you have isolated from natural sources have an action on the ion pumps of animal systems or are they totally selective for plant systems?

BALLIO

As far as I know, up to now only fusicoccin has been tested on

animals, on some mammalian organs and on mitochondria. As I stressed, the activity of this compound is very selective for plants. This selectivity might be due to the intrinsic differences between "transport" ATPases of animal cells and the Mg^{2+}-requiring and monovalent ion-stimulated ATPase of plant cells, as, according to recent data of Marrè and colleagues, the latter is activated by fusicoccin and probably represents the receptor of the toxin.

SIDDAL

One more question: do you know of any polyether antibiotics which also affect the ion pump to cause opening of stomata in plants?

BALLIO

Do you mean valinomycin and similar ionophores?

SIDDAL

Yes.

BALLIO

Well, I cannot answer this question in detail. I think that valinomycin has been tested on plant tissues, but I do not know if it has ever been tested on stomatal cells.

ABO-KHATWA

From what you mentioned regarding the mode of action of some of these phytotoxins, it seems that their action involves a change in the permeability of cellular membrane. It is interesting to note that most of these phytotoxins' molecules — if not all of them — do possess potential intra and intermolecular hydrogen bonding capabilities. These hydrogen bondings were shown in several biologically active compounds to be important in aiding these molecules to bind with the membranes. Such compounds mentioned in literature are herbicides, I think benzimidazole derivatives and phenoxyacetic acid derivatives, and also several

local anesthetics such as nupercaine and other derivatives. It might be of interest to relate quantitatively the activity of these phytotoxins to the number of potential hydrogen bondings in the molecule, thus to determine their contribution in the detrimental activity of phytotoxins.

BALLIO

I do not think that the mechanism of action of fusicoccin really involves passive permeability; fusicoccin rather influences active transport phenomena.

ABO-KHATWA

Active transport is again a membrane phenomenon.

BALLIO

Yes, of course, but I prefer to keep permeability separated from active transport.

WAIN

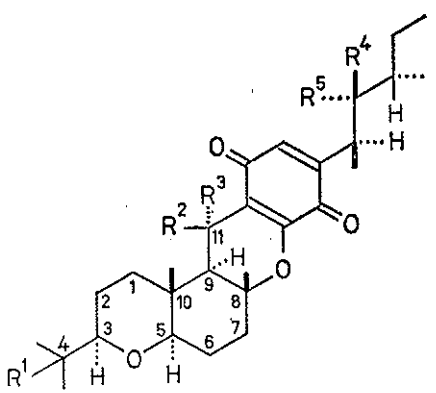
In view of the vital importance of potassium in the opening and closing of guard cells, I was wondering if any experiments are being done with plants which are suffering a mild potassium deficiency — to see whether you get the same responses with these compounds. Also since one knows that potassium can be taken in by foliar application through the leaves, whether plants so treated will respond differently to these very highly active and interesting compounds.

GRANITI

I do not know whether any experiment with potassium-deficient plants has been done. In our experiments with epidermal strips of *Vicia faba*, stimulation of stomatal opening by fusicoccin was accompained by accumulation of K ions in the guard cells from the sorrounding epidermal cells. When all the epidermal cells were disrupted by sonication and tbeir cytoplasmic residues removed, strips with only the guard cells alive were obtained. They were entirely dependent upon an exogenous supply of K^+ for stomatal movement. For this reason I think that even potassium-deficient plants contain enough K^+ to assure stomatal opening if treated with fusicoccin.

CANONICA

Only two short remarks. Professor Ballio and Professor Graniti have presented us a very interesting idea to inhibit the biosynthesis of the toxin in the fungus. I think this idea will be largely developed. Probably in some cases it will find hindrance in the extremely powerful catabolic activity of this kind of microorganisms. Eight years ago we were investigating the biosynthesis of ophiobolins, the phytotoxin of *Ophiobolus myabeanus*, and three or four years earlier we had found in a completely different work that a very simple compound, β-benzal-butyric acid, is a strong inhibitor of cholesterol biosynthesis of the ophiobolins. So we added our inhibitor to the culture of *Helmintosporium oryzae* hoping to stop the biosynthesis at a certain step, but the only result was that in 12 hours *H. oryzae* completely destroyed the inhibitor and thereafter it started to produce ophiobolin again. Another remark: very often a phytotoxin is accompanied by some minor metabolite. This is a general rule in nature — active compound is accompanied by minor metabolites. For example the *Ophiobolus* produces not only ophiobolins, but also a type of completely different phytotoxins. I have investigated the structure of these very powerful phytotoxins, the cochlioquinones, in cooperation with professor Marini-Bettòlo and his coworkers at the Istituto Superiore di Sanità (*).



(*) CANONICA L., CASINOVI C.G., FIECCHI A., GALEFFI C., MARINI-BETTÒLO G.B., SCALA A. and VACCARO TORRACCA A.M.: « Gazz. Chim. Ital. », *106*, 147 (1976).

Also the biosynthesis of these compounds is completely different from the biosynthesis of ophiobolins. It is probable that in this case the microorganism uses two different kinds of phytotoxins, and probably they are active at different points of the metabolism of the plant. About the question raised by Dr. Abo-Khatwa, concerning hydrogen bonds of ophiobolin, we have found with infrared spectra investigations an unsually stable hydrogen bond between the keto and the hydroxy group.

BALLIO

Professor Canonica has presented but one of the very many examples which demonstrate the flexibility of biosynthetic capabilities of microorganisms. In culture filtrates of *F. amygdali*, besides the family of fusicoccins just discussed, we have found a dihydroisocoumarin (5-methylmellein), namely an acetogenin, whose biosynthesis is of course totally different from that of terpenoids like the fusicoccins.

Coming back to the question of Dr. Abo-Khatwa, I could add that in the case of fusicoccin, hydrogen bonds must play a role; in fact, reactive functional groups or electrophylic centers, as for instance those identified in the compounds discussed by Professor Nakanishi, are not present in fusicoccin and the interaction of the toxin with the receptor probahly involves hydrogen bonds. Anyhow, the ion transport through the membrane is regulated by an energy-coupled process activated by the binding of fusicoccin to the receptor, probably ATPase, and is not a consequence of a direct modification of the membrane permeability.

V

NATURAL PRODUCTS IN THE PROTECTION OF PLANTS

Perspectives of agricultural applications of natural products
Industrial aspects

PROSPECTS FOR NEW CONTROL PRACTICES OF AGRICULTURAL PESTS IN DEVELOPING COUNTRIES

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Abstract

Agricultural pest control strategies in developing countries increasingly require pest control practices alternate to pesticides, in order to reduce the ever-increasing dependance on chemical pesticides and to make better use of the components of the agro-ecosystem. The need for developing more permanent pest management systems based on technical, ecological, economic and social considerations, is more keenly felt as unilateral approaches to crop protection often prove to be short-lived, apart from having several unwanted side effects. In the continuous struggle against pests, the use of simplistic approaches has resulted in the appearance of new pest problems, in the increase of genetic vulnerability of crops to pest attack, in the appearance and spreading of pesticide resistant pest species, in toxicity to man, natural enemies of pests and wildlife, and in environmental problems due to extensive use of persistent chemical pesticides.

The Food and Agriculture Organization of the United Nations has been promoting and assisting in the development of national and regional crop protection programmes based on integrated pest control concepts including biological control, pest resistant varieties, cultural methods, selective chemical pesticides and as far as possible,

natural substances. Examples of ongoing national programmes are cited.

Moreover, FAO in collaboration with the United Nations Environment Programme is currently undertaking a Cooperative Global Programme on the development and application of integrated pest control in agriculture. This programme aims to increase in various countries the capability to develop and apply integrated pest control programmes. As such, it is meant to create a structure which will allow developing countries to make full use of knowledge acquired in industrialized countries. The new developments in natural products such as pheromones and selective control means clearly fall into the category of knowledge to be transferred. There is, however, also a strong need to identify the major pest control problems in agriculture in developing countries and to see how they can be tackled with the help of research facilities which have been built up elsewhere.

Introduction

Crop protection technology developed in the last quarter century has been characterized to a very large extent by the almost exclusive dependance on the sole use of synthetic pesticide chemicals. The proliferation of these chemicals providing immediate relief to most urgent plant pest (*) problems has made farmers throughout the world trust in the effectiveness and relative ease of use. However, for the most part, the excessive dependance on chemicals has invariably led to a number of secondary problems, namely development of pest resistance to pesticides, rapid pest resurgences, and unleashed pest outbreaks. This is in addition to another set of problems involving toxicity to operators, unwanted residues in food and environmental contamination.

It is generally recognized by agricultural authorities that synthetic pesticides are and will remain, during the foreseeable future, a primary measure for combatting agricultural pests. On the other

(*) Throughout the text, the term pest is used to embody injurious organisms of animal origin, as well as weeds and plant diseases.

hand, it is evident that society cannot and will not tolerate the way in which conventional patent chemicals have been used during the last 25 years (DOUTT and SMITH, 1971). This impasse can be avoided by the development of pest management systems based on the intelligent application of pesticide chemicals, or the improved use of alternative methods of control and on the development of new ones (SMITH, 1974). An increasing array of alternative control methods are becoming available mostly in industrialized countries, to diminish the excessive reliance on pesticides. A strong trend toward development of pest management systems is being observed based on the integrated pest control approach, in order to make use of non-pesticide control measures to the extent they can provide adequate crop protection. In this approach a combination of control tactics is used and priority is given to the naturally occurring pest limiting factors in a given agroecosystem. It ensures a better-balanced system of crop protection, while providing effective pest control at lower capital inputs and less risk to the environment.

The development of pest control programmes under the latter scheme requires a good knowledge of many aspects of ecology and physiology. As WATERHOUSE (1974) has pointed out " it should be perfectly clear that, to embark on most new attempts to control without proper consideration of relevant aspects of ecology and behaviour, is to invite failure. It should also be recognized that, in general, the effective use of insecticides over the past 30 years has required far less background knowledge of the ecology and behaviour of the pest than do the majority of non-insecticidal approaches to pest control ". There is therefore an urgent need for more highly specialized national capabilities than now required for conventional pesticide-dependent practices. The transfer of technology of newer approaches based on pest population monitoring, assessment of economic injury levels, genetic manipulation of pests, and general management practices of the agroecosystem may therefore prove discouraging in its initial phases. However, efforts to improve the lack of knowledge and infrastructure are being multiplied in view of the world demand to put into practice new control approaches. In this context the Food and Agriculture Organization of the United Nations has an important role to play

to motivate developing countries and assist in the development of integrated control systems.

In order to evaluate the possibilities for practical implementation of new pest control technology and tactics, it seems justified to consider the following questions:

— what is the status of chemical control;

— what new methods are sufficiently promising as alternatives;

— what can be done to promote tbeir implementation.

Status of chemical control

Reliance on chemicals alone for the control of pests has a number of well known shortcomings. However, it is realized that most plant protection operations are based on this unilateral approach. This is particularly true in the developing countries where knowledge and structure to develop new pest control technology are generally lacking, although these countries absorb only about 8 % of the world consumption of pesticides for agricultural production.

However, in recent years major constraints have been placed upon the unrestricted use of pesticides. They are based on a number of factors:

— Food contamination by pesticide residues: better knowledge of the chronic toxicity of pesticides often leads to a review of the limits originally set. Presence of residues in agricultural produce above tolerance limits fixed by importing countries may interfere with export trade of developing countries. It has been shown also that some of these chemicals accumulate in fatty tissues and are then transmitted with milk, thus posing a direct threat to human health. The best way to avoid pesticide residues is their proper use, reduction of the number of applications and prolongation of the period between the last application and the date of harvest.

— Increased awareness of environmental pollution: persistent pesticides in particular began to accumulate in food chains and to

exert a high pressure on certain groups of organisms. For example, birds of prey have suffered considerably from these effects. Moreover, evaporation, co-distillation, condensation and precipitation distribute such chemicals to areas where they have never been used. They have even been found in animals living in the polar zones. Because of this, emphasis is now being placed on the development of pesticides which are biodegradable within a limited period of time.

— Increased costs of pesticides: prices of pesticides have continuously increased over the past years, and especially in the developing countries this has led to a situation where the farmer could no longer afford to apply the desired amount of pesticides in some crops. Even more important is the fact that current efforts to increase the production of food crops may be curtailed by these high prices.

These factors all point to the need for implementation of cheaper and safer crop protection methods where pesticides are used as rationally as possible.

New pest control methods

In recent years various new pest control methods have proven their validity within the framework of the integrated pest control approach. Most of these were originally developed in cotton growing and fruit orchards. Various pests play a major role in both of these crops and this has led to repeated pesticide applications, resulting in a number of undesirable side-effects, such as appearance of new pests and resistance to pesticides.

The first step in integrated pest control is to gain detailed knowledge of the population dynamics of the major pest species and to relate population levels to damage in order to identify the level at which crop losses are sufficiently high to economically justify the application of pest control measures. On the basis of the knowledge of the economic injury level and regular observations of population fluctuations, it will be possible to achieve better timing and thus more efficient use of pest control measures. This

alone can, in many instances, reduce substantially the number of pesticide applications, while still maintaining effective pest control.

Consequently, correct sampling of pest population in conjunction with reliable forecasting of pest development is becoming the basis of modern pest control. Any technique that can facilitate or improve surveillance and forecasting will be of direct benefit to better crop protection. As such, sex pheromones are becoming increasingly important. Used in special traps, they allow detection of the first appearance of a pest species during the season. This information combined with detailed knowledge of the bionomics of the insect, allow for an approximation of the timing of control measures in certain cases (MINKS and DE JONG, 1975). We are not yet in a position to establish the quantitative relationship between trap catches and pest incidence, but increased knowledge on the role of pheromones in the sexual relationships of the partners might provide clues.

Pheromones have also been used for monitoring population movements of the Egyptian cotton leafworm, *Spodoptera littoralis* (COPR, 1974). It might well be that in the future they will be increasingly used to assess population movements of other important migratory pests.

As mentioned earlier, correct sampling and forecasting of pest development constitute the basis for modern crop protection. Specific control strategies can then be recommended, of which the following are most important:

— biological control;
— cultural control;
— host plant resistance;
— selective pesticides and selective use.

When integrated pest control was first implemented in cotton growing and orchard crops, it soon became evident that naturally occurring parasites, predators and pathogens generally play a much more important role in the control of pests than had generally been suspected. In cotton, for example, the bollworms, *Heliothis zea* and *H. virescens*, are usually not of economic importance when beneficial organisms are not disturbed by the application of broad

spectrum chemicals (VAN DEN BOSCH, 1966; NEWSOM, 1974). The same is true for the European red mite, *Panonychus ulmi*, in fruit orchards (GRUYS, 1975). The overall importance of natural control to a large extent dictates other control methods to be used or the way in which they can best be implemented. It has also stimulated a great deal of research on the possibilities for making further use of existing biological control means. Two of these have been successfully explored:

1) introduction and adaptation of natural enemies into areas where the host has been introduced but the beneficial species concerned is lacking;

2) multiplication of parasites, predators and pathogens and subsequent release or application.

The first is particularly attractive as success, when it is achieved, is often permanent and does not involve recurring costs.

Cultural control includes, among others, crop rotation, soil preparation and destruction of stubble after harvest. A combination of these approaches was successfully used before the period of cheap and effective chemical control. Other modern agricultural requirements have also contributed to a reduced respect for these traditional methods. But changing attitudes towards pest control, especially failure to control pests with chemicals alone in a number of cases will certainly reinstate their use, at least to a certain extent. It should also be realized that they are likely to be quite inexpensive. In cotton the classical example is the control of the pink bollworm, *Pectinophora gossypiella*, through stalk destruction at the end of the season. In rice, avoiding overlap between growing cycles, which is mainly an organizational problem, will considerably reduce the impact of stemborers.

Host plant resistance is considered to be an effective and cheap method to reduce the impact of pests on crop production (BRADER, 1974). It should however be stressed that plant resistance to pest attack is mostly partial, and seldom provides complete control of pests. Optimal use of such varieties can therefore best be made within the framework of an integrated control approach.

Consideration of the above-mentioned elements will allow

using pesticides much more effectively and consequently in lower quantity and frequency. It is extremely important that their application does not reduce the action of beneficial organisms. This will increase the demand for pesticides with more selective action and techniques for applying pesticides more selectively (SMITH, 1976).

Although selective insecticides seem to present a very adequate solution to pest control, there are very few such chemicals on the market and it appears rather unlikely that many new developments can be expected in this direction. For economic reasons, industry is not inclined to embark extensively on research and development of physiologically selective insecticides. The ever-growing costs of research, of securing tolerance and registrations for use, of small economic return compared to broad spectrum chemicals, appearance of resistance to insecticides in many species and public opinion critical of the use of toxic chemicals are deterrents to their marketing. It is possible that some of the natural substances or related compounds will prove to be successful in tbis respect. The number of cases of practical use is still too limited to draw a conclusion. The discriminate application of broad spectrum pesticides to achieve ecological selectivity can be used in the development of effective pest management systems. For example, timing of application for period when natural enemies are not active. Control of the cotton boll weevil, *Anthonomus grandis*, just before it enters its winter rest avoids interference with natural enemies of the hollworm. Likewise, the use in winter of non-selective insecticides for cereal aphid control in South America when predators and parasites are still undergoing diapause or have not yet moved into the cereal fields is another example. However, such developments are very often hampered by poor knowledge of the biology and behaviour of pests and their natural enemies in a given cropping system.

Prospects for new pest control approaches in developing countries

Integrated pest control has been developed mostly under conditions of modern agricultural production, where the need for a

change was most strongly felt. It was gradually adopted on an increasing acreage and number of crops. It requires more detailed observations of pest-natural enemy-crop complexes and consequently higher specialized and more manpower inputs. But the extra costs of the inputs are largely offset by greatly reduced pesticide costs. And it has been shown in practice that this method provides cheaper and at the same time more satisfactory crop protection.

There is therefore every reason to propose integrated pest control as the best solution for crop protection in the developing countries.

However, a major limiting factor is a shortage of properly trained and capable entomologists dedicated to the development of pest management programmes based on the integrated control concept. Emphasis therefore should be laid on training consisting in fellowships and study tours abroad and in-service training at the medium and higher level. Assistance will often be needed to create the necessary infrastructure for carrying out adaptive research and for implementation of programmes. Strengthening of extension services in crop protection and communication with farmers require particular attention. It is in these areas that foreign assistance and FAO can play a useful role in the promotion of improved pest management by, for instance, assisting in the transfer of knowledge and putting into practice the results of research developments by the international scientific community concerning the use of natural substances in pest control. We should specially try to build on existing crop protection practices.

The ever-increasing awareness in developing countries of the manifold problems derived from the exclusive use of pesticide chemicals has led to a sustained although slow moving action in the adaptation of new control methods which allow for integration of all available pest control strategies leading to a reduction in pesticide usage.

The restriction or banning of several persistent insecticides in the industrial world is to some extent forcing adoption of alternative control methods in developing countries, since these chemicals have become less available or because there is no longer a residue tolerance allowed by the importing industrial countries, or the regulatory

capacity is missing in the exporting developing countries to assess the extent of permissible residues to be sure that they can export without the potential of rejection.

As a result of the world trend toward the adoption of integrated control methods, there has been an increased interest and a new drive in the use of biological control practices. The search for new entomophagous insects, the development of new mass rearing facilities, the increased studies on behaviouristic aspects of natural enemies including studies on sexual attractants, the search for new more pathogenic strains of insect viruses and bacteria, and the development of more efficient commercial formulation of entomopathogens are major highlights in modern biological control procedures. Accordingly, the prospects for an accelerated development of these methods are unique.

It is worth noting some national programmes involving biological control as an important component of integrated control methods. In Colombia, successful results have been obtained in cotton protection with the combined use of cultural methods, biological control (*Trichogramma*) and selective pesticides. Lead arsenate and mixtures of this insecticide with formulations of *Bacillus thuringiensis* have produced excellent control of leafworms, at the same time favouring the existing natural enemies. In addition, cotton growers must comply with regulations enforced by the Ministry of Agriculture regarding the use of broad spectrum insecticides, toxic baits, insect viruses for controlling *Trichoplusia ni*, sowing dates, destruction of stubble, and other cultural control methods (GONZALEZ, 1976).

Several developing countries have already been assisted by FAO in the adoption and application of integrated control programmes. These projects are focussed either on a particular pest problem or else on the whole array of pests in a given crop; major components of such projects include research, training and assistance in extension.

The first multilaterally supported project to develop an integrated control programme has concerned the coconut rhinoceros beetle, *Oryctes rhinoceros*, in the South Pacific. We will deal in some detail with this example of successful integrated pest control in developing countries. Native to Southeast Asia and India, the

beetle was accidentally introduced into Western Samoa in 1909 and from there spread to many islands including Tonga and Fiji. Beetles feed mainly in palm crowns and during the night move back and forth to breeding sites. The most favoured breeding medium is coconut wood, in the form of dead standing trees, fallen stems or trunks and stumps. The beetle damages the coconut palm by boring through the petioles of the upper and middle fronds and tunneling into the folded leaves and inflorescences of developing fronds in the crown. If the beetle arrives at the growing point and feeds extensively on its meristematic tissue, the latter may be destroyed and the palm killed.

The results of the 10-year project (1964-1975) sponsored by the United Nations Development Programme (UNDP) and implemented by FAO, initially by subcontracting to the South Pacific Commission (SPC), later under direct FAO management, have been summarized by PETERSON (in press).

Research investigations directed toward the development of useful and practical enough methods to be used by the rural populations have been undertaken in four directions: 1) cultural control; 2) chemical control; 3) biological control and 4) mechanical control (trapping). Several methods were developed and their use in a concerted manner to suppress populations of the coconut rhinoceros beetle constitutes a more or less typical integrated pest control programme. None of these methods used alone gives adequate control of the pest, but their combined application reduces beetle populations below the economic injury level. Field sanitation and a virus disease constitute the backbone of the control programmes.

General field sanitation has in the main been directed toward the destruction of beetle breeding sites, namely safe disposal of dead trees, fallen logs and stumps, and the abolishment or strict regulation of heaps of rotting organic matter. Enforced control measures however are unpopular and the disposal of coconut trunks is becoming too expensive due to increasing costs of labour. The project has therefore explored possible uses of coconut wood as timber and fence poles after treatment with a wood preservative, as firewood, or made into charcoal. Meals can be cooked on the latter material, thus saving on petroleum imports and a small

export trade has now been developed by Western Samoa and Tonga, whilst an experimental sawmill has been set-up in the latter country.

Regarding biological control, the possibility of utilizing natural enemies against the rhinoceros beetle has been extensively investigated. A number of introduced insect predators and parasites became established in islands of the South Pacific. However, none of them was found to be an important controlling agent of the beetle, although their combined effect on beetle numbers may be higher than attempts to assess their role have shown. On the other hand, the discovery of a virus disease caused by *Baculovirus oryctes* and research on the use of this disease and of the green muscardine fungus, *Metarrhizium anisopliae*, have produced significant results.

Upon the discovery of diseased grubs in Malaysia in 1963 and the isolation of a noninclusive insect virus, previously unknown to science, in a German laboratory, *B. oryctes* was introduced in 1967 with virus-infected grubs into Western Samoa, where an extensive field search had not disclosed the presence of the virus. After release in spring 1967, the virus spread rapidly and by 1969 beetle numbers and damage to coconut palms had declined conspicuously. Research showed that infected beetle grubs can transmit the virus to healthy grubs and diseased beetles to non-infected adults, and that the principal channel of virus transmission takes place in the breeding sites. Diseased beetles excrete virus in their faeces and have a much shorter life span than uninfected ones, whilst contaminated females produce fewer eggs. Those two effects of virus infection are considered to be the most important, causing reduction of beetle population. A very few beetles collected in traps, or mass reared adults, are infected by immersion in a suspension of the virus, obtained by blending virus-killed grubs in water. The released beetles carry the disease to the most remote places. By inoculating mass reared grubs and storing them after death in deep freeze, large amounts of virus are available the whole time.

Laboratory tests carried out in France to assess the possible pathogenic effects of the virus on man and certain warm-blooded

vertebrates, although not completed, have so far not detected any such effects.

Two virulent strains of *Metarrhizium* were identified. Only a small amount (50 g/m^3) of inoculum is required to treat heaps of decaying organic material in which spores remain infective to grubs for at least 24 months.

Improved trapping has been another development of the project. Split-log traps installed along the margins of coconut plantations and elsewhere are periodically examined for collection of grubs, pupae and beetles.

Another significant result was the discovery of two synthetic chemicals, ethyl dihydrochrysanthemumate, commercially known as " chrislure " and later the cheaper ethyl chrysanthemumate called " rhinolure " which are effective attractants for both male and female. A simple and cheap trap made with coconut wood and a 2.3 *l* tin containing a small tube and wick with the chemical attractant are used for beetle collection and destruction.

Despite extensive research, the large-scale use of insecticides to control the rhinoceros beetles remains largely uneconomical and impracticable. At present, the only way to include chemical control in integrated beetle control programmes is the insecticidal treatment of sawdust, dung and compost heaps.

It should be pointed out that all the above mentioned research and control programme development was carried out by expatriate staff. Training was an integral part of the project; however, only a small number of trainees with only high school education were available. As a result, a few nationals are now sufficiently trained in entomology to direct rhinoceros beetle management programmes, but not to continue research at the level it reached in the project.

In Argentina since the late 1960's, alfalfa production has been severely hampered by the pea aphid, *Acyrtosiphon pisum*. This led to an FAO/UNDP project aiming at the biological control of the pest and alfalfa improvement. The aphid parasite, *Aphidius smithi*, introduced by the project in Argentina, became rapidly established in the country and has afforded an excellent control of the pest. The project has also introduced or developed through selection and breeding, new alfalfa cultivars having better agronomic conditions

and greater tolerance or resistance to the aphid. Other alfalfa pest problems, namely the alfalfa butterfly, *Colias lesbia*, and several species of weevils (Curculionidae) are also being studied from the standpoint of their biology, natural enemies, and selective insecticides including *Bacillus thuringiensis* to develop a complete integrated pest control programme for all these pests.

In Nicaragua, an FAO/UNDP assisted integrated control project on cotton has successfully developed a pest management system based on the combined use of insect viruses, cultural control, monitoring of pests and natural enemies and establishment of economic threshold levels, thus leading to a substantial reduction in the number of pesticide applications (FALCON and SMITH, 1974). This project has been expanded to food crops, including maize, sorghum and beans and new protection systems based on resistant varieties, biological and cultural control are being developed.

On rice growing, the stem borers *Chilo suppresalis* and *Sesamia inferens* are being monitored with specific pheromones on large scale field trials conducted through an FAO/UNDP assisted project in Korea. The gathering of this data is fundamental in the assessment of pest population levels throughout the growing season in order to establish threshold levels and for timing insecticide applications. Another FAO project on rice in Thailand has been focussed on the biological control components of the agroecosystem, having studied the complex relationships between rice pests and all associated natural enemies.

Finally, a special effort to promote integrated pest control has been launched recently by FAO in collaboration with the United Nations Environment Programme (UNEP) called the Cooperative Global Programme for the Development and Application of Integrated Pest Control in Agriculture.

This programme developed by the FAO Panel of Experts on Integrated Pest Control will have a duration of approximately 15 years and will concern in priority besides cotton the main cereal crops: rice, maize, sorghum and millet.

The programme provides a global framework to increase the developing countries' capabilities for implementing these new crop protection techniques. It plans to achieve this through:

— set-up of demonstration study areas to serve as a base for training activities and to show the potential of integrated pest control, by identifying research needs and evaluating benefits to be drawn from it;

— organization of training, including training of technicians and research workers as well as organization of information seminars for extension staff and farmers;

— initiation of applied research on various factors of importance for the development of a local integrated pest control programme. This will concern, for example, detailed study of population developments of major pest species, studies of economic damage thresholds, the importance of natural enemies and studies of possible use of newly developed pest control substances.

Conclusion

The pressing need to produce more food and fibre, resulting from the tremendous increase in world population occurring during this century requires considerable intensification of agriculture. Increased production demands more inputs, particularly improved plant material, more fertilizers and efficient pest control. Improved growing conditions of crops will almost invariably lead to increased pest problems. This is particularly true of developing countries as a considerable number of them are situated in ecological conditions generally favourable to pest development. In most of them, at least three quarters of the population is dependent on farming. These countries do not have sufficient capabilities and infrastructures to face effectively the accumulating problems in pest control and other fields of agricultural research caused by the increasing intensification of crop production.

Although integrated pest control was first developed for the control of arthropod pests, it has become evident that the method needs to embrace the whole array of pest problems in the context of the total complex of activities involved in agricultural production. One can almost say that we will not be successful unless the other disciplines are also convinced of the need for a change. It is part-

icularly important that proposed new production techniques be developed within a common approach to avoid elimination of potential benefits.

It is evident that plant production and protection will require a great deal more sophistication and multi-disciplinary effort in the years to come. Within the framework of this rather unique study week, it may seem rather prosaic, but we come to the conclusion that a considerable increase in knowledge, manpower and financing is needed to achieve these goals.

Whilst current efforts in exploring new avenues in pest control and in development of research rest mainly with scientists of the industrial world, they should get more involved in the search for solutions to greater food and fibre production in the developing countries. In crop protection, aid programmes from both international and bilateral sources have hitherto been directed primarily at providing required facilities and equipment, and to training technical personnel. In the next stage, increased efforts will undoubtedly be made to assist the developing countries in carrying out and accelerating research to solve some urgent problems under prevailing local conditions. To this end, scientists are expected to give more generously their help and cooperation. It is recognised that a scientific and technological level should be reached before both human and physical resources can be made available to the more basic research work and therefore a major effort in training is required.

Agricultural education in plant protection sciences should critically examine the educational requirements of the students from developing countries. FAO has not anly had the opportunity of helping many students to obtain advanced and specialized training, but also of following their careers and activities after training in their home countries. We are convinced that in a training programme designed for those students, broad knowledge of principles involved in science and in application of scientific finding, as well as a broad outlook of agriculture and the role of pest control in agriculture, should be embodied. Unless a person is accustomed to examine plant protection problems from the context of the overall crop management system, it is unlikely that he will be able

to make full use of the knowledge he has acquired and to apply it appropriately and effectively in a different environment (BUYCKX and LING, 1973).

By joining efforts for better crop protection, developing countries and the international scientific community can contribute to the improvement of the living conditions of the small farmer and of the rural communities of the developing world.

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DISCUSSION

BELL

There is one question I would like to ask: a propos of my own type of research, which is directed toward the development of marginal crops which are themselves more naturally resistant to insect pests, I am anxious, as I told people yesterday, to obtain as many species of legumes which are grown on a small scale, to see why they are grown on a small scale and are resistant in certain areas and whether they could be developed into major economic crops, like acacias and things of this sort. Have you any comments on this? This seems to be a complementary approach to this problem.

GONZALEZ

Well, in general terms I would say that FAO has been fairly slowly moving toward that field. However, there have been instances where national and regional programs are being sponsored to develop these what you call marginal crops. At this point I should refer to the quinoa (*Chenopodium quinoa*). The quinoa is very important in the Peruvian and Ecuadorean Andes and is a marginal crop for many reasons. I would say that a major reason is because there are a number of local varieties with high content in alkaloids, but there are others, however, which have been through years selected by people which have a number of very good possibilities — and this is an important crop to replacing wheat, for example, in those areas — growing above 3,000 meters. FAO, I understand, is sponsoring a project on that, but due to the very pressing needs of major food crops, I would say that most of the FAO efforts are really oriented toward the major crops like those mentioned in my talk. However, the others are also important because that is the only possibility for subsistence under certain very marginal conditions.

SCHILDKNECHT

I did not understand exactly what is the role of the fungus last shown in control of the Rhinoceros beetle. What is the name of the fungus?

GONZALEZ

The fungus is the green muscardine, *Metarrhizium anisopliae*. The thing is that it has a very large number of strains which are sometimes very specific to a given insect species — there are two strains of the green muscardine which have been found in Western Samoa — and a very small amount of these pathogen cultures, something like 50 grams per cubic meter of the compost material where the rhinoceros beetle breeds, is enough to kill it. Now, this fungus — *Metarrhizium anisopliae* — has a very long persistence in the field because it has been found that the viable spores can last as much as 24 months in the field under those conditions. This fungus is very important in attacking the larvae, that is, the rhinoceros beetle grubs.

CHAPMAN

I think one can only applaud the sort of general aims that you are talking about — the idea of using integrated control, which clearly has to be the ultimate answer. And as you have indicated, it is a very difficult thing, in fact, to use well because of the complexities of the situation. I think the concern which I believe others probably share with me is that maybe clearly we have to go into this field, but my concern is that because we are pitched into it, as it were, we have no alternative, we must go into it now, and that as with varietal resistance as a specific instance, we get rather pitchforked into it without really knowing enough about the fundamentals. I would really like to ask two questions: first of all, is FAO as an organization in any way encouraging work on the fundamental aspects of integrated pest control? — how much effort is going into schemes to learn about the ecology of the systems before you actually start to apply any regulation? And secondly: it is always rather worried me that to use integrated pest control you obviously have to have a very good command of the situation — you have got to have a very good reporting system, you have got to have a control

system that can react readily; and it seems to me that this is an excellent thing if you are dealing with a developed country or if you are dealing with crops which are growing on a large commercial scale. I have always wondered, though, about its applicability at a small farmer level. I wonder if you could comment on that.

GONZALEZ

Yes. Well, first of all I would say that in a number of our field projects — as a matter of fact I just took as an example a couple of them — we are also dealing with basic aspects in the fundamental research in the development of integrated control schemes. I should mention, for example, the research being conducted for one pest growing on olives, the olive fruit fly in Greece, where a number of basic problems have been already tackled: the use of attractants, biological control and several other developments such as nutrient requirements for larval development; the latter aspect is fundamental to mass rearing the olive fruit fly, which is a major limiting factor at this moment, in order to produce massive numbers of fly larvae for population dynamic studies as well as for rearing natural enemies for the fly. This is just one example, but I wish also to point out that in other developing countries — I take the example of Nicaraguan cotton — there has been also basic research leading to the very immediate adoption of this integrated pest control program. Now, as to your second question, actually it is a real challenge to introduce this new plant protection scheme in a developing country at the farmer levels. I would say that what we have achieved at this moment has been in a limited number of countries. However, for example, when these programs can be adopted on a national scale such as the one on coconut rbinoceros beetle or the integrated pest control on cotton growing on a large scale basis, it means that the small farmers are able to develop these programs. You are very right in saying that there are a number of sophisticated elements which must be considered in order to carry out these programs. However, I should only regret that we are moving at a fairly slow pace, but eventually I think that we will cope with the increasingly larger number of requests — you would not believe the interest of the countries in getting rid of the conventional plant protection methods and utilizing integrated pest control schemes, aimed at diminishing the number of chemical applications — this is very encouraging.

FIELD USE OF «NATURAL PRODUCTS» FOR PEST CONTROL IN RELATION TO ENVIRONMENTAL PROTECTION AND PRODUCTIVITY

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Introduction

1) It is well known that modern agriculture has to face two absolute necessities. The first of these is to assure the feeding requirements of an ever increasing world population — economists and statisticians have estimated a population of 6,494 million by the year 2,000 as opposed to 3,632 million in 1970 [1] —; the second is to assure farmers an adequate income, comparable to the average standard of living of those working in other fields.

The farmer, like the agronomist, must remember that "an efficient economy of plant produce — both for the individual producer and for the world population — requires crops raised to a maximum and to a high level of biological properties. This must be obtained at minimum costs, taking the best advantage of natural bioregulators" [2].

The first means of satisfying such conditions — apart from the

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choice of those "crops which assure the maximum quantity of protein and calories per unit of cultivated area, per unit of fertilizer and water used . . . and a rational use of the by-products of the crops themselves" [3] — is "intensified farming" with the appropriate technical methods and, wherever possible, with subsidies for irrigation.

2) In any case, in order to farm on an economic basis, that is to say with high profits, farmers must aim towards a maximum rate in all production factors. This above all in the work which demands, among other things (as has been authoritatively confirmed above), the attaining of a high unitary yield of high quality products equal to the requirements, even the most refined, of a continually developing market.

But all this, however, has also determined the application of cultivation trends which have, in fact, proved to be factors of great modification in the natural equilibrium, with a complete innovation of biotic situations.

Crop specialization, or rather specialized farming with rapid crop rotation, has led to a rarefaction of certain trace elements necessary for the normal development of plants [4], and to a concentration on crops of damaging arthropods rather than to their dispersion, at least of the polyphagous species, on to wild plants. Phytophagous insects are also obliged to attack the same plants during the various stages of their life cycle, due to the lack of other host plants. The cultivation of specialized crops not only leads to a decrease in the number of phytophagous species, but also induces favourable conditions for the multiplication of arthropods, conditioned by the presence of one or another crop dominant in a certain area. Again the necessity of insisting on high yields has often hindered the preservation of the innate powers of resistance in vegetable crops to animal and vegetables parasites. In fact, high production of selected varieties is hardly ever compatible with the "sturdiness" that signifies resistance.

3) Therefore one resorts — often completely irrationally — to an innumerable range of pesticides (insecticides, acaricides, nematocides, mollusquicides, rodenticides, fungicides, herbicides) created by chemical science. Nearly always, however, the use of such

products does not take into consideration the negative action which they can have over the various "eco-systems". Already the natural equilibrium which presides over the preservation of nature has been upset.

It should be noted that modern agriculture bas already led to a profound modification in original eco-systems, of primary importance in ecological, phytosociological and pedogenetic fields [2], creating new and unstable ecological conditions. It is worth considering, for example, the endemicity acquired by some weeds in continuous cropped maize following the use of increasingly higher doses of herbicides. This has resulted in such a sharp drop in yield that the crop is no longer profitable. Again: the present indiscriminate use of pesticides (the so-called "second generation pesticides") has, in many cases, ended up by provoking ecological disorders and pollution of the environment. The consequences for human health become daily more serious, sometimes even beyond the limits of apparent reality. After the excellent results obtained from first applications, serious and unforeseen problems have gradually come to light. We refer to the accumulation of toxic residues in food chains, to the direct or indirect destructive action on animal organisms that have an irreplaceable part to play in the development of the vital processes of many crops (pollinator insects, microorganisms of the soil, symbions, etc.). We also refer to the "gaps" created in the eco-systems, and to the transference of toxic residues to surface water and gravitational water to such a point that it may become useless to man and to animals.

Finally. we consider the probable sterility, or the lesser degree of fecundity, or genetic alterations in anemophilous plants as a result of the toxic action of pesticides on pollen, stigmata and their very delicate papillae, as pointed out by Tonzig [5].

4) The necessity of obtaining substances that avoid such disastrous consequences in the present pest control, induces the researcher to consider, with great interest, the "natural products" — the object of our week of study. By "natural products" we mean not only substances obtained from plants i.e. pyrethrins, quassins, ryanodine, etc., or from animals i.e. pheromones, ecdysones, etc., and used directly against arthropods and harmful cryptogams, but also

substances present in the plants themselves, or eliminated by them and present in the soil. It will therefore be for the agronomist to evaluate what are the suitable measures to be taken in order to favour the formation of such substances. One must always bear in mind, however, the criterium of yield and economy from which the running of a farm cannot possibly deviate — unless, for social reasons, the state intervenes.

It has been said by S. Mansholt that "agriculture must produce at market prices and in such a way as to maintain the equilibrium of the environment, for the well-being of humanity". Also the resort to "natural products" (1) for an efficient preservation of nature must be included in well-studied productive trends. This is in order to limit their use to an indispensable minimum that is within economical limits. Preservation action can be enhanced by concomitant pest control carried out by certain traditional but still current agronomic practices. Moreover myriads of micro-organisms, fungi and mycorrhizae which emanate "natural products" are present in the soil; these natural products have fungicidal and probably also insecticidal action.

To sum up, in our report we want to point out the context in which agronomists and farmers must face the problem of protecting crops against pests. We intend to illustrate the fundamental productive means (such as fertilizers), the agronomic practices (crop rotation, water management, tillage, grafting, etc.) which favour the tolerance of plants. We also wish to indicate some trends in plant breeding that are the result of current knowledge of animal and plant physiology, biology and biochemistry.

In fact, as so well expressed by CHABOUSSOU [6] in referring to THIEM [7] "at the side of a geno-immunity there is pheno-im-

(1) We must, however, observe that not all "natural products" are able to solve the problems created by the majority of present pesticides (toxicity, persistence, etc.). To give an example, there is a natural product, well known for a long time — nicotine — which also causes a toxic reaction in mammals and which requires long intervals between treatment and harvesting of the product. It is certain, however, that the so-called "third generation" insecticides, the majority of which are "natural products", have an extremely high DL_{50} on man and other mammals and, as is already known, do not cause serious alterations in ecological systems or serious damage to human health.

munity depending on extrinsic factors (ecological conditions, structure and chemical nature of the soil, fertilization, grafting), the importance of which from the point of view of plant protection could be as great, if not greater, than that of geno-immunity".

Naturally, in our report we will by-pass all the subjects which, although within the context of general agronomy, will be dealt with in other reports during this week of study.

A - Cultural methods

1) *Crop rotation*

Certainly among the agronomic practices to be mentioned here, crop rotation holds an important place. In a vast bibliographic review recently published on crop rotation [8] there is a long list of papers that deal with the effects of rotation on phytophagous insects, on the populations of eelworms in the soil, on the cryptogamic diseases of plants, on the micro-organisms in the soil, on weeds, etc.

As for the phytophagous insects, the importance of rotation is realized for protecting crops from the damage caused by the larvae of gall gnats, *Haplodiplosis equestris* Wagn., on cereals [9], of springtails [10], of lepidopterous larvae [11] and of wireworms [12] on numerous cultivated plants. We can also remember *Oria musculosa,* which is greatly favoured in its development by cereal continuous cropping [4] but is controlled by crop rotation [13] and *Platyedra gossypiella* Saund. which can be sufficiently limited by interrupting cotton growing for two to three years [14].

Crop rotation has been found to be satisfactorily efficient in the control of eelworms, which cause fairly serious damages to cereals, potatoes, sugar beets, tobacco, tomatoes, grasses, orchards and forests [15, 16, 17, 18]. The polyphagy of many species makes it difficult to keep populations low, but careful rotation can give acceptable results.

A greater amount of data is available on the positive effects of crop rotation in the protection against various cryptogamic diseases. We mention that the rotation of wheat, oats, rice, maize with

different crops, together with correct soil preparation — to avoid water stagnation — prevents serious infestations of foot rot (*Fusarium* spp., *Leptosphaeria herpotrichoides* De Not., *Ophiobolus graminis* Sacc.) [19, 20] in wheat, as well as common smut (*Ustilago maydis* Tul.) in maize. When two successive crops of cotton are avoided, there are no serious attacks of verticillium (*Verticillium albo-atrum* Reinke and Berth) [21]. The same occurs for black root on tobacco (*Thielavia basicola* Zopf.) [22], black rot (*Aphonomyces levis* De Bary) on sugar beer [23], black root rot and wilt disease (*Fusarium* sp.) on beans, common scab on potato (*Hypochnus solani* Prill. and Delacr.), scab on cucurbits (*Cladosporium cucumerinum* Ell. and Arth.) [24], bacterial wilt disease (*Pseudomonas solanacearum* E. F. Smith) on tomatoes [25], basal stem rot (*Pectobacterium carotovorum* Jones) and black speck (*Rhizoctonia solani* Kühn) on potatoes.

But there is still more. Crop rotation is still an efficient arm in weed control in wheat, maize and minor cereals, in potato, tobacco, cotton, sunflower, sugar beet and in forage crops. In single crop farming, some weeds (*Avena fatua* L., *Echium plantagineum* L., *Alopecurus agrestis L.* for continuous crop of wheat [4]) end up by being endemic with particular virulence and power of survival, in spite of the use of increasing doses of selective or total herbicides. The same can be said for Johnson grass (*Sorghum halepense*) present in maize crops.

Lack of rotation, inducing the use of herbicides, is, either directly or indirectly, one of the sources of pollution of the environment and a further cause of rupture in the already very unstable biological equilibrium in every agro-ecosystem ([2]).

([2]) As for the use of herbicides, by now considered indispensable in modern agriculture, it must be remembered that these as well as insecticides, have a harmful influence on biocoenosis.

In fact, such pesticides, along with others, can cause real massacres of useful insects, such as the bee and other innumerable pollinator species which assure the entomophilous pollination of about 80% of the plants, among which many crop species. If, in certain areas, the farmer did not use the "pollination service" offered by beekeepers at the moment of flowering, he would risk seeing diminish the settings of nearly all his crops and, consequently, the output of the farm.

The use of herbicides, moreover, can lead to an ever increasing appearance of new dangerous phytophagous species. It is sufficient to recall the recent case of

On the other hand, crop rotation, with its natural elimination of weeds, which are often intermediate hosts of insects that are harmful to crops and of aphids and mites, which are virus carriers, is proving to be an increasingly precious auxiliary practice in pest protection of forage crops in general.

All this without mentioning the phenomenon known as soil "exhaustion": species like tomato, flax, sugar beet, alfalfa — to quote the most sensitive — as well as fruits such as the peach, cannot be grown repeatedly on the same plot, without a drop in their unitary yield. This above mentioned phenomenon is, to-day, unanimously attributed to root excretions, and therefore to natural organic products which do certainly influence the composition of the microflora present in the rhizosphere and in the surrounding soil, but not to such a degree as to not allow for normal development ([3]).

2) *Fertilization*

It is well known that intensive farming cannot be carried out without adequate use of organic and mineral fertilizers. In fact, to-day it is authoritatively accepted, on the basis of experience acquired from widely differing farming conditions, that "only combined organic and mineral fertilizers will give optimum results, both from the quantitative as well as from the qualitative point of view [27].

This is justified by a series of phenomena which originate from the humification process of organic substances. What is more, such phenomena have proved to give particular resistance to crops against

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serious damage caused to maize in Northern Italy by the ground beetle *Clivina fossor* Latr. Following the rarefaction of the soil microfauna, living on wild flora, this, by nature zoophagous species was obliged, after the use of herbicides, to modify its diet, and thus attacked the endosperm of maize seeds, causing serious damage to the crop [26].

These two examples are sufficient to clearly indicate the grave inherent dangers in the often excessive use of products which, nearly always, are considered for their primary effect only.

([3]) The fact that such excretions, usually alkaloid or glucoside, have a harmful effect on the microbial life of the soil, leads to the hypothesis that they could also have a similar effect on the life of crop damaging arthropods and fungi.

parasitic attacks in general, and also to cause an appreciable reduction in the damage caused by certain virus diseases [28].

It has been demonstrated that the most metabolically active plants possess the highest resistance to parasites. Consequently all conditions that favour "proteosynthesis" increase, at the same time, this resistance. Organic fertilization is, in fact, one of the most important of these conditions.

At the 5th symposium on "Humus et Planta" (Prague, 1971), the effects of humus on plant metabolism were well illustrated, and also reported by HAUSSMANN [2] at the meeting held at Piacenza on "Organic Fertilization in Modern Agriculture".

Certain physiologically active substances (P.A.S.) have been found in humus: humic and fulvic acids, phenols and products of their oxidization, thymo-hydroquinones and amino acids, all of which play a part in protein synthesis. In other words the humic acids and other growith substances which penetrate into the tissue through the roots, influence the synthesis of the enzymes and the enzymatic action, preventing breakdown of the amino acids and thus, as regulators, favouring the formation of protein. As a consequence the potential vigour of the plant increases. This is also due to the fact that the humic and fulvic acids stimulate the process of root formation, the solubilization of phosphate and, increasing the permeability of the cellular membranes, facilitate the absorption of mineral elements and water. But there is still more: the above mentioned assimilated organic compounds and the amino acid sequences produced during the protein synthesis, apparently cause greater or lesser alterations in the hereditary patrimony.

An increase in genetic variability is therefore offered to the plant breeders for the selection of disease resistant varieties.

It should be underlined that only by assuring the ionic equilibrium of cellular protoplasm, can one have intense proteosynthesis and then a high protein content in leaf tissues. From here stems the necessity of mineral fertilization regulated according to the availability in the soil of nutritious elements promptly assimilable and water and the potential yielding ability of the plant. An excess or deficiency in this or that element is the reason for functional disorders which favour the development of several diseases and heavier attacks

of different insects. There are ample records on this subject. Apart from various "deficiency diseases" that can be eliminated by administering microelements (B - Zn - Cu, etc.) even through the leaves [6] a number of observations of several investigators have recently been reported. According to MARKKULA and TIITTANEN [29], whilst a high quantity of nitrogen or a deficiency in potassium favours the reproduction of *Tetranychus urticae*, the supply of potassium determines a remarkable reduction of mites. An excess of nitrogen in comparison with potassium always favours the multiplication of aphids, *Myzus persicae* Sulzer in particular, responsible for the transmission of many virus diseases (cfr. also [30]).

On the other hand, it has been observed by one of us (FRILLI) that some phytophagous species — *Anarsia lineatella* Zell. and *Grapholitha molesta* Busk — cause less damage to peach fruits that have undergone nitrogen fertilization than to not fertilized plants. This is because, following such fertilization, the two lepidopterous larvae prefer to concentrate on the render shoots grown in the spring and summer months.

Balanced fertilization has proved to be even more efficient as a means of protection against different cryptogamic and bacterial diseases. Whilst an excess of nitrogen favours the development of stem rusts (*Puccinia* spp.) and of powdery mildew (*Erysiphe graminis* D.C.) in wheat, beet rust (*Uromyces betae* (Pers.) Lév.) in sugar beet, tomato leaf mould (*Cladosporium fulvum* Cke.), potato blight (*Phytophthora infestans* (Mont.) De Bary) and downy mildew of grapes (*Plasmopara viticola* (B. & C.) Berl. and De Toni), common grey mould (*Botryotinia fuckeliana,* De Bary Whetzel) in grapes apple scab (*Venturia inaequalis* (Cke.) Wint.) [6], potassium fertilization, when in ionic balance with nitrogen, hampers infection. It is the same with wilt of tomato (*Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr.) and of cotton (*Fusarium vasinfectum* Atk.), verticillium of cotton (*Verticillium alboatrum* Reinke and Berth.), sclerotina diseases in onion and carrot (*Sclerotinia* spp.), leaf spot of peanuts (*Cercospora personata* (B. & C.) Ell. and Ev.), Prunus blossom blight (*Monilia laxa* Adeh and Ruhl), certain bacterial diseases in leaves, such as "tobacco wild fire" (*Pseudomonas tabaci* Stevens) and so on [6]. Poplar can be protected against *Marssonina*

brunnea (Ell. and Ev.) Magn. with a suitable nitrogen fertilization [31].

Care must be taken, however, of the form in which the fertilizer is applied, especially for nitrogen. Calcium cyanamide, for example, has shown reasonable insecticidal action. Also anhydrous ammonia — now in ever increasing use — can not only destroy the larvae of numerous insects present in the soil, but can also control eelworms, fungi and bacteria especially when applied under the right conditions of humidity and temperature.

At this point, it might be useful to recall the agricultural technique called "biological farming" or "biodynamic farming" used in certain areas of the Maine et Loire district in France and also, more recently, in Western Germany. By this method, one tries to eliminate chemical means of production — fertilizers and pesticides — in order to obtain products of a high biological quality, that is, free of synthetic substances derived from these chemical products.

There is, therefore, a massive use of compost prepared with cattle manure produced in the same farm and distributed on forage crops grown in rotation with wheat.

After what has been said on the importance of a mixed organic-mineral fertilization for an efficient crop protection against cryptogams and phytophagous arthropods, we think it impossible, lacking nutritious mineral elements, to obtain those high unitary yields that guarantee economic productivity. "Biological farming" demonstrates how even the farmers feel the necessity to resort, although perhaps only partly, to "natural products" instead of using chemical fertilizers and pesticides.

3) *Water management*

It is well known that only soils which possess good structure, high water retention capacity and a satisfactory runoff control, allow the best development of root system, and, therefore, great crop resistance to fungus and phytophagous arthropods.

On the other hand, in fields lacking soil structure, more or less arid, an intense multiplication of aphids and mites has been observed [6]. THIEM [7] reports that the scale *Eulecanium corni* Bouché

attacks with greater virulence the trees which grow in too dry or compact soils. It has also been observed by SEGUIN and COMPAGNON [32] that excessive water absorption by grape vines (depending on the soil structure, on the consequent depth of the roots and on the available humidity) favours the development of grey mould on grapes (*Botrytis cinerea* Pers.).

Numerous other fungi are favoured by abundant water in the soil and lack of aeration. Thus drainage can hinder the development of zoospores in numerous species of *Phytophthora*, requiring water and that of the agents of black root rot and foot rot in tomato and sugar beet. In any case, good water management avoids in plants those physiological disorders which are sensitive to various diseases. A typical example is the blossom end rot of tomato and pepper which can be avoided by careful water management.

4) *Tillage*

Also to be considered are the contributions that various tillage operations — from seedbed preparation to scarification, hoeing and weeding — offer to the control of different phytophagous insects that live, at the larval stage, in the soil. Their effectiveness naturally depends on the speed at which operations are carried out in relation to the nature of the cultivated soil, on the type of instruments used, on the depth of working and so on.

Here it does not seem out of place to recall GIULIO DEL PELO PARDI's tillage system, by which — with the use of correct tools and the energy of animal traction — optimum conditions could be assured as far as structure, aeration, water retention capacity, etc. of soil are concerned, for intensive microbic life and a more healthy root system.

It is not, however, impossible that, in special cases, such as those related with heavy soils, orchards, vineyards, citrus groves, "minimum tillage" or "zero tillage" is preferable to the ordinary tillage.

In this way one can maintain the wild flora which constitute an alimentary attraction to various phytophagous polyphagous insects, potentially damaging the crops, but in practice insignificant when such a situation is preserved.

On this subject it would be useful to remember how each crop must be considered on its own, there being no possibility of generalizations. In fact, whilst on one hand correct tillage can decrease the populations of harmful phytophagous insects (see the case of *Mayetiola destructor* Say and of *Cephus cinctus* Norton, for corn), on the other it has been shown that it can be useful to leave a controlled condition of spontaneous grass growing in certain tree plantations. In peach groves, for example, wild flora can be host to certain antagonists of phytophagous mites, such as, for example, *Phytoseiulus persimilis* Athias-Henriot, an active predator for *Metatetranychus ulmi* Koch [33]. In vineyards it has been noted that, in the presence of wild flora the percentage of parasitism in grape moths (*Lobesia botrana* Schiff. and *Clysiana ambiguella* Hübn.) is greater than that found in areas where the vineyard is periodically tilled [34]. This could be explained by the fact that the lack of specialization of many parasites and predators allows them to develop on phytophagous insects living on wild flora and, at the same time, to extend their activity to phytophagous insects on crops.

Many authors have pointed out how a mixture of certain herbaceous species (in themselves repellents) and cultivated plants can hinder the attack on the crop of certain phytophagous insects [35].

Tillage technique must also be seen as a factor of the preservation of the soil flora (fungi, bacteria and protozoa) which produce various "natural products" (for example, antibiotics), inhibitors of the development of pathogens infecting the plant through its roots [31].

5) *Other cropping practices*

The above mentioned practices are not fully comprehensive of agronomic intervention in crop protection. The farmer must, in fact, avail himself of other means — long since in use in farming — which, although not favouring either directly or indirectly, the action of natural products, must nevertheless be considered as an integral part, of no uncertain importance, in pest control.

In tree cultivation, the practice of *grafting* has been shown to be of particular importance, especially with vineyards. Leaving apart what is already known about the use of rootstock in the control

of grape phylloxera, we would like to point out the positive action of some rootstocks in reducing the development of various fungal diseases or of phytophagous mites in the same variety [6].

Also the resorting to dwarfing rootstocks in certain tree cultivations (apple, pear, peach) allows one to obtain short plants with stunted growth that facilitates operations such as pruning and picking, thus rendering pesticidal measures more efficacious, even when they are applied to a lesser degree.

Another way of avoiding parasite attacks, both for certain fruit trees and for different herbaceous crops, is the resort to the early varieties of the same species, which complete their life cycle before the phytophagous insect appears. Another possibility is to have late sowing crops which begin their cycle when the adults of the harmful insects are no longer to be found. As a first example let us recall the early varieties of cherry which escape the oviposition of *Rhagoletis cerasi* L. and those peach trees whose fruit largely avoids the attacks of larvae of *Grapholitha molesta* Busck and *Anarsia lineatella* Zell. As a second example we quote the wheat sown late that is able to escape autumnal attacks of *Mayetiola destructor* Say. and the maize which avoids damage from *Hylemya cilicrura* Rondani.

Another positive means available to farmers for protecting their crops from cryptogamic and animal pests is the choice of varieties "resistant" to diseases and to phytophagous insects. Such "resistance", either temporary or permanent, or "tolerance" can be brought about, as will be said further on, in various ways, either by means of different "natural products", or by the presence of special components in vegetable juices (silicon, for example: see FIDANOWSKI, [36]; phenols and high percentages of amino acids), or by the recovery capacity connected with growth vigor [37], or by high proteosynthesis, or by the production of anti-fungal substances at the moment of the fungus attack (see the case reported by KATSUI *et al.*, [38] of potato tubers which form lubimin and hydroxylubimin, antifungus metabolites), or by the presence of glandular hairs that, by secreting special viscous substances, interfere with the movement of small phytophagous arthropods (as in the case of certain species of gen. *Lycopersicon* and of *Medicago scutellata*), etc.

Finally it must be remembered that, among the means of limiting, if not — at least in this case — of eliminating, fungal infestations

and athropod pests is that of *destroying all crop residues* (stalks, poles, cobs, pods, empty capsules, fragments of roots and tubers, etc.) either by burying, burning or breaking up. On the other hand, given that many diseases are transmitted by reproductive organs (seeds, rhizomes, cuttings, scions, etc.), it seems clear that the wise farmer will consider the choice of reproduction material to be of primary importance.

B - Genetic methods

There is no doubt that the most precious contribution to pest control (without using pesticides) is that offered by genetics. Here the farmer has at his disposal varieties specially selected for their peculiar resistance to different diseases and insect attacks, without affecting productivity. For many years, geneticists from different countries have been facing this problem with various species and with notable results. That is the case of varieties of wheat immune to various physiological races of rust (red, brown and black); of rice to blast (*Piricularia oryzae* Briosi and Cav.) and to foot rot (*Fusarium moniliforme* Sheld.) thanks to a high phenol content in the roots [39]; of sunflower resistant to blight (*Plasmopara helianthi* Novot.), etc.

The above mentioned case of rice seems to be very significant insofar as it sbows the importance of phytotoxins produced in plant species, even cultivated ones, in protection against fungal and bacterial pests (*Alternaria, Phytophthora, Pseudomonas*) [40].

Also in the forage crops there are numerous "eco-types" selected by the environment on the basis of particular resistance to adverse causes. Their capacity to synthetize natural substances is an expression of genetic combinations established under special conditions of temperature, humidity, light and physico-chemical soil composition.

In forestry, clones of certain essences (poplar, willow, elm, chestnut) have heen isolated and show less susceptibility to various serious cryptogamic diseases [31].

Also with phytophagous arthropods and eelworms, many examples have been found of crops less susceptible due to the presence

of repelling substances in the tissues, such as acrylic terpens, certain types of quinones, iridoid and alkaloid glucosides.

DE BACH [41] estimates that, at present, crop varieties having a certain resistance are known for about a hundred phytophagous insects. Research is being carried out on another 60 animal species. The same author, however, points out that very few cultivars possess together resistances for different adverse causes (arthropods, eelworms and cryptogams). In some cases it has been proved [37] that plants chosen for their resistance to a phytophagous species result more receptive to other ones.

Here, we would like to mention several varieties of potato containing phytophagous deterrents (such as demissine, solanine and tomatine) that have an inhibiting action on *Leptinotarsa decemlineata* Say [42]; varieties of potato and tomato resistant to *Meloidogyne* spp. [37], of cotton that cannot be attacked by *Anthonomus grandis* Boh [43], of *Chrysanthemum* spp. to *Phytomyza atricornis* Heigen [44] and to *Myzus persicae* Sulzer [45], of some *Rubiaceae* to lepidopterous larvae [43].

Geneticists have realized the importance of knowing the hereditary mechanism of genetic factors of resistance to various parasites [46]. Also the possibility is considered of obtaining, through mutation, crossing and selection, new varieties and hybrids able to produce — even to a limited extent — "natural substances" such as juvenoids, phyto-ecdysones, phytoalexins, antifeedants, repellents, present in a high percentage in other plant species and already well known in insect control (see, for example, quassin, rotenone, ryanodine, pyrethrins, nicotine, etc.).

It is in this sense that studies and research are being directed to-day at the Casaccia Centre for Nuclear Studies, a branch of the Comitato Nazionale per l'Energia Nucleare (Italy). It is here, for example, that some "natural substances" have been identified in olives that seem to act as repellents towards *Dacus oleae* Gmel. [47]. Among these is the so-called "vegetation water" that could, in the future, be used for the extraction of products useful against the insect.

Certainly, the ideal would be to obtain, as has been said, varieties of the different cultivated species that are resistant to different pests. Even if this result can not be attained in practice, it must be

remembered that a plant, with particular genetic resistance, can contribute to limiting infestations. Besides the presence of particular substances, also mechanical characteristics of tissues are important in determining some resistances. Here, there is no lack of significant examples as one of us (ZANINI) was able to experiment, several years ago in Sicily, on different varieties and hybrids of maize with respect to the attacks of the corn borer *Sesamia cretica* Led. This has been confirmed by experiments carried out by other researchers in different regions of Italy [14].

At any rate, plant breeding still plays an important part in the protection against various enemies by the creation of varieties with a life cycle not synchronous with the development of dangerous fungus or insect.

C - INTEGRATED CONTROL

Within the framework of agronomic and genetic interventions directed towards the maximum use of all "products of natural origin" and of all known cultivation methods, with the consequent decrease in the use of synthetic pesticides, must be included the concept of integrated control. "The corresponding expression, often used in English, 'pest management' — as observed by FRANZ and KRIEG, [42] — underlines the fact that one must learn to live with harmful organisms. The aim is no longer to kill the greatest number of pathogenic agents and harmful species within the shortest possible time (and usually by chemical means). To-day one tends to protect the environmental system, that is, to make sure that measures are taken not only in view of controlling the harmful species, but also taking into consideration the entire living community — all animals that live in the environment, the plants that grow there, and other non-living but nevertheless active factors, such as the climate and the soil".

It is not possible here to analyse the importance and complexity of integrated control. But it would be well to point out how such a modern vision of the plant protection problem forces one to consider, examine and coordinate all the data relative to the

agro-eco-system, as well as to the possible means of intervention, before deciding on the strategy to be adopted.

Integrated control must try to settle two fundamental requirements of the world to-day: firstly, to protect agricultural production and secondly to disturb as little as possible the ecosystem by avoiding or reducing environment pollution. This is perhaps the ideal towards which, in our advanced agriculture man should tend. But we are certainly only at the beginning.

This method of pest control, besides utilizing the above mentioned cultivation practices, expects much of the so-called "third generation insecticides".

Pheromones, repellents, antifeedants, ecdysones, phytoecdysones, juvenoids and toxins derived from micro-organisms, because of their relative specificity and because of their action on the behaviour of the insects themselves, make one look towards the future with confidence [48, 49]. In fact, their low level (or lack) of toxicity means that the problem of poisoning and polluting the environment can be avoided; the specificity of the majority of these products makes one think that the effects will be limited to the species concerned, while still respecting the other components of the ecosystem.

This does not mean that these new products do not present limitations or difficulties in their practical use. Here we quote only a few of them, relative to the synthetic sex attractants experimented by one of us (FRILLI) during the last few years:

a) after the recent commercialization of traps with "synthetic sex pheromones", it has been found that if, in theory, they are considerable help to the farmer in defining the population dynamics of any phytophagous insects and also in establishing the treatment time, in practice they risk becoming a means of creating "treatment psychosis" in the farmer. In fact the farmer, if new products — although innocuous — are not accompanied by clear instructions and informations, can be induced by the fact that few insects are captured to increase, instead of decrease, the number of pesticidal treatments!

b) There are, so far, available on the market, very few synthetic sex pheromones for phytophagous insects: what is more, they unfortunately do not give sufficient indications about the "intervention

thresholds" (fundamental for integrated control). In fact it has been demonstrated that the number of trapped insects is often not proportional to the populations present in the field. This means that it is difficult to indicate to the farmers numerical data on which to base the chemical interventions.

c) The limited specificity of some synthetic sex attractants, present today on the market, (e.g. the pheromone for *Grapholitha molesta* Busck [50, 51]) makes the use of such means somewhat problematical for the ordinary farmer: it is, in fact, not sufficient to count and remove the trapped insects, but it is necessary to identify the species, which is not always easy without a microscope (see, again, the case of *Grapholitha molesta* Busck and *G. funebrana* Tr.).

d) The cost of the products at present available greatly reduces the possibilities of their use both for monitoring the species (with traps) and for direct control (the "confusion" method).

We could go on much longer talking about advantages, difficulties and limitations of integrated control as seen by agronomists. But we are convinced that, after the necessary experiments for defining the most efficient methods for the various cultivations, a political choice will be necessary in order to put them into practice.

It is necessary to consider, on the one hand, production and on the other, public health. It is for man's welfare that he should induce legislators to impose different tolerance thresholds for the quality standards of various foods (e.g. fruit, vegetables) in order to reduce pollution from pesticides. But if science offers the farmer different natural products — the object of our week of study — integrated control could be established within a short period of time.

Conclusions

The description of agronomic, genetic and integrated control interventions that we have pointed out now brings us to some final conclusions.

We have already seen how, in the effort to ensure for mankind

the necessary food for survival, as well as the indispensable raw materials to satisfy life's requirements, modern agriculture must aim towards obtaining these various productions at a minimum cost. Now there is no doubt that among the means of production that weigh most heavily on the farm balance-sheet is the ever increasing use of pesticides, the cost of which is quite prohibitive in countries that are not equipped to produce them themselves.

But, apart from the farm itself, economic estimates must be seen from a much wider point of view. That is, all the currently preoccupying aspects of mankind must be taken into consideration (health, pollution, rupture of the eco-system, soil protection, energy crisis, etc.). It is in fact due to the energy crisis, that has hit so many highly industrialized countries, that a survey has been carried out by a team of investigators on any possible saving that could be made in agricultural practices if there was a considerable decrease in the use of chemical and mechanical products. They actually referred to: *a*) substitution of a fair number of chemical fertilizers with organic ones, producible in the farm thanks to an increase in livestock-breeding; *b*) substitution of a large number of herbicides with ordinary tillage and crop rotation; *c*) larger use of animals instead of machinery; *d*) recourse to varieties with increased pest resistence. This brings to light the importance of the interventions we have illustrated, and shows how they are topical insofar as they allow for a better and wider use of natural products made available for crop protection.

On the other hand, resorting to other natural products taken from animals and plants and subsequently synthetized by the industry would no doubt resolve the ecological problem, but it does not seem to be economically possible in the foreseeable future.

We would like to go over once again the concept of availing oneself as much as possihle of the natural means of protection which are already present in so many cultivated plants. We realize that this is not a problem that can be resolved immediately due to various implications involved, above all the impossibility of a rapid substitution of currently used synthetic pesticides.

In the first place, the farmer must be absolutely convinced of

the effectiveness of these new products. This means a whole series of demonstrations and explanations which cannot be carried out overnight. Amongst other things, it will be necessary to encourage and promote technical assistance from experts, as the farmers in every country desire. Farming can no longer be left to empiricism but must rest on a firm and highly scientific base.

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DISCUSSION

MARINI-BETTÒLO

Professor Zanini, as an agronomist — he was professor of Agronomy at the Catholic University, Piacenza — has a long personal experience in the field. He is in agreement with the genetic approach for the protection of plants from diseases but he also stresses the importance of the handling of the cultures which will help the resistance of plants to both insect attacks and diseases and also would lower the use of pesticides. He lays great importance on integrated control, not only of the balanced use of biological and chemical means but also on an adequate management.

This is a new approach to our problems and the philosophy of Professor Zanini's paper.

KNÜSLI

I must start by saying that I have every respect for this philosophy, but despite this statement, I think this paper would give an opportunity for debate during the whole afternoon. As we cannot do that, I want to deal only with one point. Prof. Zanini writes "Now there is no doubt that among the means of production that weigh most heavily on the farm balance sheet is the ever increasing use of pesticides, the cost of which *is quite prohibitive in countries that are not equipped to produce them*". This statement is misleading. I have tried to get some figures which really illustrate the situation. Our company carried out, in the years 1968-69 an integrated production project for rice in Indonesia and this in cooperation with the government. We have here the exact figures. We knew what the cost of the treatment was, and we knew also completely the value of the harvested rice. The cost of the treatment was 4.5% of the value. We ran similar projects on cotton in the Sudan in connection with the Gezira project. I have the figures 1974-1975 and 1975-1976: here

the cost of the treatment is 7.7%. Cramer of Bayer gave figures for Germany and he says — these figures now refer to the production costs, not to the value of the crop — that the average is in Germany, in the temperate climate, 1.3% of the production costs. For fodder plants it is 0.4%, for small grains it is 1%, and the maximum is for soft fruit 7.8%. And a further witness that the statement is wrong is, I think, our chairman himself. He gave the value of the world crop production as 150 billion dollars. Now it is known that the cost of pesticide — the application cost not included — is in the range of 5 billion dollars worldwide. So this gives you the true proportion. I would say that the correct paragraph should read rather: "In the present time one of the opportunities for the production of crops and other materials (for example cotton) is represented by the low cost of pesticides. This fact constitutes a great advantage also for those countries which do not possess industrial production of pesticides".

MARINI-BETTÒLO

All right, I think that the figures you gave are quite illustrative to clear this point and will appear in the proceedings. Now some other comments.

WILLIAMS

I would like to get back to this question of the possibility of a juvenile hormone being systemic within plants. If any compound has an opportunity of being so, it would be this remarkable new juvenile hormone insecticide epofenonane — and I wonder whether Dr. Silvia Dorn can tell us whether it has been tested for systemic action.

DORN

Yes, we did a few studies on the systemic activity of epofenonane. Indeed we got some positive results. After application of very high dosages of epofenonane to the roots of the host plant, the morphogenesis of the insects feeding at the leaves got disturbed. Interfoliar translocation, however, obviously occurred already after application of normal dosages to the leaves: The cotton leaf perforator and also the beech leaf-mining

weevil were well controlled by epofenonane in field trials (the larvae of both species are feeding in the leaf). So I would not call epofenonane a systemic agent, but it can at least penetrate the epidermis of the leaf.

WILLIAMS

And translocate from the applied place to somewhere else in the leaf?

DORN

I don't have the exact data, but I don't think so — I wouldn't remember.

CHAPMAN

I was slightly wondering whether Professor Zanini as an agronomist would think, as I tend to do, that really the problem is producing food in many of these cases — clearly we do have a pest problem as he indicates — but I am surprised that he does not say a little more about the problem of food production. It seems to me that in many marginal areas of the world we have not yet given the pests anything to eat, and if we were actually to provide food for the pests — if we could produce plants — we would overcome a lot of the problems of food shortage, without having to worry about the pests. If plant production was good enough — as I think it could be — then it wouldn't matter if the pests took 20% or whatever.

ZANINI

We can increase the production of plants for food purposes, but these plants would grow not strong enough to defend themselves from diseases — they would grow weak — and one should take into account all these means which were indicated here to grow plants as healthy and strong as possible and give them resistance. We should have a balance between the production, the productivity and the resistance of the plant to disease, because sometimes there are also some varieties which are quite resistant but they give a very low yield in crops.

CHAPMAN

Yes, of course, I agree and I understand that point. It does just sometimes seem to me that we do not place enough emphasis on plant production — and maybe that is just because of the people that I am acquainted with — maybe in other circles — I would feel they did not say enough about plant protection.

GILBERT

I am referring to the possibility indicated by Professor Zanini that not only substances obtained from plants, like pyrethrins and so on, or from animals may be used directly against anthropods, but also substances present in the plants themselves or eliminated by them and present in the soil. My question is: is anyone seriously looking at plants which eliminate substances in the soil that themselves destroy nematode larvae and probably eliminate other small parasites — is anyone seriously trying the intercropping of such plants? I mentioned some plants, like *Tagetes minuta,* lemon grass and mint and rue — but there are also a number of plants which do eliminate nematodes from the soil. These are small plants that are easily handled, everyone has got access to them. Is there any serious attention being paid to the intercropping of such plants with crops that suffer from nematodes, particularly in Italy?

ZANINI

A certain amount of research was done in this field, but so far the results which would be expected on the theoretical basis are not at the practical point.

STAAL

Yes, I believe also in answer to this, the tagetes story has been looked upon by very competent practical nematologists for at least 25 years, I think. Attempts have been made to identify the substances responsible. They were identified, but they have been found not good enough for nematode control as such. And the intercropping, of course will work on a very small scale but whether you can still carry on econo-

mical agriculture on a large scale with something like that is highly doubtful I think.

KNÜSLI

I have a further point in which, however, I am less strict than in the first I mentioned. Professor Zanini says "The use of herbicides, moreover, can lead to an over-increase in appearance of new dangerous phytofage species". I can not agree in principle here, but I wonder, here again you have the link between herbicides and the consequence and I think this is not the right link because the right link would be weed control. In consequence, if by herbicide treatments you eliminate weeds which fed before certain parasites, wben you eliminate these weeds, then the parasites attack the crop. Now, this could occur also when you do hand weeding.

I think this could be the consequence of the decision to carry out weed control chemically or mechanically. And so I think nevertheless it is a little nuance in the picture.

ZANINI

It is a question of quantity. The continuous use of herbicides leads to a complete alteration of the microflora of the soil and this fact gives a lot of trouble in the resistance and in the growth of the plant. So herbicides should be used hut not in excessive quantities and not even continuously because they may lead to serious inconvenience. For instance it was necessary in many cases to stop the herbicide treatments on maize cultures just for these reasons. In the case of sugar beet the excess of herbicides led to the outspreading of nematodes which overwhelmed the flora.

KNÜSLI

I agree but I believe that the same may happen if you weed by hand.

USE OF NATURAL PROCESSES FOR CONTROL OF INSECT PESTS OF SUGARCANE IN NORTHEASTERN BRAZIL

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Brazil is the greatest world producer of sugarcane. The production in the 1974/75 harvest was 6,720,000 metric tons of sugar. Although production is high, productivity has not reached the desired level, being estimated at about 1/3 of the productivity of Hawaii and 60 % of the average in South Africa, Australia and United States. This low productivity results from several factors, such as low genetic potential of existing species, varietal susceptibility to infection of diseases, deficiency of cultural operations and attack of insects.

Among the insect pests of sugarcane in Brazil, mothborers *Diatraea* spp are the most important because of the damage they cause. They are responsible for high losses throughout the country. Recent estimates show that for the infestation of 8.75% (average in the country) losses of sugar in the period 1971/75 were 1,236,633 metric tons of sugar corresponding to US$ 335,077,000 in international market prices [1]. The froghopper *Mahanerva posticata* causes great damage in northeastern Brazil, especially in the states of Pernambuco and Alagoas. To these losses are also added losses caused by the giant mothborer *Castnia licus* Drury (Lep,

Castniidae) whose natural habitat is the Amazon region. Adapted to the Northeast, it attacks mainly ratoon crops and is an insect difficult to control. During the 1970/71 harvest it caused an estimated loss of 31,000 metric tons of sugar in the state of Alagoas. The root froghopper *M. fimbriolata* although in lesser degree, also causes problems in sugarcane regions of Rio Grande do Norte, Sergipe, Rio de Janeiro and São Paulo [2].

The use of insecticides to fight these pests is not advisable. In the case of mothborers, owing to climatic conditions in the Northeast which favor continuous propagation of these insects in their ecological niche, and in the case of froghoppers because while insecticides are effective against adults, the same is not true witb regard to nymphs as a result of their physiology, which protects them from the action of insecticides.

In order to fight efficiently the most important pests, PLANALSUCAR (*) started in 1974 the National Integrated Control Project divided into three specific sub-projects, namely:

— Integrated control of the giant mothborer *Castnia Licus*.

— Integrated control of the froghoppers *Mahanerva posticata* and *Mahanerva fimbriolata*.

— Biological control of the mothborers *Diatraea* spp.

The integrated control of *Castnia licus* is subdivided in three processes:

1. Tests with insecticides through applications on leaves of ratoon crops.

2. Mecbanical and chemical control.

3. Search for and capture of natural enemies from the Amazon region (thus far undetermined) and their multiplication in laboratory conditions.

The main purpose of the second sub-project is the biological control of froghoppers. Its objective is to attain reduction of the use of chemical products which are considered not effective against

(*) The National Sugarcane Improvement Program.

nymphs and that can cause biological unbalance to other species, especially when used carelessly. Dependence on insecticides should drop drastically in Northeastern plantations of sugar cane with the introduction of entomogenous fungus *Metarrhizium anisopliae* which is highly specific to nymphs and adults of *Mahanerva posticata,* and other natural parasites of froghoppers — PLANALSUCAR has a central laboratory for the production and nation — wide distribution of this fungus. At the same time, studies on other natural enemies are being made, such as microhymenoptera *Acmopolynema hervali, Oligosita sanguinea,* and the diptera *Salpingogaster nigra* and *Salpingogaster pygophora* predators of nymphs.

The biological control of mothborers *Diatraea* spp has the purpose of reducing the populations of *Diatraea* (*D. Saccharalis* and *D. flavipennella*) to the economically allowed minimal limits (5 % of infestation). Investigations aimed at adaptation of the parasites introduced are being carried on. These parasites are *Lixophaga diatraea* T. (Dip. tachinidae) and *Apanteles flavipes* C. (Hym. braconidae). The latter is already completely adapted to the different ecological regions of Brazil; until now 2 million artificially bred wasps have been released. It is estimated that the present incidence of parasitims is 20% in average. Moreover, other investigations are being carried out with native parasites, namely: *Metagonistylum minense* T. (Dip. tachinidae), *Paratheresia claripalpis* (Dip. tachinidae) and *Leskiopalpus diadema* (Dip. tachinidae).

The initial results with *L. Diatraea* T. and *Apanteles flavipes* C. have been satisfactory in the states of Alagoas, Pernambuco and Amapá, while *Metagonistylum minense* T. shows optimal possibilities of being used on a large scale in the South of Brazil; thus far 50,000 insects have been released in the field. *Paratheresia claripalpis* W. is very efficient as a parasite, but it presents difficulties of artificial multiplication in laboratory. For this reason, the introduction of a Peruvian race of *P. claripalpis* which affords better conditions of artificial breeding is being tried [3].

In considering the problem of *Diatraea* in Brazil and the alternatives of biological control of this pest presently used, it is highly desirable that this be rationalized through the use of methods which

will allow the gathering of very precise informations on their populations movements and definitions as to where and when parasites of *Diatraea* spp should be distributed so that they may act with greatest efficiency.

Two alternatives were tested. In the first one, luminous traps were used and it was seen that the trap with green light was the most effective for *D. saccharalis* [4]. In the second one, traps baited with virgin females of *Diatraea saccharalis* and *D. flavipennella* were used. Results obtained were extremely interesting. Comparison of attraction by green light with the attraction exerted by virgin females has shown that in the second case the efficiency is 30 times higher when the number of males collected is considered. Furthermore, it was seen that traps baited with females are specific at the species level, that is, females of *D. saccharalis* only attract males of *D. saccharalis* and females of *D. flavipennella* only attract males of *D. flavipennella.* During the period of observation a strong dominance (2:1) of *D. flavipennella* was observed. Continuation of investigations has the purpose of determining population curves for both species.

The practical importance of these observations is self-evident. On the basis of the obtained information, the Sugar Cane Experiment Station and Chemistry Department of Federal University of Alagoas have prepared a research project whose purpose is to accomplish chemical and entomological studies in order to rationalize the use of pheromones in the control of *Diatraea* spp. This project is being presented to Brazilian agencies for the needed financial support.

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NEW TRENDS IN RESEARCH FOR THE PROTECTION OF THE COFFEE PLANT AGAINST COFFEE RUST

MARCO QUIJANO-RICO

Laboratorio de Investigaciones sobre la Química del Café y de los Productos Naturales de la Federación Nacional de Cafeteros de Colombia.
Bogotà

The problem of the protection of plants is a very important one for developing countries and in particular for a "green country" like Colombia, which depends on agriculture for its economy.

I believe that developing countries must actively participate in research in this field, approaching a solution which should be compatible with the protection of the environment.

For example, it is possible, investigating in this field, to obtain important clues for the most efficient use of the known control methods, in particular environments such as many tropical areas.

Coffee, without being properly a food product, because of its large use all over the world constitutes in various countries and mainly in Colombia (*) a product of basic economic, and thus social, importance [1].

In most producing countries the production of coffee is maintained through a continuous control because of the several pests and diseases which affect more or less seriously the coffee plant.

(*) In Colombia fifty percent foreign exchange currency is due to the export of coffee. Three million people are living directly from coffee. A large proportion of the farmers involved in coffee production are small farmers, who produce the coffee themselves. People living in coffee producing areas have one of the highest standards of living of the country.

The most important disease, the so called coffee rust, *Hemileia vastatrix*, which affects mostly the *C. arabica* species, is widespreading all over the world. In 1970 it had appeared in Brazil and it has been calculated that the reduction of the production in affected areas can reach 40%. Colombia is not yet affected by this disease but due to the imminent contagion, measures should be envisaged in order to coexist with it. It is known that many chemical products may be used with quite good results to control the coffee rust. Nevertheless, without taking into account other problems associated with their use, it seems that the cost of the protection may constitute for some countries, a limiting factor.

Another method would be to grow resistant varieties, but, facing the high probability of hybridization and production of new races of the parasite, one cannot exclude their failure in the course of time. It is too difficult to obtain resistance and to conserve organoleptic quality simultaneously, especially for the Colombian varieties of *Coffea arabica* which finds important markets for its particular features. In order to face these problems the Research Laboratory of the National Federation of Coffee Growers of Colombia is now working on chemical problems related to coffee technology and will do research on selected topics of the Chemistry and Physics of the coffee plant protection. Research in this latter field should include the protection of coffee against pathogenic attack and the chemistry of resistance to diseases and pests. Recently J. GONZÁLEZ at the Universidad Nacional de Colombia in Bogotá, demonstrated the different patterns of flavonoid contents in varieties of coffee from Oeras (Portugal) which are resistant and non-resistant to coffee rust. The first results show that only resistant varieties contain quercitrin, the glycoside of the pentahydroxyflavone, quercetin [2].

We have determined 27 microelements by thermal neutron activation analysis of different coffee. We found that the elements distribution pattern was correlated with the different species [3].

The knowledge of the content and distribution of bio-elements in plants can be used as an important key for the understanding and utilization of several biochemical phenomena.

Trace elements play a vital role as enzyme constituents and activators in many cellular and metabolic mechanisms.

Because of their role in the enzymatic catalysis they are asso-

ciated with very particular, genetically determined, biochemical pathways.

It could be possible to obtain an idea of the enzymatic state of a living organism through the analysis of the trace element content and distribution. This may be an easier way to attain the enzymatic state because of the relative simplicity and sensitivity of modern analytical methods like the thermal neutron activation analysis, that allows the simultaneous determination of many elements.

The simultaneous study of the bio-elements and selected organic compounds may give interesting information for the protection of plants against pests and diseases by the production of better products, since bio-elements may play a role, due to their organoleptic and nutritional properties, in obtaining better yields by designing more complete fertilizers.

Coming back to the protection of plants, from the viewpoint of inorganic biochemistry, one should take into account that chelation and deactivation of the enzymatic catalysts are often the principle of action at the molecular level of many drugs and products used to protect living organisms from infectious agents.

Essentially, the bio-elements participate in the biochemical mechanisms that are responsible for the synthesis of natural products of the plants and thus also of those useful for the protection of plants. They have thus a place in the natural product research for disease control as a kind of biochemical precursor.

Because of these reasons we plan the parallel investigation of the inorganic and organic biochemistry of the coffee plant.

I thank you for the opportunity you gave me to take part in this study week, the results of which are most important for my country, and for the possibility of calling your attention to the great problems involved in the protection of coffee.

I hope that I may have in future your cooperation for our researches.

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DISCUSSION

MARINI-BETTÒLO

I should like a few comments on the report presented by Alves de Lima in conjunction with Risco Briceño about the researches in Alagoas, northeastern Brazil, on the ecology and the possible control through pheromones of the parasites of sugar cane.

Sugar cane is one of the major cultivations in the world, of great economic importance. It is also known that it is attacked by a number of parasites like *Mahanerva posticata* and various *Diatrea* species.

The experiments carried out so far are made with traps baited with female *Diatrea*, but the aim is to isolate the specific pheromones.

I think that this is just the beginning of an important research in this field, which I believe may also be supported by the countries interested in producing sugar cane because it involves the supplies of sugar for a great part of the world.

I think that it was important in this study week to have raised the problem of sugar cane protection, so important for tropical countries, and to consider it in our discussions.

Now the problems of coffee have been raised by Dr. Quijano Rico director of the Laboratory of the Federation of Producers of Coffee of Colombia. This Laboratory possesses very good facilities for pure and applied research.

Also coffee has a great importance in the economy of many tropical countries: Brazil, Colombia, Central America and many African States, and all approaches directed towards the protection of the plant must be envisaged in order to avoid diseases.

It is very interesting that resistant coffee strains have been found to contain flavones, whereas they are not present in non-resistant strains. The biogenetic linkage between flavones and pterocarpanes

and isoflavonoid phytoalexins is most interesting, and we must consider if these flavones may represent a sort of pro-phytoalexins.

These two short communications have the merit to draw our attention not only to cereals, leguminosae, solanacee but also to these typical cultures so important for the economy of developing countries.

GILBERT

Is there any juvenile hormone or anti-juvenile hormone that can be fed to the roots of sugar cane so that it comes in contact with the insect during its larval stage?

STAAL

Juvenile hormone and most analogs are not taken up, or at least not transported, as intact molecules in the plant. The only exception so far has been the peptide JH analog of K. Slama, which is very selective in that it is only active on pyrrhocorid bugs. As a matter of coincidence, the structure of this peptide analog is surprisingly similar to the juvenile hormone antagonist that I described yesterday, but which has an entirely different spectrum of selectivity.

BOWERS

There have been one or two other compounds that have shown some small systemic activity — principally some of the methylene dioxy compounds — which have been put in the soil in pea plants and picked up by the peas. They may induce morphogenetic effects on insects fed on the upper leaves, though the amount that is translocated is extremely small.

BALLIO

About flavones and resistance, I think in Israel they have performed some work on citrous plants and have demonstrated that flavones are responsible for resistance of some trees. This is quite unrelated to coffee of course.

GRANITI

Have you tested the susceptibility of the Colombian coffee varieties against the rust in Colombia or were you compelled to do this genetical work abroad?

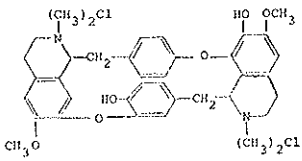
QUIJANO-RICO

All these studies were done in the Portuguese Center of Oreras.

INDUSTRIAL ASPECTS OF THE PRACTICAL USE OF NATURAL PRODUCTS OR DERIVATIVES IN THE PROTECTION OF CROPS

E. KNÜSLI
Ciba-Geigy Ltd., Basle - Switzerland

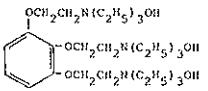
You will, no doubt, be rather surprised, that I start my formal contribution to this Study Week with a projection of the structure of d-Tubocurarine,



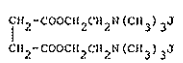
a material which has really nothing to do with plant protection.

d-Tubocurarine, component of preparations used as arrow poisons, has cholinergic and nicotinic properties and acts on the neuromuscular transmission; in the forties it was introduced into surgical anaesthesia as a muscular relaxation agent. Now I ask you to give your attention to the two quaternary ammonium functions, as DANIEL BOVET and coworkers did at the Pasteur Institute in Paris and at the Istituto Superiore di Sanità of this great city of Rome in the late forties. Working around this model they were

able to develop much simplified structures which offered improved qualities and which reached the level of clinical use, as



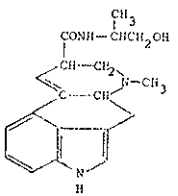
Flaxedil (R) Sincurarine (R)



I.S. 362 Succynilcholine

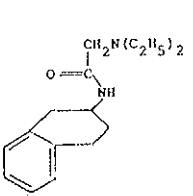
(BOVET D. *et al.*, 1949)

A couple of years later G. B. MARINI-BETTÒLO, DANIEL BOVET and coworkers, again at the Istituto Superiore di Sanità, became interested in the structure of the alkaloids derived from lysergic acid:

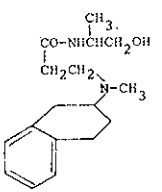


Ergobasine

This time they gave their attention to the tetrahydro-β-naphthylamine moiety of the molecule. They found that such simplified structures as I.S. 621 and I.S. 833 possess still substantial oxytocic activity which is even observed in tetraethyl glycinamide, I.S. 1062:



I.S. 621



I.S. 833

$(C_2H_5)_2NCOCH_2N(C_2H_5)_2$

I.S. 1062

(MARINI-BETTÒLO G. B., BOVET D. *et al.*, 1952)

I have made this detour, which I ask you to excuse, for two reasons:

— in order to give due credit to the genius loci and for expressing my feelings of deep gratitude for that with which he provided me;

— to express my firm belief, my credo, that a look at the chemical infrastructure of nature can be extremely stimulating and rewarding also in the field of the protection of plants against undesired biological vectors.

By analysis of the work which has already been carried out along such lines it seems that, principally, two approaches can be recognized:

— to search in nature for exogenic structures, which may have a desired activity against a target organism. Examples: nicotine, pyrethrines, rotenone;

— to search in a target organism for endogenic structures, which regulate its biological sphere. Examples: juvenile hormone, insect sexual attractants, pheromones, ethylene, indolyl acetic acid.

As a result of these approaches, structures suitable for use may be found and brought forward to the level of practical application. Very often, however, the structures lack some prerequisites indispensable for practical use and in this case the structures will serve as a lead and induce the chemist to structural variation. The fruits of such endeavours have then lost the innocence — if this ever was innocence — of a naturally occurring product; it may however be that their chemistry and their properties have still much in common with the original natural product.

In this characterization of the role which natural compounds can play in providing useful tools in plant protection I mentioned expressions such as: lead; structural variation; prerequisites indispensable for practical use.

These terms are all so familiar to somebody who has been involved for many years in research and development activities also for structures which do not show a link to naturally occurring compounds. Therefore I cannot escape the temptation to put these two strategies side by side and to compare them, and this I intend

to do now. It will be interesting to see the differences — there are differences — and to see the similarities — there are tremendous similarities — in the aspects which have to be considered in the course of the development of a substance for practical application. To this end I will deal briefly with the following chapters:

Ways to new structures;
Persistence of structures;
Biological activity;
Toxicological evaluation;
Evaluation of the environmental behaviour;
Economic competitiveness.

Ways to new structures

The chemist searching for new, biologically active structures has an infinite number of possibilities to combine atoms to molecules, and this forms a part of the fascination of his profession. In the field of insecticides he has encountered interesting natural models from the very beginning. But during the past forty years the chemist has devoted much of his attention to anatural structures, and the fact that some of these have shown spectacular activity is not the least reason for this. Such anatural structures have reached and will maintain a major practical importance and there is, in my opinion, still a long way to go using this strategy.

In some fields, however, his imagination reached an impasse. Despite the fact, that members of a large variety of chemical classes were examined, in the field of insecticides, for example, only four classes led to useful compounds. You all know them: thiocyanates; halogenated hydrocarbons; P-esters; carbamates.

The chemist may also be trapped sometimes by tradition and his ideas may suffer the negative consequences of intellectual in-breeding. At such times, impulses which could bring motion to a static situation by suggesting new structural matrices are more than welcome. The creative suggestiveness which emanates from the knowledge of and the familiarity with the chemical infrastructure of nature is, besides other factors which I will mention later, a major strength of this strategy.

Persistency

On the practical level the solution of problems by the use of chemicals, however they are conceived, presents an inherent dilemma: in the majority of cases a certain duration of the effect is desired, because the target organisms need to be controlled over a certain period of time. A campaign against tropical disease carrying insect vectors should not necessitate more than one treatment per season or year. A selective herbicide should control emerging weeds until the crop will no longer suffer from weed competition. But then, as soon as the job is done, the compound should disintegrate as quickly as possible. In the face of this dilemma of apparently incompatible requirements any compound must be a compromise.

On the average natural structures have a better chance of being degradable in a reasonable period of time, and the probability that their terminal residues are simple, basic structures is great. Since they were built up under natural conditions they will usually also degrade under natural conditions. Often their degradation is even too fast so that antagonistic measures have to be taken, for example through special formulation techniques.

Modified natural structures have to be examined case by case. It has to be remembered that only the introduction of a methylene group can change drastically the degradability of certain structures as illustrated by the example of a couple of alcohols:

$$CH_3(CH_2)_9CH_2CH_2OSO_3Na$$

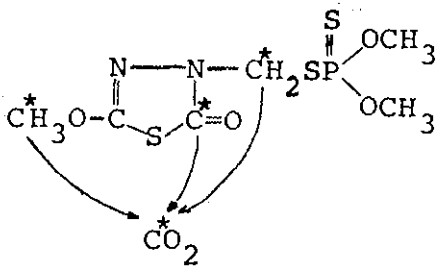
$$CH_3(CH_2)_9\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2OSO_3Na$$

(FISCHER W.K., 1967)

The speed of the biodegradation of the branched chain C_{13}-detergent is drastically reduced when comparing it with the C_{12}-analogue.

The postulate of degradability is fulfilled also in many anatural structures. They, too, can degrade to terminal metabolites which are very compatible with the environment.

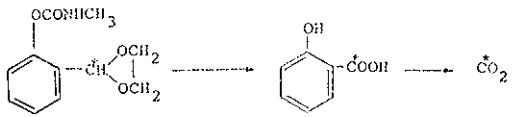
Example:



in soil 45% metabolised in three weeks

(DUPUIS G. *et al.*, 1971)

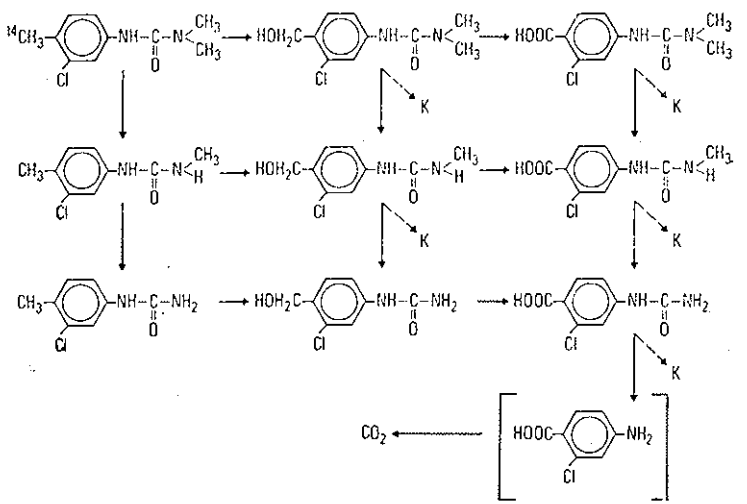
Example:



in soil 42% metabolised in 12 weeks

(BURKHARD N. *et al.*, 1975)

The chemist now dealing with anatural structures can and will consider the postulate of degradability in the compound conception phase much more deeply than in the past:



(MÜCKE W. *et al.*, 1976/77)
(GROSS D. *et al.*, 1976/77)

In the case of the respective 3,4-dichloro analogue the speed of the mineralization is much slower.

For all chemical structures which are to be applied in plant protection and related fields, be they of natural or anatural character, persistence can only be measured by analytical means. In each case it is compulsory, therefore, to develop very sensitive micro-analytical methods. Sometimes a bio-assay may also give useful, although not fully conclusive information.

The metabolic behaviour and the nature of the terminal degradation products have, again, to be studied case by case. The working with radiotagged materials provides invaluable help in such projects.

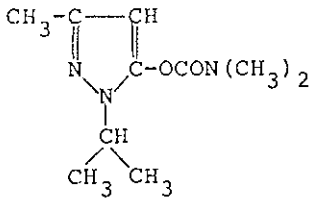
Biological activity

Activity is the first and decisive requirement which a compound has to satisfy. It may be a trivial statement to say that the nicest, most untoxic, easily degradable, cheap material is of no use when not sufficiently active. And when I say active, this means active under practical conditions. I have recently defined that the aim of our efforts is in the end not to kill or to eliminate destructive vectors but to protect the crop plant. It follows that also a per se active insecticide which would kill for example the larvae in the end but only after it had destroyed the crop could hardly be offered in this form to the practitioner.

A further overriding principle in the search is, of course, selectivity; a herbicide should eliminate plants and be of low risk for warm blooded organisms, for the microflora, for the microfauna; but even more: it should selectively destroy some plant species leaving some others unharmed. In the field of insecticides a compound should eliminate insects and cause no phytotoxic symptoms on the respective plants; but in the recent past an even refined selectivity requirement has been established: harmful insects should be killed while predator insects should remain untouched.

I believe that, as far as insecticides are concerned, the natural and the anatural strategy offer similar opportunities for reaching this superior level of selectivity.

Like many of the phosphate insecticides also pyrethrine and, if I am right, some of the newer pyrethroids have a very broad spectrum, not differentiating too much between injurious and useful insects. On the other hand natural attractants and juvenile hormone mimics are very specific. Also representatives from the series of carbamates have a quite selective behaviour. As an example the carbamate Isolan(R) may be quoted:



(GROB H., 1952)

It has an outstanding selectivity pattern in so far as it eliminates Aphidae only, leaving other insects and particularly such predators as Coccinella and Calcididae unharmed. The LD_{50} of the compound is quite low, but the formulation which was offered to the farmer did not present any special risks. However, despite the fact that scientists from various state experimental stations supported for environmental reasons enthusiastically such a very selective product, the compound was a failure. The practitioner does not usually like products with too narrow a spectrum, because they force him to increase the number of bottles on his shelf. The behaviour of the grower is a factor which must always be kept in mind. There is also no doubt that new concepts and new approaches need an intensive educational effort.

A further aspect of activity is the build-up of resistance. Resistance against herbicides has been observed occasionally; more important is, in this field, the selection of resistant species out of a stand with a mixed weed population. In the field of fungicides resistance has become a problem only recently in connection with the use of a particular structural class. In the field of insecticides the development of resistance has been an unpleasant phenomenon for a long time, although it has materialised in many cases much slower than expected. A. W. A. BROWN from the Pesticide Research

Center of Michigan State University made at the ACS-Meeting in New York, in April 1976, the quite depressing statement, that there is evidence that with respect to all chemical weapons introduced up to now cases of the build-up of resistance have been observed. He included in this comment Pyrethrines, Pyrethroids, *Bac. thuringiensis*, JH-mimics. Natural and anatural agents seem to have the same limitations in this respect. It looks likely that in this field an enduring, tough fight between insect and man will continue.

Attractants may perhaps be a way out of this unpleasant situation, as they allow the luring of the insects at a place where they can be destroyed by physical means or heavy dosages of chemicals. This technique would also offer important environmental advantages. However, the limitation of this method could be in its extreme specificity and in the fact that the degree of efficiency is quite far from 100 %, so that enough females are fertilized to ensure procreation.

Toxicological evaluation

Here again there is no principal difference between natural and anatural compounds with regard to the need for evaluation and the evaluation methods. The unhappy record of extreme toxicity is shared by representatives of both classes:

TCDD	LD_{50} rat	0,023 – 0,045 mg/kg
Botuline	LD_{50} mouse	0,00005 mg/kg

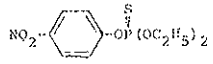
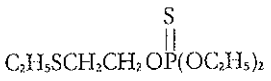
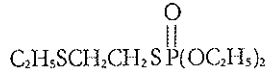
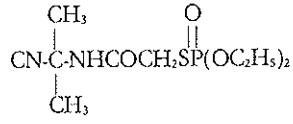
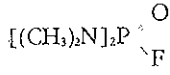
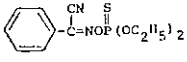
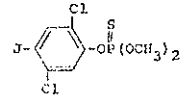
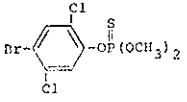
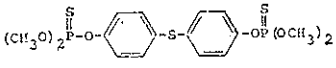
(SPARSCHU G. L. *et al.*, 1971; LAMANNA C., 1959)

As a consequence, the carrying out of the usual evaluation programme which is very demanding today, is necessary for each compound which is expected to be developed for practical use. Also the conclusions which can be drawn from analogies are limited. Let me remind you once again of a trivial example which shows what a difference of only one methylene can mean:

$$CH_3OH \quad : \quad CH_3CH_2OH.$$

In the search for compounds with an improved toxicological picture compounds of natural origin suffer strong competition from

anatural compounds. For the general public the word pesticide is linked, unfortunately, with the image « highly toxic or persistent ». This image was coined by representatives of the pioneering phase. Since then much progress bas been achieved, as shown in the following picture:

Parathion		LD_{50} rat 3,6 – 13 mg/kg
I Systox (R)		30 mg/kg
II		1,5 mg/kg
Tartan (R)		3,2 mg/kg
Dimefox		1,2 mg/kg
Phoxim		1900-2200 mg/kg
Jodfenfos		2100 mg/kg
Bromophos		3750-7700 mg/kg
Abate (R)		8600-13000 mg/kg

(MARTIN H. and WORTHING C. R., 1974).

The wide range of acute toxicity values also offered by products of natural origin is shown in the wollowing Table:

Scilliroside	LD_{50} rat	0,5-0,7 mg/kg
Strychnine	LD_{50} rat	1-30 mg/kg
Blasticidin S	LD_{50} mouse	39,5 mg/kg
NRDC 143	LD_{50} rat	1'500 mg/kg
Validamycin A	LD_{50} rat	>20'000 mg/kg
Altosid (R)	LD_{50} rat	>34'600 mg/kg

(MARTIN H. and WORTHING C. R., 1974)

With regard to the chronic administration, the chances for a favorable toxicological picture may be superior for a product of natural origin with a specific, narrow activity, again in view of its, generally, easier biodegradability. The classic example of the tumorigenic action of Aflatoxin is, however, a warning against generalization.

Evaluation of the Environmental Behaviour

Some important aspects with regard to the compatibility with the environment have been mentioned already, such as the toxicological behaviour vis-à-vis warm blooded organisms and the persistence and biodegradability.

It remains to mention that the behaviour of a compound towards other non-target organisms must also be clarified, for example the fish toxicity and the influence on the soil microflora and microfauna. Although compounds of natural origin, again with a very specific, narrow activity pattern, may have better prospects in this respect, the information must be obtained by experimentation case by case. There is a difference whether a compound is localised endogenously in a target organism or whether it is spread over a wide acreage.

Economic Competitiveness

Expressed in a simplified way the economic competitiveness can be judged by contrasting the sum of the cost of the product, the necessary profit and the cost of the distribution with the willingness of the potential user to pay this sum. The amount the farmer, for example, is willing to pay depends on the economic utility provided by the product. In the case where a crop protection agent or technique is already available, he will pay a higher price for a new product only if it brings him an advantage. He will also buy the new product when it does the same job as that established but at a cheaper cost. In many countries he has learnt to handle relatively toxic materials; therefore, he will generally not pay a substantial premium price for a compound whose only advantage is safety.

The following factors play, of course, a major role in the establishment of the economic picture: the doses to be applied; the number of repeated treatments needed; the size of the potential market; the cost of the product; the possibility of protecting the industrial property.

The expenses of the development of a new compound, be it natural or anatural, are so high, that small markets would hardly justify them.

The cost of the product will depend on the raw materials, on the simplicity or complexity of tbe synthesis, on the number of synthetic steps necessary, on the optimisation of the yields. The optimal product cost picture is reached very often only on the level of large scale production. The large scale production, in its turn, necessitates large investments.

Without a system which protects the industrial property, as for example patents, the means necessary for research and development could never be generated. A drastic reduction of research for new products and new techniques would be the consequence.

I dared to mention a moment ago a word which is very often under heavy attack by the public: profit. The area around industrial profit has been taboo for far too long, and this has led to completely unrealistic ideas. Only profits allow an enterprise to operate and to survive, and this is clearly illustrated when in a period of rough economic conditions an organization fails to reach the profitability

level and suffers the respective consequences. Profits permit the payment of salaries, the establishment of institutions for social welfare, allow the carrying out of applied and basic research, allow the equipment, allow the construction of plants and laboratories. In the company in which I am active, in 1975 1/3 of the turnover was accounted for by raw materials, 1/3 by salaries and social benefits, 1/9 by industrial investments, but only 1.2% went to the stockholders. That this is the dimension which leads to the adverse image of profit making, will probably be a surprise to you.

Epilogue

Biologically active principles occurring in nature and the biochemistry of the target organisms offer an outstanding opportunity for developing new tools for plant protection, and in this definition I include the protection of man against desease carrying insect vectors. The approach is rich in creative suggestiveness. A particular attraction lies in the fact that related chemical structures may be easily biodegradable with all the positive aspects of this property. Offspring from this concept must, however, satisfy the same severe requirements as agents conceived along other strategies: to be efficient under practical conditions; to be toxicologically acceptable; to be compatible with the environment; to be economically competitive.

The way from the interesting laboratory observation to the level of broad practical application is long and troublesome. By no means would I wish to demotivate anybody by making this sober, realistic statement; on the contrary. The seriousness of the problem of providing the nutritional needs of an underfed, steadily increasing world population and the mercilessness of the antagonists justify efforts along all lines which serve the purpose and need the enthusiasm of all scientists who can offer, through their brains and their hands, useful contributions.

And in this connection I can, for concluding, rely again on the genius loci: NON ENIM PARANDA NOBIS SOLUM SED FRUENDA EST (Cicero de Officiis III, 15).

This appeal can be read in the entrance hall of our meeting place.

I would like to translate this sentence as follows: « We should not only aim at wisdom but also be aware of the enchantment of wisdom ».

From the contemplation, we have, however, to find our way to the practical utilisation of our wisdom and knowledge that the problems of our age are awaiting.

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DISCUSSION

CANONICA

In an authoritative evaluation I heard some months ago that today it is necessary to prepare and to test more or less 12,000 new compounds in order to find a really useful new pesticide. Of course everybody is impressed by the great expense which accompanies this kind of approach, and many efforts are being devoted to the finding of less expensive solutions. An idea now popular is the idea to use the computer in order to predict the molecules which can show the better properties for a biological activity. So you can imagine putting into the computer the molecular parameters of many pesticides and of other compounds which are inactive as pesticides. The computer will work and will present us the best possible structure of this type of pesticide. What is your opinion about this?

KNÜSLI

Of course there are always attempts — and highly welcome attempts — to improve this really miserable relation of one eventually successful compound per ten thousand compounds tested. Now, the analyses you mentioned usually start from a hindsight base. When, for example, 5000 urea derivatives have been synthesized and tested already and when a certain structure-activity relation has been recognized, then you may eventually deal with an additional parameter like the solubility of distribution between lipophylic and lipophobic media. In such a case the computer will tell you which compounds you possibly omitted to consider and then you may follow this advice. For such an exercise the computer may be helpful, but I don't know an example where on the green desk a computer designed a fundamentally new active structure. And I can eventually add: also the leads of natural origin discussed during this study week were optimised first by the trial and error approach.

CANONICA

I agree completely with you: the computers are merely machines which are able only to elaborate in a perfect form the information we put into them. I can imagine what would be the situation in 1934, for example, while the scientists were investigating drugs useful against coccus infections. What could one do with the computers in the case they existed at that time? He could only put into the computer the parameter of sulfamides, no more. Probably under this condition, it was possible to find the best sulfamide, but it was impossible to foresee that there was an excellent anticoccus agent whose name is penicillin.

KNÜSLI

May I add the following: when you work with large numbers of natural or anatural compounds then the computer can be a most valuable tool for the storage and retrieval of chemical and biological data. You may be able to select out of a data bank compounds with a special activity, check whether you can detect common structural elements and design respective working hypotheses. In this respect I would not be so negative as you, but I understood, of course, the point you wanted to make.

WIGGLESWORTH

At the end of my talk the other day I ventured to hope and to predict that in the future entomologists would be playing a more active part in the procedures of insect control. But here we are faced with a dilemma: the entomologist would like, if possible, to avoid using insecticides; to substitute biological and cultural methods. Such procedures cannot appeal to the manufacturer of insecticides. And when the entomologist needs insecticides he would like them to have specific activities. But no manufacturer wants to make insecticides with specific activities — nothing could be less profitable! Indeed in all circumstances the entomologist aims to use minimum quantities of insecticides and to avoid flooding the environment with them. That again can hardly be welcome to the chemical industry. We seem to have a clash of interests.

KNÜSLI

May I make two comments? *a*) I must say that our entomologists were and are also stimulated — and I think our colleagues from FAO can confirm that — to participate in the thinking along the idea of integrated control, and also to consider already on the developing level compounds which may fit into such an integrated approach. Now you said that the companies don't want to develop such compounds. I just quoted one example where we wanted to do it and where not industry did not want it but the practitioners did not want it. We introduced this compound in Switzerland, and in other places, and then I think we sold 120 kilograms or so a year. We went on for two years and then we decided to drop it. After that we had hig protestations from our colleagues at the federal experimental stations. They said "now we once have such a wonderful agent which is really selective, please reconsider your decision". We did so, we reintroduced it, but after two further years, for the same reasons, we had to confirm our conclusions that a keeping of the compound was not justified.

WIGGLESWORTH

I have much sympathy with what Dr. Knüsli says: selective insecticides are not commercially attractive from the manufacturer's point of view. My experience has been that the senior people in industrial concerns are wholly sympathetic and understanding of the point of view of the entomologist. But these are not the salesmen who meet the farmers. We all remember how, during the post-war years, the advisory entomologists had to spend much of their time trying to counter the harmful advice of salesmen urging the use of chlorinated insecticides for undesirable purposes. If the type of co-operation which Dr. Knüsli mentions can be brought down to the "grass root" level, that would be splendid.

KNÜSLI

I don't think that the salesmen of my company make a tough approach to sell DDT and halogenated hydrocarbons nowadays.

KARLSON

We have discussed the matter from the economical point of view, which is quite obviously necessary, but we have only taken into account two sides: the producer, i. e. the chemical company, and the farmer who is going to apply the pesticide. Now, with a number of more sophisticated compounds and approaches, and I would like to mention in this respect especially the pheromone approach — I don't know if Dr. Shorey will join me here —, it seems to be necessary that it's not left to the decision of the individual farmer. In order to apply a pheromone preparation efficiently, all farmers in a certain area should use the same pheromone at the same time. So there is a necessity for some agency telling the farmers what they have to do. This agency may even have to use government money for the control operation as is done with the biological control measures, which are not handled by the farmers themselves.

On the other hand, we have seen that the concern of the public has already resulted in the banning of some insecticides formerly on the market. Here is a second point where government comes in. This should be very carefully taken into account with the aim of giving ecological needs a higher priority. In the present situation with the competition of firms for a market, and the need of the farmer for a rather cheap product, it may be very difficult to introduce more sophisticated, ecologically "safe" methods.

KNÜSLI

Well, I can even have some sympathy with what you said. But I wonder whether you were not already a step too far advanced with your reasoning. You spoke already about the practical introduction and about recommendations. Sometimes by asking during the discussion I wanted to have a clear answer, whether the respective systems, for example pheromones, would work under practical conditions, and practical conditions to me means under large field conditions. I think Dr. Chapman said it too: we had no convincing yes answer to this point. So I think the first link in such a development would have to be the showing that there is practical usefulness. And when you can show that, then I think the money will be found to develop such a system further.

VI

FINAL DISCUSSION AND CONCLUSION

GENERAL DISCUSSION

MARINI-BETTÒLO

Now begins the more difficult part of our meeting, that is, the discussion and the evaluation of the results. What are the conclusions of our actual work?

I think that in order to have a useful discussion we should focus on some points. I have prepared some questions and I have also asked some colleagues to express their opinion to see if we can put together a limited number of questions, let us say, five or six. And after that, we shall try to answer these questions. I will begin now by asking Dr. Gonzalez.

GONZALEZ

In my view we should stress a few points which I have actually prepared in a very general way. I am very much interested in seeing some of the practical uses of all these new developments. As I pointed out yesterday, the gap between the new developments and the field applications may be a wide gap if we don't at least suggest the means to put the people who are actually working on these new developments and those who are utilizing the new applications into closer contact. I would suggest the same for the basic research. I think that the basic research in the various fields we have been discussing should also be in close contact with those who are utilizing the research. For example, in the field of insect attractants and pheromones, I think that the results of research should be immediately communicated to those who are utilizing them in order to accelerate the actual field testing of all these products and to determine their feasibility and economics. This applies to pheromones and their efficacy, safety, testing, cost and further applied research on field application. Likewise, for other natural plant products

there might be a practical use in plant protection. I would also point out that we should stress the need to develop further integrated pest control studies because this is the best possibility for utilizing all these various new developments. In effect not one of them can be utilized alone but must be studied in multi-approach.

And the best foundation for this would be the integrated pest control approach. Therefore I think that we have also to stress that point This is what I have so far developed.

MARINI-BETTÒLO

These are the two points you have stressed: interchange of information and integrated pest control.

SHOREY

All right. I have two general areas, although I also have a group of diverse notes here which I may be able to summarize some more information from. But I think that one of the aspects that we should consider now is asking ourselves what particular areas of study are not sufficiently investigated, so as to explore the potential of those areas for alternatively providing natural products for pest management. In particular, I would like to mention one area that was discussed during the Study Week and that I particularly think should receive considerably more emphasis, and that is the area of host plant resistance to insect and pathogen and nematode attack.

MARINI-BETTÒLO

This is plant resistance?

SHOREY

Plant resistance mechanisms, but with the emphasis on "mechanisms" because... ... it does appear that this area has received very little attention as yet, and I visualize that this is an area where a team approach by physiologists, behaviourists, natural products chemists, and individuals who have a direct interest in pest management applications could very

profitably work together. I have one other point which is not very well defined and is related to the first one: let us further ask ourselves how our particular talents can be directed so as to make practical control systems, or practical pest management systems operational. It has been stated around this table that in many instances the sorts of things that we are talking about are still essentially laboratory games. For instance, in the area of pheromones, there is not a single system that is truly operational for the direct use of pheromones in pest control, although there are survey usages. Well, what can we do to improve such a situation? I don't have any good answers. I would like to propose, however, that in relation to this, the types of insects upon which we do our experiments might be very carefully selected and, when possible, those insect species that serve as our model species should be key insect pests. I know that this often is not accomplished or is not done and people frequently pick organisms. I keep saying insects, excuse me, those of you who are not necessarily working with insects understand that this is my own bias and we could be talking about fungi or anything else. But anyway, key pests should be selected rather than what is often done and that is that people frequently will work with the laboratory animal that is easiest to culture in a particular group of animals or organisms that we may be insterested in. But I think that is quite important and it is also important to continue to keep in mind this concept of key pests as one even tries to make a pest control system operational. If one tries to develop a pest control system for what I would call a secondary pest, it is very possible that such a pest control system would never be used against that organism. If the organism is only one organism out of a complex of organisms that attacks a crop, and if that one organism by itself even if it were removed would not lessen the necessity for using more standard pest control practices, then I think that you can he guaranteed that the method that you have spent a great deal of labor in helping to develop for that particular organism will probably never be used. And so I think that even those of us who work on some of tbe most fundamental aspects should try to keep in mind that if we want our work to have direct relevance to pest management, we should remember this concept of key insect pests. I fact I will make it even stronger: I think that an immense advantage could be accomplished if a fairly extensive group of individuals with different talents could, in some manner, identify the same pest so that they are bringing their

diverse talents to bear on that same organism and perhaps thereby be able to really further the development of these modern techniques for pest control.

MARINI-BETTÒLO

Thank you Professor Shorey. Now I will ask the opinion of Dr. Jacobson.

JACOBSON

I agree of course with what Dr. Shorey has said. I especially agree with what Dr. Gonzalez has said with regard to integrated control. I think that we cannot hope to get insect control using either pheromones or hormones or predators and parasites alone. I think that it will take a combination of these. Now I have written down some thoughts on the possibility of using insect pheromones and hormones in pest control and so on and I would like to read what I have.

1) *Possibility of using insect pheromones*

Now as far as the possibility of using insect pheromones is concerned: despite the success reported by Dr. Shorey for his work on gossyplure as a confusion agent for the pink bollworm, I do not feel that the use of insect pheromones alone for mass trapping or confusion is practical in areas of high insect infestation. This has been clearly demonstrated in the case of gypsy moth in the United States. They may work well in lightly infested areas. In my opinion the use of an integrated method is called for, in which parasites and predators, bacteria or viruses can be used to reduce the size of the infestation, followed rapidly by the atmospheric release of pheromones as confusion agents or of other type of mating inhibitors.

An excellent example of a mating inhibitor is (Z)9-tetradecenyl formate, which we have found will reduce mating by 96-98% for insects such as the corn earworm and the tobacco budworm in the United States and 3 species of pest insects in Israel.

Hormones: As mentioned in my presentation the critical timing, short persistence, lack of immediate control, and reinvading populations tend to argue against the possible practical use of hormones or hormone

analogs to control agricultural pests. In addition, the obstacles to be encountered from Agencies such as the Food and Drug Administration and the Environmental Protection Agency in the United States against the broadcasting of such material would be formidable.

However, the use of the Zoecon product « Enstar » may be promising. Also experimental results recently obtained from the use of a juvenile hormone analog known as JH-25 by the USDA to keep flour free from the flour beetle appear to show definite promise, and it is for the purpose of controlling insects in stored products that compounds such as this may well find use. Such application should also be quite useful in tropical areas for the protection of stored agricultural grain and especially for stored agricultural products not destined for human consumption, such as jute, sisal and animal fodder. Of course safety of such applications would still need to be checked very carefully.

2) *Possibility of using resistant plants in pest control*

A good example of the practical use of resistant plants is the case of cotton. Extensive research with varieties of cotton susceptible and resistant to boll weevils in the cotton-growing areas of the United States, such as Texas, Louisiana, California, Arizona, and Arkansas, have shown clearly that the resistant varieties have high contents of gossypol.

Introduction of cotton varieties among the farmers by Federal, State, and County agents will help immeasurably in increasing cotton production. This should also be done in the cotton growing areas of Central and South America and the countries of the Middle East, despite the fact that the boll weevil may not occur in those areas, since gossypol also appears to confer some resistance against other cotton pests such as leafhoppers, aphids, etc. In addition, the successful translocation of gossypol into susceptible cotton plants and other hosts for cotton pests cannot be ruled out.

3) *Possibility of using natural products for insect control in the tropics.*

An excellent example of this is that of fruit fly survey and control with methyleugenol, a constituent of many species of plants (especially Labiatae) in tropical islands of the Pacific. The specificity of traps baited with a small amount of methyleugenol in Hawaiian coffee, citrus and other fruit growing areas has been shown clearly. Males of the Oriental fruit fly strongly attracted by the methyleugenol, enter the traps in large num-

bers and feed on the compound until they become so engorged that they hurst. The methyleugenol therefore draws the males to their death. The large amounts of this compound now used for this purpose are prepared synthetically hy commercial concerns in the United States.

MARINI-BETTÒLO

Thank you, Dr. Jacohson. I think you have already opened the discussion, but I think that now we must also find new items for the discussion, not answer them. So I will ask the group I consulted yesterday, to continue to suggest questions to pose to the audience.

KNÜSLI

Well, gentlemen, I think we have, if I am right, three hours and a quarter at our disposal, and yet you would like to have either tbe base or already the conclusions.

MARINI-BETTÒLO

We will have the conclusions in due time. I need now the base, that is, the questions. I will try to summarize the questions and put them to you and then we shall open the discussion on these questions.

KNÜSLI

Well, but I wonder whether we will achieve our objective in this way. I would like to offer the following proposal for consideration: that we should split in various groups. One group should write a preamble to the whole, then one further group for insect hormones, pheromones, attractants, a group which covers the possibilities of using natural resistance breeding, etc., a group dealing with natural resources for producing pest control agents, a group reviewing toxins, of living organisms, bacteria and viruses. And these groups should be confronted with definite questions, and the questions should be the following: the prognosis as to practical usefulness, the limitations in the approach itself and the recommendation on what has to be done in the future. To these groups should be given some indication as to the review space at their disposal, let us

say one page or two pages. Let us divide into groups, in a corner of the garden or in one of these rooms, and sit together after one hour with these documents prepared. Then we could eventually, at the end, take the general points together, which are common to all chapters. This is a suggestion, I don't know what you think of this.

MARINI-BETTÒLO

I think it is very good but I should like to make a time table. I will ask first the opinions of Professor Williams, Professor Nakanishi, Professor Bell and Professor Ballio. At this moment we shall have a number of questions, and then we shall ask other proposals from the other participants and after that — let us say at 10.30 — we shall split in small groups and draft a report. At 11.30 — or 12.00 — we shall come together and read what we have done. Will that be suitable for you? There are no objections? So now please Professor Williams, what are the questions, the more important ones you wish to bring up for discussion?

WILLIAMS

The questions I have written, jotted down here, are as follows:

Who is to produce and market the new generation of bio-rational pesticides?

Who is to finance the new generation of pesticides?

What can be done about the steady increase in the disincentives to the production and registration of the bio-rational pesticides?

MARINI-BETTÒLO

Thank you, Professor Williams. Now may I ask the opinion of Professor Nakanishi?

NAKANISHI

Can I just make a general statement? Probably this is going to rub everyone here the wrong way, but, excuse me, I feel strongly the necessity of interdisciplinary collaboration as I think that every one of us has shortcomings. I could start with the academic people. They are, in

general, including myself of course, occasionally overcompetitive and this leads to petty competition if I may say so. Now with respect to industry, I think it is far too profit-oriented, as suggested yesterday by Sir Vincent. The administrative people in industry — I am not speaking of the younger people — are far too egocentric and think too much about their profits. The third category I would like to criticise is Organizations like FAO and other international Agencies. The first impression I get from such organizations is negative. That is sometimes I think their realization of the seriousness of the problems is insufficient or superficial and they tend to be more like diplomats. I feel from them a general funding agency syndrome. Excuse me, I have been criticising every one, but I have no personal grudges of course. Now I would like to discuss some aspects of I.C.I.P.E. This institute has been receiving most generous support from the United Nations, which channels the funds through FAO and various other agencies. A definitely positive point about ICIPE which I have enjoyed and profited from is its interdisciplinary character which is provided by the research scientists and the so-called Research Directors.

I have personally been able to make many fruitful contacts at and through ICIPE which have led to collaboration. There are of course problems at ICIPE also but this is not suitable for discussion here. I think it will do us good if we just extract good points from this rather unique international organization consisting of a real multi-disciplinary nature. I think we can consider things like the Apollo project, for example, if you want. In this case most of the people who are involved in the Apollo project are more or less anonymous. And nevertheless, we are achieving some results which would have been absolutely impossible if any industry, any personal scientist were to develop them him or herself and so I think that is a rather good example of some anonymous collaboration, nevertheless filling the demands.

I would like to see a closely integrated collaboration between industry, Academia and international agencies in some of the crucial problems. Another point I would like to raise, just for the sake of discussion, is the setting up of a sort of central organization, non-political, which could coordinate the interests and stimulate this international collaboration; and I am not speaking of the entire pest-control area (it would be too gigantic), but it could be pest-oriented or it could be oriented towards some discipline.

Another point is, although we are all sick and tired of having too

many journals, it would nevertheless be beneficial if there were an international newsletter in which each individual gives us an abstract of his paper or results. A very short abstract, I am saying, and then those who are interested in collaboration or who think they could contribute to its solution, could get in touch with the authors, provided we have some organization which could occasionally coordinate a project. It is only through meetings like this that we start realizing that there are many people in other disciplines who have a common interest in the same problem.

MARINI-BETTÒLO

Thank you, Professor Nakanishi. Professor Bell, please.

BELL

At the risk of being considered other-worldly and academic, let me just put a few thoughts to you on the subject of the development of agriculture in the world. At the present time, there are a limited number of cultivated species which are used for human food. Most of these have been developed in the Western world and they have been developed by trial and error methods, the less toxic and more pleasant tasting varieties having been selected, and those producing sickness or having an unpleasant taste having been rejected. Man has been his own experimental animal and the development of crop varieties has been slow and painful. Using thse methods it may have taken thousands of years to develop a relatively non-toxic species in the Northern temperate region from a native species which was highly toxic. I suggest however that if we can identify the toxins in a species and develop a systematic screening programme — we can with our present knowledge bring new species into cultivation and improve minor crops in a relatively short period. This work should, I believe, be pursued side by side with the work of protecting and improving crops which are already of major importance. To do this we, the academic scientists, must provide information on the nature and distribution of secondary compounds which are physiologically active in insects and/or other animals, so that geneticists and breeders can use these data to develop crop plants which are both resistant to insect attack and at the same time acceptable as food for man and his domestic animals.

Now Prof. Chapman mentioned to me that we should have the same sort of information centre with regard to biological assays. Where does one get material tested, who is willing to see whether this is active in a microorganism, or in an insect, or in a plant? I now know more about this, since I came to this meeting, than I did before and I suspect that all of us do, but there are many people outside these walls who have not had the opportunity of listening to our discussions and are totally unaware that people are working in these particular fields and I honestly believe that this matter of communication is a matter of knowing where particular things are being done. If we can do something to disseminate this sort of information so that A knows that he can write to B when he has a need of help and B knows that he can write C, I feel this is an important matter which we should consider at this meeting.

BALLIO

I agree about most of what has already been said by Professors Nakanishi and Bell, but I should like to add a few further comments. I think that one should encourage chemists working on secondary metabolites of plants, especially those belonging to academic institutions, to collaborate more closely with biologists. In the field of natural substances it is becoming more and more important that chemists dedicate more work to projects of biological significance and not limit themselves to structural investigations unrelated to a biological problem.

My second comment refers to the very important work carried on by plant breeders to improve quality and/or quantity of certain chemical characters considered useful to man. It is my opinion that chemists can make important contributions by investigating plant substances which are responsible for natural resistance to pests. These characters ought to survive genome manipulation, as their loss might produce undesirable consequences.

Finally, I think that the characterization of a novel bioactive substance isolated from plants should, whenever possible, involve not only its chemical structure, but also its biological role and its mechanism of action; until now these aspects have been much less frequently and thoroughly investigated in plants than in animals and microorganisms.

MARINI-BETTÒLO

Now that we have had this first round table discussion, I should like to summarize the questions raised by me and by the other participants and then we shall discuss whether all the points are covered.

I think that the final document should begin with a preamble, then explain the results of the present Study Week, discuss critically these results, forecast evolution of pest control management, try to focus the question of integrated control and then stress the cooperation between chemical and biological means and also chemical and biological research for pest control, introducing the idea of further research on plant resistance and considering the role of the Countries situated in the tropics. Thus the questions I should suggest are:

1) Present possibilities of the use of insect pheromones and hormones in field pest control, although limited to some plant species of economic importance. We should consider under this item the limitations due to the effectiveness, the costs, the safety and finally the necessity of further research.

2) Present practical possibilities of the use of natural products as crude extracts in the tropical areas as suggested by Professor Gilbert. I think this point cannot be extended to the industrial cultures of the other parts of the world.

3) Practical possibility of the use of natural products extracted from plants or their synthetic analogs in agriculture, as the case of pyrethrins, precocenes and so on.

4) Practical possibilities of the use of bacterial toxins and viruses in plant protection.

5) Possibilities of using naturally resistant plants or plants in which resistance has been chemically induced, in order to protect crops from pests and diseases.

I believe this is an extremely interesting point. I think also that we cannot expect immediate results but that further research is necessary. This should be underlined very clearly.

6) Another point: as national legislation, in various Countries, for licensing new pesticides is the same both for natural and unnatural products, would it not be advisable that at the international level a guide-

line should be established to facilitate the use of these compounds all over the world?

7) Would you think it advisable to recommend that in addition to the field research, also basic research on the biochemical mechanism of plant protection should be thoroughly studied?

Professor Zanini is suggesting that some institution should prepare a list of the various species of cultivated plants which present a particular resistance to the attack of the parasites and molds. This with the aim of indicating those plants to agriculture, but also to gather a very rich material of study which could be interesting for specialists and would lead to learning more about the compounds that give this resistance. I think this is very important; we should eventually try to see which are the resistant species of the most common crops and try to direct our attention to discovering what is the chemical and biochemical difference between the non-resistant and resistant varieties.

Well, this is more or less a guideline for the conclusions and the final document. We have heard a certain number of colleagues who made their proposal, now I should like to open the discussion on these points to all of you.

HEIMPEL

I have three points I would like to make this morning. In order to carry out integrated control of insects one must have an intimate knowledge of the insect and its habits. We must carry out extensive studies of the life cycle of the insects that are causing damage not only in food and fiber crops, but in stored product and forest insects, as well. These are long term studies and the sooner we start the better. Point 2: I was impressed by the results of the African methods of evaluation of potentially useful fauna and flora. Perhaps these studies would be worthwhile continuing in Africa as well as in other developing countries. Point 3: Recently I was interested to find out that there are extensive studies on the culture of plant cells and in some cases a single cell can grow into a complete plant. It occurs to me that we might be able to use this technique to grow plants resistant to insects. I know that resistance studies take from five to ten years to really select a variety resistant to a particular insect species. I am suggesting that we might propose that this technique might be more rapid and should be intensively investigated.

MARINI-BETTÒLO

Thank you, Dr. Heimpel. I would like to hear the opinion of Prof. Cruickshank on this point because here not only the genetical variations but the induction of these elicitors, may play an important role.

CRUICKSHANK

In my view the recommendations that have been discussed are directly relevant to plant protection whether it be in relation to insect pests or fungal diseases. The principles involved are similar irrespective of whether insects or fungi are involved as the control methods are based on the use of externally applied toxic chemicals. However, if we consider control measures based on natural mechanisms of pests or disease resistance in plants there emerge several important differences between the two.

An insect is mobile. It chews a leaf, enjoys it or is poisoned by it. The insect then moves to another leaf or is killed. Fungi are not mobile. They infect a leaf and grow in the leaf tissues. In the former case, plant responses play a minor role, if any. In the latter case, plant responses and the net accumulation of phytoalexins are believed on current evidence to be very important.

I am interested in two approaches to disease control based on our knowledge of the physiological mechanisms involved. Both are based on the rate of net accumulation of phytoalexins and the differential toxicity of these compounds. The first is an approach through plant breeding based on the use of a "phytoalexin index" as a selection parameter. The second is the use of fungal metabolites or functionally equivalent compounds as elicitors of phytoalexin formation. Both procedures depart from orthodox practice and if successful could lead to an important procedural revolution in this area.

In the light of acknowledged genetic vulnerability of our major crops under existing plant improvement and crop management practices it is suggested that the second approach, based as it is on the activation and control of the plant's own defense system in genetically susceptible plants must be considered as a serious possibility for the future.

CHAPMAN

Prof. Marini-Bettòlo, I am not altogether clear what the object of

this document is going to be, but I rather fear that if we really want to have some impact we are really getting a little too complicated. I think that if we really want to make a mark it has really got to be a rather simple and not too long document which tries to make two or three or certainly not more than four key points. I think if we try to include all these things that everybody is talking about we should simply reiterate the whole of this symposium and the impact on other people would be negligible. And I would suggest we really try and concentrate on a couple of what people would generally regard as key areas, rather broad generalisations within which we might want to go into a little more detail as we might implement things. Two things that seem to me to have come out from the general suggestions are: what do we need to do, to get integrated control on a functional basis, what exercise has a need to be carried out and secondly what can we do about comunication, and within that second context I would add are there things which we can in fact even round this table set in motion? I believe there are things that we can actually do, and not just put down on paper. So I would make a plea tbat we do not put too much in this document but we keep it very simple and try to select a very small number of topics that we could discuss.

MARINI-BETTÒLO

Thank you, Professor Chapman, I think it is very important that we have a short document with few but clear ideas.

NAKANISHI

I fully agree with what Dr. Chapman mentioned. Industry can go on pursuing its profits but the important thing is to have a better understanding. Now what I would like to suggest here is an improvement in general communication between funding agencies, industries and Academia.

It is a rather unique opportunity that we have all these experts from various disciplines assembled in this room.

KNÜSLI

I take up the communication and I think there is a common agreement

that this is a lack in our field. Now it was proposed to create new means for communication: offices, newspapers etc., and here I would even warn that we already have too much but we do not make use of the possibilities which already exist. I come back to the famous example of the neem tree. I was surprised to see that even in this case certainly these neem tree possibilities were not known to various researchers. I just asked Professor Nakanishi whether his findings were published. He says yes. Now I think in a certain way everyone of us has to follow literature, so that this work on neem tree active principles should be known and then everybody would have the possibility to write to Nakanishi and come in contact with him for further developments of the research.

I think that without the personal collaboration on a particular item no organization and no news-letter will help anything.

ELLIOTT

Following Professor Chapman's remarks, I think that the problem has been presented in an unduly complicated way, so that the solution is not necessarily as clear as it might be. We need chemical compounds with appropriate properties, whether they be natural or synthetic. One source of such active materials will be naturally occurring compounds which will provide lead structure for syntheses. There is not necessarily any special merit in natural materials, some of which, like aflatoxin and various plant pathogens, are very harmful. Other leads may come from random screening, although experience has shown that to be not a very productive source. However, whatever the starting material, biological activity should be developed to the highest level economically possible.

BUYCKS

In your opening speech Mr. Chairman you pointed out the possibilities presented by natural substances to improve pest control and you also stressed the need for improved pest control to increase crop production and to satisfy the increasing needs in food, in fiber particularly of developing countries. On the other hand, there is a consensus here that integrated control seems to be the best way to integrate in plant pest control the new findings in the field of biochemistry and plant chemistry. We all know that integrated pest control requires in-depth studies in the field of ecology,

physiology and behaviour and also an interdiscipline reapproach. This requires a lot more of research capabilities than the simple application of chemical controlling the pest, and of course this is a very very important problem for the developing world. Therefore I would suggest in connection with what Dr. Knüsli has already proposed that there be a small working group to examine the prospects of integrating these new control practices in pest control, particularly in developing countries: what are the needs and requirements and how these could better be achieved. On another subject just to follow up on what Prof. Nakanishi mentioned, I would like just to say that we at F.A.O. have tried already to have this type of international collaboration that he pointed out. We have several panels which are composed of a certain number of persons known for their knowledge in plant protection and who are only appointed in their individual capacity but we have a panel of experts on integrated pest control, we have a panel of experts on resistance of pests to pesticides, we have also a panel of experts on pesticide residues and environment which are working in close collaboration with the W.H.O. and I would also like to point out that we have an industry cooperative program. In that industry cooperative program we have a pesticide working group where we regularly meet and discuss matters of common interest with the pesticide industry and also take action. For instance one of the actions we are taking is the possibility of sort of unifying the registration requirements for pesticides all over the world. This is a big problem for industry as Dr. Knüsli has pointed out in his talk and we are working in collaboration with the pesticide industry to try to come to an agreement and next year we are planning to have an international meeting here in Rome in F.A.O. to precisely try to come to some practical proposal in this field. I would just like to point out that F.A.O. is not a funding agency and that of course our action is not always as broad or as much in depth as we would like it to be but we must not forget that F.A.O. is an organization set up by the will of its member governments and therefore we can only do what those governments request us to do.

WIGGLESWORTH

I agree that we should get down to practical politics; but I should like to spend a moment on the philosophical approach of Professor Nakanishi, which may be central to our problem. I agree with his remarks

about competitiveness in the academic world. On the other hand, in associating with applied entomologists I have been impressed by the altruism of their approach: they seem to devote their lives to furthering the profits of manufacturers who supply the chemicals and of farmers who grow the crops — and get little out of it themselves. This altruistic component is of immense importance for the consumer.

A second philosophical component we require, as has been mentioned, is co-operation. We must all have had mixed experiences in this regard. Some chemical firms are able to co-operate in a most liberal fashion with academic workers; but others will absorb all the information they can get on joint committees but are reluctant to give out anything that *might* conceivably have some economic value in the future. I believe there is an increasing trend toward openness.

A third criticism by Nakanishi concerned the International agencies. Here again it is impossible to generalize; but in the past the Agencies seem sometimes to have been slaves to rather narrow dogmas when, in dealing with insect problems, what is required is a broad integrated approach.

These are generalities but they should form an important background to any recommendations we have to make.

WAIN

I think we should agree with what Dr. Chapman has said this morning, not to continue trying to educate each other. I think we have had a whole week for this and so I think we really should get down to what have we achieved, what is the basic feeling of the meeting at this stage? I would just like to read a sort of understanding which people might like to consider.

"The basic aim of the study week has been to review and discuss the present position in regard to fundamental research which might lead to the more efficient control of plant pest diseases. In spite of control measures which are at present available such as the use of established insecticides and fungicides the world losses of human and animal food due to these causes, especially if the growth and storage phases are taken into account, are enormous. The aim of our study week, which has been abundantly achieved, has been a stimulation of new ideas, the sharing of knowledge and educating each other in the various aspects of pest control research which lie in fields different from our own. As a result many of the participants will return eagerly to their laboratories to initiate experiments whose

conceptions arose directly from intensive discussions carried out in a calm and relaxed atmosphere between scientists from widely different fields. One aspect of our program was related to studies on the chemical basis of plant disease resistance. Recent research has provided much information on why all plants growing in nature are completely resistant to most of the fungal diseases to which they are continuously exposed in the field. There is now much evidence that defensive chemicals within the healthy plants are chemicals produced within the plant, which, when under stress conditions, operate natural disease resistance. The isolation and indentification of this group of natural compounds have given a new impetus to research on the chemical control of plant diseases. Such research might also prove a benefit to medicine. The study group recognised the importance of these developments and supported them. They also became aware of the many problems which are common to both pest and disease control. The desirable close collaboration and improved communication between research workers in these fields was well recognised. The serious disadvantages associated with the use of synthetic pesticides: build-up of resistance, destruction of beneficial species of insects and so on, were fully discussed. There was full agreement that the new approach indicated in the title of our study week "Natural compounds for the protection of plants" provides much promise at the present time. As a result of our deliherations our individual program of research will be stimulated and positive achievements toward the welfare benefit and happiness of mankind could be realised".

GILBERT

Prof. Nakanishi suggested an international organization. I would like to suggest that the U.S.D.A., would be a central world clearing house to whom we could send natural products or synthetic products. They are used to registering and numbering compounds and collecting results and if they were willing to do this they would then redistribute data to the appropriate laboratories that would carry out biological tests. And they could suggest field tests, collect the results and make a yearly report perhaps to this Academy which would be distributed to all the persons present. In Brazil we have a central Agency which receives all synthetic and natural products for biological testing, ships off to 20 testing laboratories and receives and collects results.

MARINI-BETTÒLO

I should like to add that National Cancer Institute in Bethesda is doing a similar work in order to test all possible antitumor substances sent by people from all over the world.

BRADER

During this study week we had a rather unique occasion to discuss various aspects of host-plant resistance. I would suggest however that we avoid making the final conclusions too all-inclusive. For example, we have looked into the chemical basis of the pest-plant relationship, but the best known examples of resistance are mostly based on changed morphological characteristics. More intensive information exchange is certainly needed, but I agree with Dr. Knüsli that we should avoid setting up new organizations or mechanisms to do this. A good deal of progress can certainly be made by making full use of existing systems.

MARINI-BETTÒLO

I agree with Dr. Brader we must make better use of the existing bodies because they are very flexible and they could help us.

BOWERS

I also would like to avoid just sitting here holding forth with our special viewpoints, many of which we know already, but I think that it is important that we try to apply the compounds and natural products which we do have to practical problems where possible.

Natural products of botanical, microbial and animal origin have either proven useful in insect control or provided chemical structural leads for the development of useful products. We should seek to expand our knowledge of natural product chemistry, especially as it relates to insect-plant interactions. I believe that plants have evolved many chemical defenses, some of which we could develop into safe and useful insecticides and fungicides.

To seek out these natural plant defenses requires a great deal of imagination. I believe it was in this city many years ago that Da Vinci

made some drawings of an airplane with long wings in which he imagined that man might some day fly. I am sure this dream was considered nonsense in his day, but through the controlled combustion of liquid petroleum we now fly from New York to Rome in a few hours in an aircraft very similar to the drawings of Da Vinci.

I am sure few of us came to Vatican City believing we would develop some new methods of plant protection. Rather, we were brought here to consider the problems of the past and present and encouraged to dream and reason together over the approaches to take in the future.

In this study week I have received a great deal of stimulation from people I would not ordinarily come into contact with, and I am going back with many fresh ideas and with some experiments in mind which began here. It should be an important part of our report that we have interacted here and that we have genuinely educated ourselves and not just engaged in polemics.

SIDDAL

I would like to echo Professor Bowers' and Dr. Chapman's words, but to make it short let us be original; one original idea is worth a hundred statements of general value. I think we have all been charged up like a battery by the last four days of discussions. If we simply sit quietly here and produce perhaps one or two original ideas and throw away the stuff which has been written five hundred times in literature, then the Study Week will perhaps achieve more than any twenty page document, which would do no more than reiterate what is already known. I would like to end by saying that I do believe that 90% of what has been presented here is already in the published literature; we simply have not read it.

We have all agreed that we should make a better use of the available information and available organisations, but I still feel that it is our duty to be original and I would therefore plead that we simply do not waste time on stressing the value of integrated control; it has been stressed a thousand times already. Let us find some original way to do one small part of this instead of trying to solve the total problem.

I should like to make one more operational suggestion: I believe the purpose of the Pontifical Academy of Sciences in part is, if possible,

to reach agreement in areas where disagreement has existed. That was in the document which you gave us before the meeting. It is my feeling that there is no essential disagreement in the area of natural product chemicals which provide leads for normal development in the conventional sense of industrial or other fields. We can pass over this area and simply agree that, as Dr. Elliott said, there is no fundamental difference between the chemical structure of a natural and an unnatural product, the latter obtained by a screening or a synthesis system. And let us give our attention to the other areas of natural product use; for example, Dr. Gilbert mentioned the use of natural products in situ.

MARINI-BETTÒLO

Thank you, Dr. Siddal, for these suggestions. I think they exactly correspond to what many of us believe. But we know these facts ourselves and we must also let other people know them. For this reason I should like to have some answers to the questions I have put introducing this meeting.

I think that here we have all interacted between us, we have learned very much of what has been done and published in some laboratories, but perhaps in the discussion new ideas came to our minds.

Now that so many doubts arise about the safety for man and environment of new products, we must ask ourselves if the line we are following is good or bad.

Some of the products we have examined may have an application in the future strategy of integrated control.

I think we should say something briefly here about the dissemination of information; and I think our mutual contact here has been very useful to learn about other complementary fields of research.

For example one of us may send a substance isolated from a plant to be tested on the insect behaviour, this fact may raise some interest which can lead also to significant results.

For the final document I think we should suggest a line somewhere between the two suggested. I think that Professor Chapman's suggestion is one of the best: just make an assessment of what we have done without writing these long papers stressing and underlining things that have already been said everywhere.

NAKANISHI

What I meant by international organization is not a real institute; I am simply suggesting a news-letter.

What I meant by this more concrete term is: when someone feels that he has some data even negative, or when someone publishes a paper, just send to a certain office one or two paragraphs of the results; no detail but just conclusions or results. The results can then be circulated to people who are concerned with pest control. And as an organization I would suggest places like the Rockefeller Foundation or any such international organizations. They will just print these one or two paragraphs of results received from the various research workers and then this is sent to interested individuals in Industry, Academia, Funding Agencies, etc. After that I think contact or cooperation, if necessary, would be generated spontaneously.

If I may say so, I was myself very surprised to see that there still was interest in the neem tree and its product azadirachtin. If I had known this, I would have been willing to cooperate in terms of sending them pure compounds. This is just a small example, but I am simply suggesting some sort of a very simple news-letter which will not put any extra burden on each individual worker.

MARINI-BETTÒLO

Thank you very much, Professor Nakanishi, I think this question of a news-letter could be easily solved. I think that either FAO or the USDA could promote this news-letter.

Now to return to our conclusions they should consist of two parts: one part to answer the questions in a very general way, and the second part to make some recommendations. Some have been already proposed here and they are especially for the research programs; then we have the question of the dissemination of information and eventually the cooperation, which is an important item. To resume, we have: first, recommendations for future research; second, dissemination of information and eventually cooperation; third, eventual collaboration between all the groups working in this field.

Now I should like to go back to Professor Chapman's suggestion which indicates, I would say, the simplest way to prepare this document.

For example: integrated control is the ultimate goal of the present work, but I think we should first answer some of the questions many people asked me outside here "Will these pheromones replace the present conventional pesticides?" I answered no. At present we are only trying to prepare new defenses for plant protection strategy using new scientific approaches.

I should like to have now more precise answers on the various points raised before in a qualitative and when possible in a quantitative way. Therefore I think that following Dr. Knüsli's suggestion we should split in three or four groups to draw up these very few pages.

A first group could prepare something about pheromones and insect hormones in general and the practical possibility of their use.

A second group could make a draft on plant disease protection. A third one could point out the research which is now necessary in the different fields. A fourth group could suggest recommendations about the dissemination of information and cooperation programs. Do you agree on these proposals or would you like some other suggestions?

SIDDAL

I think it would be helpful to have a suggestion for choosing the groups.

MARINI-BETTÒLO

Well the group members can be chosen easily practically automatically.

SIDDAL

I would like to suggest that the group on resistant varieties for example should include only one person who is working in that area and that all the other people should be from totally different disciplines. And I think that there is a possibility that there may be some original suggestion evolved from this approach.

HEIMPEL

You have the suggestion, that we form a small subcommittee on biological control of insects.

MARINI-BETTÒLO

Well now let us choose these committees.

KNÜSLI

I find Dr. Siddal's proposal outstanding, but I must say that we have no time for that. So I propose the following. I want to put the questions to be answered by these groups.

A - Where we are.

B - Prognosis, that is where we can go and what is possible.

C - Limitations which have to be overcome.

At this point I would propose the following groups.

A) Hormones, pheromones, attractants: Siddal, Bowers, Wigglesworth, Williams, Staal, Shorey, Jacobson;

B) Living organisms: Somerville, Heimpel;

C) Natural resistance, breeding etc.: Gilbert, Bell, Chapman, Cruickshank, Graniti;

D) Natural products, chemicals, extracts, raw materials: Schildknecht, Elliott, Nakanishi, Ballio.

Then eventually a group formed by Dr. Brader, Dr. Gonzalez and Dr. Buyckx on integrated control. The maximum length of a single report (because you could write a book) will be for example two pages, as we have all decided.

The remaining persons would devote themselves to the preamble. Of course Professor Wain is highly qualified for the natural products group, but I think we need him here as he is a good stylist, so I would propose that he write with the Chairman, and eventually with me, the draft of the preamble.

MARINI-BETTÒLO

I think we all agree on these points and that we can begin to work.

DISCUSSION OF THE REPORTS

CHAPMAN

Chemical Resistance to Plant Pests and Diseases.

Numerous examples are known of plants which are resistant to pathogens and herbivorous animals. This resistance may depend on static mechanisms of protection either chemical or physical, or on dynamic mechanisms involving the production of specific chemicals in response to attack. The precise mechanism of resistance is known in only a few cases. A knowledge of these mechanisms is important to enable us to take a rational approach to the development and use of varietal resistance and to enable geneticists to take full advantage of the enormous pool of resources available in plants. It is recommended that emphasis should be placed on:

a) studies of the mechanisms of resistance of existing crop plants and of mechanisms of pathogenicity of plant parasites in close collaboration with plant breeders, so that the most productive effort can be focused on the development of new relevant varieties;

b) studies of pest bost plant relationships in naturally occurring populations of wild plants and minor crops with the objects of developing potential food crops which are already adapted in other respects to a tropical environment, and of establishing the underlying principles involved in pest host plant relations which can then be applied in agricultural eco systems;

c) the investigation of the mechanisms and potential of induced resistance to disease, especially as a possible safeguard against the progressive reduction of gene pools, which is recognized as having occurred in some major food crops;

d) improving communication between scientists of different disci-

plines involved in the study of plants and their pathogens and pests. Only in this way is it possible to achieve maximum utility of the findings in different areas of research.

SIDDAL

Hormones, Pheromones and Attractants.

The report begins by dividing all uses of natural products for plant protection into two major categories:

First: natural products which have provided lead structures for operational practical use in plant protection. Under this heading a few examples could be found: Nereitoxin led to the development of Padan insecticide; juvenile hormone I led to the development of Epophenonane, juvenile hormone III led to the development of methoprene and kynoprene; indolacetic acid led to the development of 2-4-D and many other herbicidal analogs; the hormone ethylene led to the development of the analog ethephon.

Second: natural products which are used per se.

1) Ecdysteroids have an operational use which is not yet achieved or is pending.

2) Many pheromones of pests are used for monitoring as survey tools and are very successfully used in numerous countries with great economic benefit.

3) Living organisms and viruses will be treated by a different subgroup.

4) A very limited number-less than ten — of pheromones of key pests, which are now being explored on a semioperational scale — in other words, greater than 100 hectares.

Pheromones - The status and limitations are listed as follows:

a) Pheromones for direct control are likely to be used operationally only for key pests. A key pest is here defined as a pest species which, if reduced to noneconomic levels, would lessen the need for pest control measures on the given crop.

b) The need for low pest population density as a prerequisite for successful pheromonal control imposes obvious limitations, such as:

i. full season usage but not usage for spot treatments;

ii. areawide cooperative program usage but not for individual grower use.

Juvenile hormone analog insecticides. Status and limitations:

Status

a) Current operational use of only three different chemical compounds is known. These are methoprine, kynoprine and epophenonane.

b) The total current sales of all operationally used juvenile hormone analogs is less than 0.25% of worldwide insecticide sales, which total approximately 1.2 billion U. S. dollars. (*)

c) The chitin synthesis affecting compound diflubenzoron also known as diflurone, was not derived from a natural product and is therefore outside the scope of this Study Week Report.

Most significant limitations:

a) High selectivity with low economic return on the high investment, which is also true of other selective insecticides.

b) Selective useful activity on insects which represent only minor markets (**).

c) The activity observed on major pests is not of an operational useful nature.

Prospects for juvenile hormone analogs in crop protection:

Only a few areas in plant protection appear to be suited for ready control by morphogenetic agents. This is primarily because of the problems of critical timing in application, short persistence in the environment, lack of immediate control, re-invading populations, and economic evaluation.

Prospects for the use of anti-juvenile hormone agents for crop protection. Major limitations are as follows:

1) Activity is so far limited to non-economic insects.

2) Internal homeostatic mechanisms for control of natural hormone levels within insects are both very efficient and virtually unexplored.

(*) *Farm Chemicals*, September, 1975.

(**) Selectivity here refers to selectivity within insect species.

This lack of knowledge is greatly hindering plant protection by use of anti-JH chemicals.

Recommendations for needed research:

1) Intensive research on the natural mechanisms of control of endocrine organs is needed so as to provide an understanding and a rational basis for discovery and design of agents which will control young larvae of insects.

2) This is more important: economic research on mechanisms for providing the means for both development and production of selective insecticides which are conventionally described as uneconomical in terms of return on investment.

That is the end. The original ideas are coming later.

WAIN

There are just two points I would like to make — they are really points of detail — but you cannot talk about ethephon as an ethylene analog. Ethephon is betachloro-ethyl phosphonic acid and actually yields ethylene, so it is an ethylene generator, if you like, but it is no ethylene analog. At the end of the document, the very last sentence, I suppose; you talk about insecticides — I would like to add the words: " and fungicides " there, so that you bring in the disease aspect as well as the pest aspect.

KNÜSLI

We all can qualify the figures, the relation to the world turnover of pesticides which these compounds have. Now I wonder whether the addressees who are completely out of the knowledge we have may qualify it in the same way; they may eventually conclude: well, here there would be suitable means but some adverse forces hinder these compounds from getting 50% of the market. I wonder whether here not a certain precision should be included, because I think that the limitation is from the actual biological activity and not by other forces.

SIDDAL

I believe that we listed the limitations ascribable to biological activity

and the inference was that these are economic limitations. If it is not sufficiently clear, we can revise this section of the report to reflect your point. But it was clearly intended that the major limitation is economic and the cause of this limitation is the biological mode of action. Does this answer your point?

KNÜSLI

Well, it answers because I am fully aware of the background, but I wonder whether all the other addressees who will get this report from Professor Marini, whether they can make this connection.

BELL

One very minor point: the order in which you are presenting the report — you dealt with the modified derivatives first and the natural products, let us say, second. Would it not be logical to do it the other way around?

SIDDAL

Yes. Inversion. Just from the logical point of view.

BELL

When you got the things from the plants, and then you modified them, or from the insects, or from whatever you...

SIDDAL

We did it this way around because I did not think of any natural products which are actually used per se in operational crop protection.

MARINI-BETTÒLO

Yes, but they must be considered as the models.

SIDDAL

Well, it is just a matter of...

MARINI-BETTÒLO

A matter of editing.

SIDDAL

Yes, the report should be edited to reverse the order as you suggested, Prof. Bell.

MARINI-BETTÒLO

Is there any further comment?

SIDDAL

There is one addition which Dr. Bowers pointed out: the activity of anti-juvenile hormones is not totally limited to noneconomic insects. We shall modify the report to include activity on two species of economically important insects.

BRADER

Maybe just a technical point, Mr. Chairman. I do not want to bring up any discussion on that. It is the emphasis on the key pests. Many of the key pests which are key pests now are so because they were created by use of certain chemicals. If we take them away, we see that other pests become key pests, and they might need then again the use of pest control measures, so I think we should be a little bit careful about the use of that word "key pests".

SIDDAL

I believe that our definition overcomes your objection. Read the definition again: "a species which, if reduced to noneconomic levels,

would lessen the need for pest control measures on the given crop". I agree that many key pests are a consequence of earlier insecticide usage.

SOMERVILLE

Microbiological control agents, microorganisms for use in plant protection can be divided into two groups, based on application procedures. Those groups are: those that will be applied in the same way as chemicals and those for which specialized knowledge will be required. There may be a broad parallel between these two groups, respectively, and groups which require repeated application and those which can be called semi-permanent. Future production of biological pesticides will probably be carried out at two levels: (a) the large scale production using fermentation technology and (b) production within host species. In both of these cases, research is needed to develop inexpensive and effective methods of production. The latter approach shows more immediate promise for new biological control agents. However, it is important that adequate safety checks are undertaken on each production batch of material; and caution should be exerted before the use of any nonindigenous control agent. Despite this, the second approach may be more practical and may be of practical value to developing countries.

The following are examples which are in varying stages of research and development; we exclude products such as *Bacillus thuringiensis* and viruses which have been referred to in the Study Week. There are two Microsporidia, several species of nematodes, and a spore-forming bacterium that show promise in controlling populations of insects known to transmit human and animal diseases. Several protozoa, viruses and fungi are currently being developed to control phytophagous insects harmful to food and fiber crops and several protozoa, viruses and bacteria are available for control of stored product insects. For the future it is recommended that any observation of epizootics should be processed, if possible, as follows: Contact should be made with a competent microbiologist, who should isolate the causative agent and at least demonstrate that Koch's postulates are satisfied. The organism or virus responsible should then be deposited in the most accessible culture collection and the observation published. Further development may depend on strain selection and host spectrum studies and in this connection the international program on

Bacillus thuringiensis, which has already been referred to, could serve as an example.

It is possible that detailed study of the molecular mechanisms involved in the efficacy of such control agents will reveal new target mechanisms for the biochemist; when toxic products of microorganisms can be identified, we believe that research should be directed at elucidation of their mode of action.

BRADER

I would have liked that in this report was included — I know there were not instructions — parasites and predators, in general — and it only needs very little rewording. I don't see after all why they are not in.

KNÜSLI

Again, with the objective of this report, I think you should at least mention the realization already in operation.

ABO-KHATWA

I have just one comment here: so far all indications show that insect viruses attack only insect cells. Now the molecular basis for this specificity is quite unknown, and I think it must be mentioned that this specificity ought to be investigated: that is, why particular insect cells are attacked by viruses, and not other cells.

HEIMPEL

I should like to answer Dr. Abo-Khatwa. This is not true. There is a viper cell line that does support the virus — even if it does not replicate it — the virus does influence the cell line to produce proteins that are associated with the virus. Again there was an experiment by Himeno, *et al.* at Kyoto, Japan; they reported an FL cell line (human amniotic cells) was infected with extracted DNA from the nuclear polyhedrosis virus of the silkworm. I don't think this experiment was ever repeated successfully. A possible explanation might involve a contaminated culture of FL cells by insect cells from a silkworm cell line. On another subject,

Mr. Chairman, I would like to make a plea that it might be of tremendous advantage in this report, to include the recommendation that a central collection point be established for germ plasm of organisms and cell cultures that are currently being developed in microbial control of insects and other pests. Here they could be preserved permanently, with reasonable certainty that they never would be lost. There have been tragic losses, in the last 30 years, of the organisms involving the life work of several famous investigators. I refer to a Prinsloo's collection and that of Paillot among others. I think this might be the responsibility of one of the international organizations — to provide funds for specialists, in various countries that have culture collections, to insure their permanent retention. It is something that should be mentioned at any rate.

SHOREY

I believe the comment on other biological control organisms — parasites and predators — was very well made. But I would rather suggest that perhaps a brief statement concerning them belongs in the more general portion of this document and the emphasis might be somewhat as follows: One of the most pressing reasons for our emphasis on selective natural products is the protection of naturally occurring parasites and predators, which should be fostered by all possible means to enhance their capability to keep most potential pests at non-economic levels.

BUYCKX

I would just like to offer a brief comment in connection with what Dr. Heimpel said. There is an international board on plant genetic resources which has the same objective, namely to see to it that important genetic plant material is preserved for future work. The work of this board is financed by several donors, multilateral and bilateral donors. For the moment — since the last two years — FAO has been serving as the secretariat for that board. But I would like to point out that it is working with funds from multilateral and bilateral donors and not with FAO funds. Now, maybe the idea could be put to this board to also consider extending its activities to the field that Dr. Heimpel has just mentioned.

ELLIOTT

1) We consider that natural materials are one of the best sources of pest control agents or of leads to their development. Despite many studies of natural products, consideration of their potential use is largely neglected.

2) The most appropriate natural products for attention are those containing compounds which act endogenously or exogenously or in both ways.

3) We recommend that the collection and extraction of natural products should be oriented towards demonstrated or suspected chemically controlled biological phenomena and that therefore isolation should be guided by biological activity.

4) We recommend that a list of laboratories willing to perform biological assays be drawn up and that FAO be asked to act as a medium for communication.

BELL

Just one minor point: when you say biological activity — biological activity in what? This is an enormous concept because different compounds are reactive in different organisms to different degrees; they may be active in one and inactive in another.

ELLIOTT

We intended to indicate there that the biological activity should be that which had been demonstrated under 3-a. And we will make an appropriate alteration with agreement to indicate that.

NAKANISHI

I would just like to explain a little bit more to answer Dr. Bell's comment. The reason we put that phrase down is: natural products is one of the oldest branches in chemistry. In the past many natural product chemists have worked simply by taking a plant, for example, any plant in the garden, and working on the crystals, the easiest to get their hands and then doing the structure determination; that is what we are

warning against. Nowadays structure determination itself is getting much easier, and so we should concentrate our efforts more on the extraction of the biologically active principle. Now, this can come from two sources — I'm just going to take two typical sources. One is folk medicine — the plants which have been used. In that case we don't know, but this has to be started by screening, sending, for example, to a wide as possible screening — the simplest I envisage is one of these commercial places where they will run a variety of bioassays for $ 400. When we get the lead, we follow that biological activity and if during the course of extraction we encounter crystals which are not bioactive, they could either be neglected or given to graduate students for their training, to be able to handle the more difficult biologically active substances which quite often exist in only submilligram quantities. The second type of biological problems are those which are carried out in collaboration with biologists. An example may be the termite queen-building pheromone which Glen Prestwich (chemist at ICIPE) studied in collaboration with the ICIPE biologists. They teamed up and came up with palmitoleic acid as being the major component of this remarkable pheromone. Such a discovery is only made possible through close collaboration between scientists of different disciplines.

ELLIOTT

I will just read the modification — let me read 3 once more although it has been read, so that you can get the meaning of this modification. "We recommend that collection and extraction of natural products should be oriented toward demonstrated or suspected chemical controlled biological phenomena. *As a consequence, isolation should be guided by biological activity rather than by suitability for chemical investigation*".

BELL

Thank you, Professor Nakanishi. I entirely subscribe to your philosophy. I do the same thing myself. It is just that this report is going to be read by people outside this room and I throught we might try to make it a little clear. That was all.

BUYCKX

I would just like to offer a brief comment on the proposal of possible FAO involvement. Of course this gathering can make any recommendation or give any advice they may think useful to FAO. The only thing I can say is that certainly such advice or recommendation will be considered within FAO, and I would like to remind you that in these times of broad financial stringency and so on we have our constraints on possibility to implement any recommendation. So I would like you to bear this in mind and I think, if I may just later on have a look at the way it is worded — so that it is in very suitable form for our organization.

MARINI-BETTÒLO

I think that dissemination of information is very important and thus I believe that the best way to realize this should be the Food and Agricultural Organization of the United Nations.

KNÜSLI

I want only to mention a practical point. Of course for being able to examine material there is a need for a certain amount of the material available and this at the address of people who will submit the material for testing. The intensiveness of testing of course depends on the material available.

CRUICKSHANK

The success of biological assays is dependent on the test compounds being in solution or molecular dispersion in a solvent which is not toxic to the test organism or the target host plant. To study the dosage-response of a new product in a biological assay the biologist needs to know certain physical characteristics of the compound. Firstly, how can he measure it? Secondly, what can he dissolve it in? Data on the wavelength maxima of the compound in visible or ultraviolet light along with extinction coefficients normally are sufficient to answer the first question. If the compound is sparingly soluble in water, as many natural products are, information on its solubility in the solvents ethanol, acetone, or dimethylsulphoxide

is usually extremely valuable and may supply an answer to the second question. Tbis information should always accompany samples submitted to biological laboratories for testing.

BUYCKX

I think that we have tried to dissociate ourselves from FAO, to take off our FAO hat and just to be part of tbis gathering. So we have tried to keep it as concise as possible.

It was agreed that crop protection should preferably not be based on any lateral approaches and that the results of research on natural products and their analogs could best be used within the framework of integrated pest control. Integrated pest control has so far been mainly developed and implemented in the industrialized countries in a limited number of situations. Its further extension in these countries depends on the availability of research and extension capabilities as well as adoption by farmers. Regarding the future role of crop protection in increasing food and fiber production in the developing countries, it is considered important that these new approaches be made available to their rural communities. In spite of the apparent complexity of integrated pest control, it has been demonstrated that it can be implemented progressively on a step-by-step basis. This approach offers therefore a unique opportunity for introducing new research findings to improve crop protection. Integrated pest control requires a certain degree of infrastructure to exploit current scientific information as well as research capabilities to improve knowledge and develop more advanced pest control techniques. Furthermore, local knowledge in crop protection should be studied and considered for inclusion in crop protection programs. To overcome the present limitations in development and adoption of integrated pest control programs, more research and extension work is required. This is particularly so for the developing countries, where special efforts are needed to strengthen national capabilities. Emphasis has to be placed on training in crop protection, research and extension; and in this connection close collaboration of the scientific community is needed.

ZANINI

The use of natural products in the protection of plants and in par-

ticular in the control of parasites, should always be in association with cultural practices (use of fertilizers, irrigation, cropping patterns, etc.), adapted to local environment characteristics.

The different cultural techniques may themselves enhance or inhibit the extent and the intensity of the parasite attacks (infestations).

BRADER

I think, Mr. Chairman, that this would give more details, but that it was taken care of by the sentence that local control practices should be studied so that optimal use can be made of them.

ABO-KHATWA

If the FAO is going to adopt this integrated control approach, I think there are two corners really we have not mentioned, in this report. The first one is: we really need to know the basic principles of population suppression and how different approaches of insect control affect insect population trends. Now this requires a good understanding of insect population dynamics, insect ecology and behavior. These aspects we have not discussed so far. Another points is the ways and means to produce reliable information to assess crop losses. I don't think there are effective methods to get this reliable information so far. So maybe you ought to look into this.

BUYCKS

I may answer to Dr. Abo-Khatwa that FAO — as its key policy in promoting improved pest control — has adopted the integrated pest control approach. And we are making an effort not only by programs in the field but also by a series of publications, like Guidelines for Integrated Cotton Pest Control, Guidelines for Rice Pest Control. We are of course very well aware, and this has been, I think, also stressed in our communication, of the need to have in depth studies of the ecology, physiology and the behavior of pest species in order to gather the necessary information for population dynamics. And we have pointed out in this summary for the report the stepwise approach. In the developing countries, particularly, we have to go step by step and build up. Our

programs generally are conceived for 10 to 15 year periods. I think this has covered both points that you made.

MARINI-BETTÒLO

I should like to ask Dr. Knüsli to read the preamble.

KNÜSLI

No. I think it is not yet the time to read that because we said the preamble has to be modified following what has been already said now in the various contributions. For example, Prof. Wain put various items in which may now all be covered. And we said that it would need now to be the homework of our chairman to assemble that in a way that nothing is said twice. And so I think for that reason it wouldn't make much sense to read it now.

(After a discussion it is decided that Dr. Knüsli should read it).

KNÜSLI

(Reads with slight modification the text of the preamble drafted by Professor Wain and reported at page 793-794).

MARINI-BETTÒLO

I think now, hearing once more after the discussion what was read, that it could be the basis not for the preamble but for the final part of our report. What do you think about it? What is your opinion?

WAIN

I think, Sir, this requires very careful thought perhaps on your part; the separate reports that are being presented have got to be welded into the other one and it would be impossible really to decide whether to accept what Dr. Knüsli has just read as a preamble or as a sort of final for now, until this exercise has been done. It would seem to me that

the Knüsli report we just heard will have to be chopped up and part of it could be in the preamble and part at the end.

MARINI-BETTÒLO

I think the best thing is that you leave it to me and, before publishing anything, I am sending out a draft of this final report to be approved by you, so you can make all the suggestions.

CONCLUSIONS

The basic aim of the Study Week has been to review and discuss the present position in regard to fundamental research which might lead to more efficient control of plant pests and diseases.

In spite of control measures which are at present available, such as the use of conventional insecticides and fungicides, the world losses of human and animal food due to these causes — especially if both the growth and storage phases are taken into account — are enormous.

The seriousness of the problem of providing the growing needs of the underfed, steadily-increasing world population and the aggressiveness of plant insect pests and disease organisms justify making intensive efforts to combat these enemies. Scientists from all the relevant disciplines must work together towards achieving this objective. The following requirements, however, have to be satisfied before any method or measure can be generally adopted in crop protection:

it must be efficient;

it must not be disruptive or harmful to man and his environment;

it must be economically acceptable.

There are various ways in which pest and disease control can be achieved, for example by:

adopting proper cultural practices;

using disease-free seed and planting material;

breeding and selecting resistant varieties of crop plants;

utilization of biological control measures and use of chemicals.

Studies on the biochemistry of host plant, pest and pathogen, the recognition of sophisticated chemicals which play a unique part in insect behaviour and other developments of this kind have led to a particularly attractive strategy for pest control which involves the use not only of these naturally occurring molecules but also of some of their structural analogues.

I. - Naturally occurring products

1. We consider that natural materials are one of the best sources for providing agents for pest control or for providing leads to their development. Despite long and extensive studies of natural products their potential use has been largely neglected.

2. The most appropriate natural products to be studied for potential practical use are those containing compounds acting endogenously and/or exogenously.

Endogenous compounds are defined as those which are produced by the particular plant, animal, microorganisms, etc., for the purpose of sustaining its life, either overtly or covertly, e. g., toxins, hormones, pheromones.

Exogenous compounds are defined as those which are produced by the various organisms which seemingly are not involved in life maintenance but, nevertheless, exert physiological activity on other living organisms, e. g., antibiotics, alkaloids, phytoecdysones, and numerous other odd natural products.

3. (*a*) We recommend that collection and extraction of natural products should be oriented towards demonstrated or suspected chemically controlled biological phenomena. In this way such research would develop from the naturally occurring biologically active compounds rather than from a less precise " screening " approach.

(*b*) As a consequence, isolation should be guided by biological activity, rather than by suitability of compounds for chemical investigation.

4. It would be most important to have a list of laboratories willing to perform biological assays, with details of amounts needed, and preliminary information required for testing.

One aspect of this Study wrek program was related to the insect hormones pheromones and attractants. The natural products belonging to the group which can be used for plant protection may be divided into *two major categories.*

A) Natural products used *per se*:

1) ecdysteroids (operational use is pending);

2) pheromones of pests used for *monitoring*, as survey tools. These are used very successfully in numerous countries with great economic benefit;

3) a very limited number (about ten) of pheromones from *key* pests, which are now being explored on a semi operational scale (100 Ha). On the basis of present knowledge pheromones for direct control are only likely to be used operationally for *key pests.* A key pest is here defined as a pest species which if reduced to non economical levels would lessen the need for pest control measures.

The need for a low pest-population density prerequisite for successful pheromonal control imposes obvious limitations; thus, for example, they should operate during the whole season and be applied over wide areas in a co-operative program not involving individual growers.

B) Natural products which have provided a lead towards structures for practical use in plant protection.

Some specific examples already developed and used in operational practice are listed below:

Nereitoxin	led to the development of	PADAN insecticide
Juvenile hormone I		Epofenonane
Juvenile hormone II		Methoprene; kinoprene ...
Indoleacetic acid		2,4-D and many analogues
Ethylene		Ethephon (an ethylene producing compound)
Pyrethrines		Pyrethroids

In the case of analogues of Juvenile hormones only 3 different compounds are in current operational use as insecticides at the present time; these are methoprene, kinoprene and epofenonane.

The total current sales of such analogues in less than 0.25 % of the worldwide insecticide sales which amount to approximately U.S. $ 1.2 10^9 (Farm Chemicals, Sept. 1975, data source for total). The compounds affecting chitin synthesis (diflubenzoron, difluron) were not derived from a natural product and are considered outside the scope of this Study Week.

The limitations which operate with chemicals possessing juvenile hormone activity are:

1) high selectivity for insect species which give a low economic return on the high industrial investment made (this is also true of other selective insecticides);

2) useful selective activity on insects which represent only minor markets;

3) activity exerted on major pests is not high enough.

Prospects for using antijuvenile hormone agents in crop protection are so far limited by the fact that they act, with two exceptions only, against non economic insects.

Internal homeostatic mechanisms for control of natural hormone levels within insects are both very efficient and virtually unexplored. The lack of knowledge is greatly hindering the possible use of anti-JH chemicals in crop protection.

The *prospects for using juvenile hormone analogues in plant protection* are also limited. Only a few areas in plant protection appear to be suited for control by morphogenetic agents (juvenile hormone analogues). This is primarily because of lack of economic return on grower's investment arising from lack of immediate control of young larvae, the necessity for critical timing of application and the fact that re-invasion can occur.

Recommendations for future research may be focused on two points:

1) intensive research on the natural mechanism of control of endocrine organs of arthropodes is needed so as to provide an understanding and a rational basis for discovery and design of agents which will control young larvae of insects;

2) economic research on the provision of funds for both development and production of selective insecticides which are conventionally described as uneconomical in terms of return on investment.

II. Biological control agents (living organisms and viruses)

Biological control agents for use in plant protection can be divided into two groups based on application procedures:

a) those agents that will be applied in the same way as chemicals;

b) those for which specialized knowledge and methods will be required.

There may be a broad parallel between these two groups, respectively, and those which require repeated application and those which are semipermanent in effect.

The future production of biological pesticides will probably be carried out at two levels: production within host species and, for some micro-organisms and viruses, large scale production based on fermentation technology.

In both of these areas research is needed to develop inexpensive and effective methods of production. The latter approach shows more immediate promise for new biological control agents. However, it is important that adequate safety checks are carried out on each production batch of material and caution should be exerted before the use of any non indigenous control agent.

Biological control agents that have been used successfully include *Bacillus popilliae*, in control of Japanese beetle, *Bacillus thuringiensis*, which is used in the control of many lepidoptera; and virus and nematode preparations which have recently been made available commercially. The following are examples which are in varying stages of research and development.

There are two *Microsporidia*, several species of nematodes and a spore forming bacterium that show promise in controlling populations of insects known to transmit human and animal diseases.

Several protozoa, viruses and fungi are currently being developed to control phytophagous insects harmful to food and fiber crops and several protozoa, viruses and bacteria are available for control of stored product insects.

For the future it is recommended that any observation of epizootics should be processed as follows:

Contact should be made with a competent biologist who should isolate the causative agent and, at least, demonstrate that Koch's postulates are satisfied. The organism or virus responsihle should then be deposited in the most accessible culture collection and the observation published.

Further development may depend on strain selection and host spectrum studies and in this connection the international program for *B. thuringiensis*, mentioned elsewhere in this volume, could serve as an example.

It is possible that detailed study of the molecular mechanisms involved in the efficacy of such control agents will reveal new target mechanisms for the biochemist and, when toxic products of micro-organisms can be identified, we believe research should be directed at elucidation of their mode of action.

III. Disease resistance

Numerous examples are known of plants which are resistant to pathogens and herbivorous animals. This resistance may depend on static mechanism of protection, either chemical or physical, or on dynamic mechanisms, involving the production of specific chemicals in response to attack. The precise mechanism of resistance is known only in a few cases.

A knowledge of these mechanisms is important to enable us to take a rational approach to the development and use of varietal resistance and enable geneticists to take full advantage of the enormous pool of resources available in plants.

[One aspect of the programme was related to the studies on the chemical basis of plant disease resistance.] Recent research has provided information on why all plants growing in nature are completely resistant to most of the fungal diseases to which they are continuously exposed in the field.

There is now much evidence that defensive chemicals present in healthy plants and chemicals produced within them when under stress conditions, operate in natural disease resistance.

Isolation and identification of the chemicals in this group of

natural products have given a new impetus to research on the control of plant diseases. Such research might also be of benefit to medicine. The importance of these developments was recognized and supported. One became well aware of the many problems which are common to both pest and disease control, and the desirability of close collaboration between research workers within these fields was well recognized.

1) It is recommended that emphasis should be placed on studies of the mechanisms of resistance of existing crop plants, and of mechanisms of pathogenicity of plant parasites, in close collaboration with plant breeders so that the most productive effort can be focused on the development of new relevant varieties.

2) Studies should be made of pest/host plant relationships in naturally occurring populations of wild plants and minor crops with the objects of developing potential food crops which are already adapted in other respects to a tropical environment and of establishing the underlying principles involved in pest/host plant relation which can then be applied in agricultural ecosystems.

3) Investigation should be made of the mechanisms and potential of induced resistance to disease, especially as a possible safeguard against the progressive reduction of gene pools which is recognized as having occurred in some of the major food crops.

4) Communication should be improved between scientists of different disciplines who are involved in the study of plants and their pathogens and pests. Only in this way is it possible to achieve maximum utility from the findings in different areas of research.

IV. Integrated pest control approach

Crop protection should preferably not be based on unilateral approaches and results of research on natural products and their analogues could be used within the framework of integrated pest control.

Integrated pest control has so far been mainly developed and implemented in the industrialized countries and only in a limited number of situations. Its further extension depends on the avail-

ability of research facilities and capabilities as well as adoption by the farmer.

If crop protection is to be fully utilized in increasing food and fibre production in developing countries, it is important that these new approaches be made available to rural communities. In spite of the apparent complexity of integrated pest control, it has been demonstrated that the system can be implemented progressively on a step by step basis. This approach therefore offers a unique opportunity for introducing new research findings to improve crop protection.

Integrated pest control requires a certain degree of infrastructure to fully utilize current scientific information and research capabilities and to develop more advanced pest control techniques.

Furthermore, local knowledge in crop protection, for example, the impact of cultural practice, should be studied and considered for inclusion in crop protection programmes.

In order to overcome the present limitations in development and adoption of integrated pest control programmes, more research and extension work is required. This applies particularly to the developing countries where special efforts are needed to strengthen national capabilities.

Emphasis must also be given to training workers for crop protection research and extension; in this connection, close collaboration with the scientific community is needed.

V. Information and Communication

The importance of sharing knowledge and the need to perform highly specific biological assays was also emphasized.

Suitable media for documentation exist already; they could be used in a more consequential way, in so far as printed information should be considered more than in the past as a bridge to direct personal contact between researchers engaged in related topics.

In order to strengthen the flow of suitable information the FAO and also other appropriate Agencies are suggested to consider possibilities for a coordination of the interchange and for acting as mediators.

VII. Conclusions

There was general agreement that the new approach indicated in the Title of our Study Week, "Natural products and the protection of plants" provides much promise at the present time.

One of our aims — which has been abundantly achieved — has been the stimulation of new ideas, the sharing of knowledge and educating each other in the various aspects of pest control research in fields different from one's own. As a result, many of the participants will return to their laboratories to initiate experiments whose conception arose directly from the intensive discussions carried out in a calm and relaxed atmosphere between scientists from widely different fields.

As a result of our discussions and deliberations, it is hoped that further achievements towards the welfare and benefit of mankind will be realised.

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