Insect-Plant Interactions (1969)

Pages
101

Size
7 x 10

ISBN
0309344255

Environmental Physiology Subcommittee; U.S. National Committee for the International Biological Program; Division of Biology and Agriculture; National Research Council

INSECT-PLANT INTERACTIONS

Report of a work conference

Environmental Physiology Subcommittee

U.S. National Committee for the International Biological Program

Division of Biology and Agriculture

National Research Council

NATIONAL ACADEMY OF SCIENCES
Washington, D.C.
1969

PREFACE

In 1967 the U.S. National Committee for the International Biological Program issued a report that described actions by which the national committee and its subcommittees hoped to develop the U.S. phase of the International Biological Program.*

One of the proposals, made by the Environmental Physiology Subcommittee, was that entomologists and botanists hold a working conference on insect control by plants. The report stated (page 17):

> This conference will center on chemical interactions between organisms. It will emphasize control of insects by plants and control of plants by other plants. The fact that terpenoids are implicated in both of these control phenomena suggests that entomologists and botanists might well be brought together to consider mechanisms and applications.

Pursuant to this proposal, conferences on insect-plant interactions and plant-plant interactions were held at the University of California, Santa Barbara, March 18-22, 1968. About 40 scientists attended the insect-plant conference, and about 30 attended the plant-plant conference. The two groups held joint meetings in the morning and met separately in the afternoons and evenings.

This publication is concerned with the plant-insect conference. It consists of "Introduction, Highlights, and Recommendations," summaries of the papers presented, and bibliographies. The report on the plant-plant conference is entitled Biochemical Interactions among Plants.

Plans for the conference on insect-plant interactions were developed by the following committee: Edward F. Knipling (chairman), U.S. Department of Agriculture, Beltsville, Maryland; Reginald H. Painter, Kansas State University; William E. Robbins, U.S. Department of Agriculture, Beltsville, Maryland; and Carroll M. Williams, Harvard University.

The summaries in this report were prepared by the authors of the papers. The bibliographies were prepared by Fowden G. Maxwell and Reginald H. Painter.

*U.S. National Committee for the International Biological Program, National Academy of Sciences-National Research Council. 1967. U.S. Participation in the International Biological Program. National Academy of Sciences, Washington, D.C.

CONTENTS

I Introduction, Highlights, and Recommendations 1

Introduction 1

Highlights of the Conference 3

Juvenile Hormones and Juvenile Hormone Analogues 3

Ecdysones and Ecdysone Analogues 6

Plant Chemicals Affecting Insect Behavior 9

Pheromones and Pheromone-like Interactions 10

Neurophysiological Studies of Responses to Plant Chemicals 11

Recommendations 14

II Report Summaries 16

Biology and Biochemistry of Insect-Plant Relationships 16
Stanley D. Beck

Natural and Synthetic Compounds with Juvenile Hormone Activity 19
William S. Bowers

Electrophysiological Studies of Food-Plant Relationships of Lepidopterous Larvae 20
Vincent G. Dethier

Insect Exocrines 21
Thomas Eisner and Jerrold Meinwald

Effect of Juvenile Hormone and Ecdysterone on Rectal Pad Development during Adult Development of *Hyalophora cecropia* 24
Lawrence I. Gilbert and K. Judy

Effects of Juvenile Hormone on *Sarcophaga bullata* 25
Lawrence I. Gilbert and U. S. Srivastava

Chemical Components in the Cotton Plant and Their Effect on Boll Weevil Behavior 29
Paul A. Hedin

Investigation of Insect Hormones 31
Peter Hocks

Natural Products Chemistry 34
D. H. S. Horn

Some Population Aspects of Plants in Insect Suppression 36
Carl B. Huffaker

Behavioral and Chemosensory Background of Host Specificity in Phytophagous Insects 38
Tibor Jermy

Effects of Ecdysones and Analogues on Insect Development and Reproduction 40
John N. Kaplanis and William E. Robbins

Host Plant Resistance to the European Corn Borer 42
Jerome A. Klun

Development of Cotton Plants Resistant to Heliothis spp. and Other Cotton Insects 43
Maurice J. Lukefahr, T. N. Shaver, and A. A. Guerra

Biologically Active Substances in Cotton and Related Plants That Affect Boll Weevil Behavior and Development 45
Fowden G. Maxwell

Ecdysones in Plants 50
Koji Nakanishi

Biological Basis of Insect Resistance in Crop Plants 53
Reginald H. Painter

Bark Beetle Manipulation with Natural and Synthetic Attractants 56
Gary B. Pitman

Artificial Diet for Saturniid Silkworms 59
Lynn M. Riddiford

Oak Leaves and the Mating and Ovipositional Behavior of the Polyphemus Moth 60
Lynn M. Riddiford

Effects of Juvenile Hormone on Embryonic Determination 61
Lynn M. Riddiford

Research on Insect Hormones and Pheromones 62
Herbert A. Röller

Studies on Timber Beetles 64
Julius A. Rudinsky

I INTRODUCTION, HIGHLIGHTS, AND RECOMMENDATIONS

INTRODUCTION

Insects and allied pests constitute one of the major obstacles to maximum food and fiber production. They destroy a high percentage of the world's timber resources, they destroy manufactured goods and clothing, and they affect the well-being of man by transmitting many serious disease organisms.

Great progress has been made during the last two decades in developing the highly effective insecticides that now provide the chief means for controlling insect pests, but adverse effects have resulted from their use. Virtually all insecticides now available are nonselective in action and may be toxic to a wide range of organisms in addition to the target pests. Insects have the capability of developing strains highly tolerant to insecticides. When such resistance occurs, larger amounts of insecticides must be applied or alternative insecticides must be developed. Conventional chemical approaches to insect control leave much to be desired as economical, effective, and permanent solutions to many insect problems.

It is estimated that losses due to insects in the United States amount to $5 to $6 billion annually and that agricultural pests take a 30-percent toll of the world's food production. Many health problems created by insects and related pests remain unresolved. Malaria, filariasis, and other diseases transmitted by insects continue as critical health problems in many parts of the world.

The hazards associated with the use of broad-spectrum chemical control agents are matters of continuing concern. Conservationists are apprehensive over the possible immediate and long-range adverse effects of pesticides to wildlife resources. Biologists and entomologists recognize the adverse impact of conventional broad-spectrum insecticides on the natural beneficial-insect complexes that are vital to the management of insect populations. Pesticide residues in foods must be avoided or held to a minimum; they may affect international trade in agricultural products because of differences in policies as to appropriate tolerance levels.

Research efforts on a number of alternative means of insect control have been intensified. These include research on (1) more selective insecticides and more selective means of using those we now have; (2) new, highly selective chemical agents, such as sex pheromones; (3) natural biological agents and more dependable ways to use them; (4) crop varieties that resist insect attack; and (5) various genetic techniques, such as induced sterility by radiation, by chemicals, or by genetic principles.

Other approaches to insect control may materialize as a result of basic research on fundamental life processes in insects. As was revealed in the work conference, progress has been made in isolating and characterizing natural agents in insects that regulate development and behavior. Moreover, some of the natural growth-regulating chemicals that occur in insects may also occur in plants and in other animals. Chemists have succeeded in synthesizing several of these natural agents, including the juvenile hormones and ecdysones. New chemicals are being synthesized that may possess greater biological activity than their naturally occurring parent compounds.

Rapid progress is also being made in research on substances in plants that affect insect behavior. Plants contain highly active substances that may attract insects for feeding, for mating, or for oviposition. Such substances may be highly selective in their biological activity. Although full knowledge of the toxicology of these new types of chemicals derived from plants and animals is lacking, they appear to be highly selective, often acting on a narrow range of closely related insects. Thus, the hormonal chemicals and other new types of growth- and behavior-regulating chemicals offer opportunities for developing new classes of biologically active agents that are selective in action to destructive insects, without hazard to man and his environment. Moreover, the unique biological properties of these agents, such as delayed disruption of insect development, interference with reproductive processes, or changes in the behavior of insects, could lead to highly effective, safe, and novel approaches to insect control.

Reports by participants in the work conferences revealed that a dozen or more kinds of chemical compounds have been identified that have a wide range of biological effects on insect development and behavior. These and other new agents with similar properties need to be evaluated against economically important insects and other pests. Results of preliminary biological tests on laboratory insects suggest the need for mission-oriented research projects on a number of economic species. These would include insects affecting stored grains and other foods; major field crop pests, such as the boll weevil and bollworms; fruit pests, such as the codling moth and fruit flies; and key pests of man and animals, such as tsetse flies and mosquitoes.

The International Biological Program could play a prominent role in advancing an expanded research program in insect-plant interactions by coordinating international programs and by lending financial support. Fundamental investigations must continue before applied research and development can begin. Sufficient basic information indicating the potential of these new types of chemicals for insect control is already available. The selection of suitable economically important insects of wide distribution as the test species for further basic research would help advance research findings into practical insect control.

HIGHLIGHTS OF THE CONFERENCE

Juvenile Hormones and Juvenile Hormone Analogues

Novel means of disseminating hormonally active pesticides were reported by Karel Sláma. In the experiments described, he exploited his earlier discovery that certain juvenile hormone analogues can block the embryonic development of eggs laid by treated female bugs without interfering with the viability of the bugs themselves (Sláma and Williams, 1966). He applied an enormous dose (1 mg) of synthetic juvenile hormone analogue to individual male adults of *Pyrrhocoris apterus* (L.). When the treated males mated with normal females, the latter received sufficient juvenile hormone to sterilize them. Moreover, in the manner of a venereal disease, the contaminated females were able to "infect" normal males that in many cases passed along sufficient hormone (ca. 0.1 μg) to sterilize a further group of normal females.

Preliminary field tests of juvenile hormone pesticides were among the numerous findings reported at the conference. Herbert A. Röller reported that 20 mg of synthesized cecropia juvenile hormone were fully effective in controlling codling moth infestations of individual apple trees. Tests on other species also show promising results.

Röller described the isolation and identification of the juvenile hormone of the cecropia moth as methyl *trans, trans, cis*-10-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate (Röller *et al.*, 1967a, 1967b; Dahm *et al.*, 1968). The question of the *dl* isomerism of the hormone was not solved. A synthesis of the racemic juvenile hormone was described that allowed separation and stereochemical assignment of *cis-trans* isomers after the introduction of each double bond. By this means, *dl* juvenile hormone was obtained stereochemically pure (Dahm *et al.*, 1967) and was found to possess all the morphogenetic, prothoracotropic, and gonadotropic activities associated with the secretion of the corpora allata. Authentic juvenile hormone as well as the *dl* hormone was active at submicrogram levels when assayed on diverse insects, including representatives of the Coleoptera, Lepidoptera, Hemiptera, and Orthoptera. Strangely enough, the synthetic ethyl ester analogue is eight times as active as the native methyl ester. Röller also described the recovery of authentic juvenile hormone from the medium in which active corpora allata had been cultured *in vitro* for five days.

Though the optical isomerism of the molecule has not been resolved, the bioassay results on *Tenebrio* indicate that the *d* and the *l* forms have similar specific activity. A number of compounds with the carbon skeleton of juvenile hormone were synthesized. Röller reported that the trans-configuration at the Δ^2 and Δ^6 double bonds is extremely important for biological activity; by contrast, the stereochemistry of the oxirane ring seems to be of secondary importance.

In the discussion of these findings, Carroll M. Williams suggested that during the millions of years of insect evolution the detailed chemistry of juvenile hormone has probably evolved and diversified. Consequently, the day may be near at hand when one can no longer speak of the hormone isolated by Röller as the juvenile hormone. Later in the session, Howard A. Schneiderman reported that André S. Meyer has indeed isolated a second juvenile hormone from cecropia oil (Meyer et al., 1968a, 1968b). The molecule was shown to be identical in biological activity and in structure to that described by Röller except that a methyl (rather than an ethyl) group is present at C-7. The new hormone is responsible for about 15-25 percent of the endocrine activity of cecropia oil. In the discussion of this important work, some participants indicated that the new hormone is merely a stage in the biosynthesis of the previously known hormone. Be that as it may, the molecule shows great endocrine activity in the biological assay and is an important constituent of cecropia oil.

William S. Bowers reported that the synthetic juvenile hormone analogue, trans, trans 10,11-epoxy farnesenic acid methyl ester, successfully terminates adult diapause in several species of Hemiptera, including the boxelder bug and *Pyrrhocoris apterus* (L.). This duplicates his previous findings on Coleoptera. He also reported that a number of insecticide synergists (e.g., sesoxane and piperonyl butoxide) have substantial juvenile hormone activity when assayed on mealworms and milkweed bugs and that several hybrids of farnesol (i.e., farnesyl piperonyl ether and 10,11-epoxy farnesyl piperonyl ether) also show high juvenile hormone activity. The diversity of some of these compounds requires a re-examination of the relation of chemical structure to hormonal activity.

Bowers reviewed the isolation and identification of juvabione from the balsam fir. Sláma reported that he and his co-workers were able to confirm the presence of juvabione in fir wood and, in addition, they found a related compound, dehydrojuvabione, that displayed the same selective action on insects of the family Pyrrhocoridae, although it was somewhat less active. Though juvabione and dehydrojuvabione are to date the only compounds with juvenile hormone activity that have been isolated and identified from plants, Bowers reported that among 52 species of plants chosen at random, six have provided extracts with juvenile hormone activity in the *Tenebrio* assay, with two of these extracts showing considerable activity.

The site and mode of action of juvenile hormone were considered by Lynn M. Riddiford, who described preliminary radioautographic studies of the localization of tritiated juvabione--a juvenile hormone analogue with selective action on bugs of the family Pyrrhocoridae. The larvae of the sensitive species *Pyrrhocoris apterus* showed definite localization of radioactivity at or near the nuclear membranes of cells of epidermis and fat body. By contrast, in parallel experiments carried out on the insensitive lygaeid bug, Oncopeltus fasciatus (Dallas), no localization of label was evident at the nuclear membrane or anywhere else.

In additional experiments performed on silkworms and on two species of bugs, Riddiford documented the ovicidal action of synthetic juvenile hormone analogues when they are administered to adult females or to freshly laid eggs. Eggs treated in later embryonic development produced apparently normal larvae that, surprisingly, showed aberrant development days or weeks later during the larval stages or at the time of metamorphosis. Evidently, in addition to its ovicidal action on the early embryo, juvenile hormone is able to interfere with "embryonic determination"--that mysterious programming and prepatterning of the embryonic cells for postembryonic development.

Sláma described ongoing studies of juvenile hormone that are being conducted in Prague, including the synthesis of compounds with selective action on certain kinds of insects. Moreover, the biological activity of dehydrojuvabione was enhanced tenfold by aromatization of the alicyclic ring and removing the keto group at C-10. A further tenfold increase in activity was realized when one of both aliphatic double bonds were hydrochlorinated (Sláma et al., 1968). Like juvabione and dehydrojuvabione, all these materials show selective activity on bugs of the family Pyrrhocoridae. By contrast, the synthetic mixture described by Law et al. (1966) is highly active on virtually all insects since it contains many different materials with juvenile hormone activity for diverse insects. From this mixture the Prague investigators isolated and characterized the ethyl ester of trans-2-dihydrochlorofarnesoate -- a substance with selective action on pyrrhocorid bugs when administered at nanogram levels.

Sláma also described a number of ingenious experiments examining the basis of the selective action of the hydrochloro compounds. This material rapidly penetrates the unbroken skin of both sensitive and insensitive species of bugs and is stable for many days after entry. In certain resistant species (pentatomid bugs) but not in others (Galleria, Tenebrio), the hormonally active material can be shown to circulate in the blood without any effects. So, at least in the case of pentatomid bugs (and perhaps in most insects) insensitivity to the hydrochloro compounds is apparently due to the failure of the cellular receptor mechanism to bind the hormone analogue.

The muscoid Diptera have been thought to be insensitive to all presently known materials with juvenile hormone activity. This is not so, according to experiments described by Lawrence I. Gilbert. Gilbert reported that when larvae of a blowfly (Sarcophaga bullata Parker) were treated with synthetic cecropia hormone, they showed a prolongation of larval life and the formation of abnormal puparia. Moreover, when the synthetic hormone was dissolved in olive oil and topically applied to freshly pupated individuals, as little as 0.4 μg caused the formation of nonviable flies whose abdomens showed the formation of a second pupal cuticle. These experiments were carried out in collaboration with U.S. Srivastava.

Little information is presently available as to the possible action of juvenile hormone on animals other than insects. Invertebrates of many different classes and phyla are known to undergo extensive metamorphosis. The question therefore arises as to whether materials with juvenile hormone activity for insects can interfere with the metamorphosis of some of these other invertebrates.

An affirmative answer to this query was suggested by Röller in a brief report of ongoing research demonstrating that synthetic cecropia juvenile hormone, when topically applied to a mouse's tail (Mors et al., 1967) protects the animal against schistosomiasis.

Ecdysones and Ecdysone Analogues

A second major theme at the Santa Barbara conference was ecdysone--the growth and molting hormone of insects. During the past two years, amazing amounts of ecdysone-like materials have been discovered in certain weeds, ferns, and evergreen trees.

Koji Nakanishi presented a comprehensive summary of these surprising developments. Thus far, a total of 15 phytoecdysones, including authentic α- and β-ecdysones, have been isolated and characterized. With a few exceptions all possess the same chemistry in the sterol ring system: hydroxyl groups at C-2 and C-3, a ketonic function at C-6, a Δ^7 double bond in ring B, and an α hydroxyl at C-14. Moreover, in all materials except ponasterones B and C, the hydroxyls at C-2 and C-3 are β. Nakanishi explained that by adopting a "boat conformation," ponasterones B and C achieve an exposure of the α hydroxyls of ring A that approximates that displayed by the "chair forms" of the other phytoecdysones.

Nakanishi emphasized that the absolute configuration of the side chain is known only in the case of α-ecdysone. However, J. B. Siddall reported that by the synthesis of β-ecdysone (crustecdysone, ecdysterone) and ponasterone A from the same parent compound, he and his co-workers have found that all ten asymmetric centers are precisely the same in the two materials.

The number of phytoecdysones is certain to increase. During the past year and a half the structures of 15 have been determined. Nakanishi described a massive research effort under way in Japan, on which a preliminary report has been published by Takemoto et al. (1967). Very extensive screening has been carried out by the Takeda Pharmaceutical Industries; 186 of the 188 families of higher plants in Japan, including 738 genera and 1,056 species, have already been examined for ecdysone activity. Forty species have provided active extracts. Screening operations have also been carried out on 350 plant-derived drugs, with many positive findings.

These investigations have been greatly assisted by the Takeda Company's development of a quick method for separating the ecdysones and a simple assay for determining ecdysone activity. The separation consists of column chromatography on XAD-2 resin (200-400 mesh) in which a linear gradient elution of 20-70 percent aqueous ethanol is used. In the biological assay, abdomens of fifth-instar larvae of the Asiatic rice borer (Chilo suppressalis (Walker)) are isolated by ligation and dipped for 10 sec in a methanolic solution of the plant extract. The presence of ecdysone activity is signaled by pupation of the abdomens after 24-48 hr. In a calibration of the assay, 0.5-1 μg ponasterone A per individual provoked a 100-percent response. Moreover, when a dosage of 0.5-1 μg was fed to individual silkworm larvae any time after the fourth day of the fourth molting, all individuals initiated spinning within 12 hr.

Peter Hocks and J. B. Siddall described a number of ecdysone derivatives that have been synthesized by Schering and Syntex in following up their independent syntheses of α-ecdysone. Hocks stated that the Schering laboratory had found no detectable effects of α-ecdysone in tests performed on mammals, including tumor-bearing mice. Siddall reported that Syntex found α-ecdysone devoid of estrogenic or androgenic action in tests performed on chicks and mice. However, a definite mineralocorticoid effect, including sodium retention and potassium excretion, was detected in mice injected with α-ecdysone.

D. H. S. Horn announced the isolation of 2 mg of β-ecdysone (crustecdysone) from a ton of shrimp. This same ecdysone was also isolated from kilogram lots of carefully timed larvae of a blowfly, Calliphora stygia (F.). Surprisingly, no trace of α-ecdysone was detected.

Malcolm J. Thompson reported new or improved synthesis from cholesterol of approximately ten 5α- and 5β-analogues of ecdysone containing the cholesterol side chain and various combinations of functional groups in the ecdysone ring system. The aim of his synthetic program is to determine the structural requirements for biological activity. John N. Kaplanis reported that he and William E. Robbins have tested a number of these synthetic analogues, as well as authentic α- and β-ecdysones, on five species of insects by feeding them in the diet. The α- or β-ecdysones were found to have little if any inhibitory effects in the larval-growth tests. By contrast, certain of the synthetic analogues, including Δ^7-5β-cholestene-2β,3β,14α-triol-6-one, were found to disrupt larval growth and metamorphosis when ingested. In some insects, the analogues caused high mortality and inhibited development when present in the diets in the ppm range. The triol mentioned above was also found to inhibit ovarian development and reproduction when fed to the house fly or the confused flour beetle. These compounds, therefore, have a potential as chemosterilants. The corresponding 5α-analogues are hormonally inactive in the house fly assay and are not inhibitors of growth and development. This suggests that the deleterious effects of the 5β-analogues are related to their hormonal activity.

James A. Svoboda reported investigations of the insect's mechanism for dealkylating phytosterols to cholesterol. In many phytophagous insects, this conversion provides the major source of the insect's essential cholesterol. Two steroids have been isolated, identified, and found to block the conversion of β-sitosterol to cholesterol by inhibiting the Δ^{24}-sterol reductase. In addition, certain synthetic hypocholesterolemic diazasterols, such as 20,25-diazacholesterol, block the conversion of β-sitosterol to cholesterol. Studies carried out on the tobacco hornworm suggest that these materials interfere with the normal functioning of the endocrine system. Growth is retarded, abnormal pupae are formed, and larvae often initiate metamorphosis an instar earlier than normal.

Nakanishi described Japanese studies that demonstrated a rapid inactivation of cyasterone in the silkworm gut; the loss of activity in this case was shown to be due to an opening of the lactone ring by the alkaline conditions of the gut (pH 9).

Sláma found that polypodine B, an ecdysone isolated from ferns, is completely inactive when fed to _Calliphora_ larvae. However, the crude fern extract proved to be a potent inhibitor of growth.

Williams reported on biological studies of seven ecdysones that he and T. Ohtaki have carried out. All materials were highly active in the standardized puparium assays as well as in assays performed on brainless diapausing pupae and on isolated abdomens of the cynthia moth, _Samia cynthia_ (Drury) (Ohtaki _et al._, 1967; Ohtaki and Williams, 1968). Moreover, studies carried out in collaboration with Roger M. Milkman have demonstrated the ability of the phytoecdysones to provoke the characteristic pattern of chromosomal puffing in the cells of the salivary glands of _Drosophila virilis_ Sturtevant. Despite this fact, Milkman's preliminary radioautographs show no trace of selective binding of tritiated 25-deoxyecdysone to the chromosomes or the puffs.

In additional unpublished studies, Ohtaki and Williams found that α-ecdysone is swiftly inactivated after its injection into fly larvae or silkworm pupae. Its half-life in _Sarcophaga_ larvae injected with 1 μg is 1 hr; in diapausing _Samia_ pupae, 8 hr. Inactivation is opposed or prevented when the injected larvae are placed at low temperatures (1-2°C) or in the absence of oxygen. The inactivating mechanism could not be demonstrated in fly homogeantes or in cell-free preparations of either the blood or the tissues.

Williams suggested that the short half-life of ecdysone requires a new theory of ecdysone action that emphasizes the accumulation, not of the hormone itself, but of its covert effects. These undergo, as it were, spatial and temporal summation within the target organs and finally trigger the overt effects -- for example, molting or metamorphosis. If this process is interrupted short of completion, then the covert effects are subject to decay.

The question naturally arises: Why have certain plants gone in for the synthesis and accumulation of such exotic sterols as the phytoecdysones? Williams suggested that the plants make use of the materials to protect themselves from insects and possibly also from nematodes. In this connection, he cited experimental evidence that the ecdysones can penetrate the insect cuticle when topically applied in nonvolatile solvents such as undecylenic acid, α-tocopherol, or caprilic acid. This being so, one may conjecture that in its native dissolved state within the plant, ecdysone can penetrate the insect on contact. In that event, diapause would be terminated, and metamorphosis might be hastened to completion at an unpropitious season.

Williams emphasized that in addition to enforcing this kind of "ecological suicide," the uptake of excessive ecdysone would cause a lethal derangement of development. This fact has been documented by Kobayashi et al. (1967a, 1967b), by Williams (1968), and by unpublished experiments that Lawrence I. Gilbert carried out with K. Judy on the cecropia moth.

Plant Chemicals Affecting Insect Behavior

Fowden G. Maxwell and Paul A. Hedin described the complicated chemical signaling that goes on between the cotton plant and the boll weevil. They reported the extraction, assay, and characterization of numerous active materials, including attractants, repellents, synergists, arrestants, and phagostimulants, as well as assembling scents of the pheromone type. Hedin concluded that no single plant component evokes the full feeding response. However, combinations of compounds have been formulated that are as active as the most active plant extracts.

Maurice J. Lukefahr described research directed toward utilizing both the morphological characters and the naturally occurring substances of cotton plants as a means of protecting the plant against the attack of two species of *Heliothis*. Varieties of cotton lacking extrafloral nectaries and trichomes caused reduced insect fecundity and longevity and decreased oviposition because of lack of food and favorable oviposition sites. In addition, certain selected experimental lines contain gossypol and related compounds that may effectively limit insect populations. Lukefahr also reported that the boll weevil feeds on the buds of male *Hampea rovirosae* but that the mature female tree appears to be protected by one or more volatile deterrents.

The importance of insect deterrents was further emphasized by Tibor Jermy, who reported that narrower food specialization, at least in chewing insects, is associated with a higher sensitivity of the insect's chemoreceptors to inhibitory agents present in nonhost plants. Thus, the acceptability of a plant is determined more by the absence of deterrents than by the presence of stimulants. This sensitivity of the chemoreceptors to inhibitory stimuli makes possible the use of natural and synthetic antifeeding substances for plant protection. Two promising systemic feeding deterrents have been discovered in his research: 2,4,6-trichlorophenoxyethanol and 2,4,6-trichlorophenoxyacetic acid.

Both olfaction and vision guide tobacco hornworm moths to oviposit on the host plant, Robert T. Yamamoto reported. Although oviposition preference for tobacco and tomato rather than wild solanaceous plants was found in nature and confirmed in the laboratory, this preference of the adult female is not always related to larval-feeding preference. For example, the larvae chose potato and *Petunia* (a toxic plant) over tobacco. Yamamoto also reported a finding of great theoretical interest--that larvae fed on various solanaceous plants other than the two preferred hosts produced adult moths that retained a pronounced tendency to oviposit on the larval host plant. The presence of a water-soluble oviposition stimulant in the tobacco plant and studies of its extraction and assay were also discussed.

Additional experiments on the tobacco hornworm were described by Jermy. When presented with leaf discs cut from tobacco, tomato, and Solanum, the larvae always showed an induced preference for the particular plant on which they had previously fed. This same result was observed when tests were performed on larvae reared on an artificial diet and provided only token exposure to one of the three food plants. Final-stage larvae were conditioned by pulsed exposure as brief as 2 hr.

Carl L. Tipton and Jerome A. Klun reported that the resistance of certain corn varieties to the European corn borer is a function of the concentration of benzoxazinone derivatives of which 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (DIMBOA) appears to be the most important. Studies of the biosynthesis of DIMBOA, its lactam, and their corresponding gludosides are under way.

Thomas Eisner and Jerrold Meinwald pointed out that certain insects have exploited the tools and tactics of chemical defense in protecting themselves from predation. The chemicals include a number of materials that plants also use as deterrents. Therefore, the question arises as to whether the insects get some of these materials ready-made from plants. In a few cases (e.g., the monarch butterfly and certain grasshoppers) this is indeed true. The defensive secretions of insects are commonly discharged in solvents, such as caprylic acid and nonylacetate, which show great ability to penetrate the unbroken skin of predatory animals. It is interesting to note that caprylic acid was among the solvents that Williams reported as facilitating the penetration of topically applied ecdysone.

A. J. Thorsteinson described the use of simplified model systems in studies of the behavior of the diamondback moth. In analogous experiments, Stanley D. Beck found that wheat-germ oil contains a potent phagostimulant for the cabbage looper. When the oil was subdivided into neutral and phospholipid fractions, all activity accompanied the phospholipids. Despite this fact, a combination of neutral lipids and potassium ions greatly enhanced the response to plant phospholipids.

Pheromones and Pheromone-like Interactions

Chemical signals between plants and insects include pheromone and pheromone-like agents. Thus, according to Julius A. Rudinsky, storm-damaged Douglas-fir trees release volatile terpenes that attract "pioneer" female Dendroctonus beetles. After tunneling into the phloem, these beetles signal their presence by secreting a pheromone that attracts additional males and females from afar.

Gary B. Pitman reported studies of a related species of Dendroctonus in which the pheromone was isolated and identified as trans-verbenol.

An assembly pheromone is also used by another scolytid beetle, Ips confusus (LeConte), in its attack on the ponderosa pine. According to Robert M. Silverstein, the tree is first attacked by a few male beetles. As these tunnel into the bark, they produce frass containing an extremely potent attractant for both male and female Ips confusus.

From 4.5 kg of frass Silverstein and his co-workers extracted and characterized three different terpenoid alcohols that were active in the biological assay only when recombined. One of the three materials was cis-verbenol; the other two were previously unknown compounds (Silverstein et al., 1966). Small amounts of the three materials were synthesized and were shown to be active only when combined. This phenomenon was discussed in further detail by David L. Wood.

In additional discussions of pheromone-like agents, Lynn M. Riddiford summarized her recent studies of polyphemus moths. Under laboratory conditions these moths mate only in the presence of a volatile emanation from oak leaves (Riddiford and Williams, 1967). The active material was extracted and shown to be trans-2-hexenal (Riddiford, 1967). Vapors of a dilute solution of this substance were found to act on the female antennae. The resulting nervous input to the brain provokes the release of the sex pheromone by glands at the tip of the female abdomen. This chain of events leads to the sexual activation of the male.

Riddiford reported additional experiments on Antheraea pernyi Guérin-Méneville. She found that after brief exposure to the vapors of formaldehyde, male moths showed a reversible loss of sensitivity to the female sex pheromone. The same was true after rinsing the male antennae in saline. These observations suggested the presence on the male antennae of a proteinaceous receptor mechanism for binding the sex pheromone. Therefore, additional experiments were carried out in which male antennae were washed at 6-hr intervals. The resulting extract, when dialyzed, lyophylized, and examined by acrylamide gel electrophoresis, showed the presence of several different proteins. Material collected after the first washing showed one main band that increased in density as the interval between washings was prolonged.

Neurophysiological Studies of Response to Plant Chemicals

Vincent G. Dethier described how to "bug" an insect by placing electrodes in or near the primary sensory receptors. The receptors in question consist of bipolar neurons. Through a pore in the overlying cuticle, one end of each neuron communicates with the outside world; the other end proceeds without synaptic delay to the central nervous system.

Insects are equipped with both volatile (olfactory) and contact (taste) chemoreceptors. According to Dethier, the receptors are but slightly affected by the physiological state of the insect; therefore, the electrophysiological techniques permit one to avoid the ups and downs of "central excitatory state," such as was described by K. N. Saxena.

Dethier summarized the state of the art. The contact chemoreceptors are often discharged by chemicals of only one class or subclass, and there may be little or no spontaneous firing. By contrast, the olfactory receptors, in the terminology suggested by Schneider (1964), consist of "generalists" sensitive to many kinds of chemicals and "specialists" sensitive to individual chemicals such as the sex pheromones. In the absence of stimulation, the olfactory receptors usually show substantial levels of spontaneous firing. Each of the general olfactory receptors appears to have its own repertoire of sensitivities in terms of the molecules to which it will respond. The response, in turn, consists of either an increase or decrease in the rate of firing and changes in the latency of firing.

Dethier described recent efforts, both in the United States and abroad, to crack the code of the sensory receptors (Schoonhoven, 1968). The vast majority of receptors are sensitive to numerous chemicals. Moreover, as previously mentioned, the response of a particular receptor can consist of either an increase or decrease in firing. Therefore, it appears that the sensory information is coded in terms of the temporal and frequency patterning of discharge. Dethier suggested that if the insect brain can understand these messages, we too may hope to do so.

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RECOMMENDATIONS

Types of research programs on insect-plant interactions that would be appropriate for support under the auspices of the International Biological Program were considered in detail. Participants at the work conference agreed that it would not be desirable to select a limited number of research proposals and to propose principal investigators or institutions to conduct the research. It seemed more appropriate to recommend areas in the broad field of insect-plant interactions that need research attention and support. Many clearly defined project proposals for different physiological or biochemical investigations dealing with different insects, host plants, and chemicals, could be developed within the following research areas:

1. Isolation, identification, and structure determination of natural products

   a. Affecting behavior of insects and other related economically important pests and parasites;

   b. Affecting insect growth, metamorphosis, and reproduction;

      i. Nonhormonal agents,

      ii. Hormonal agents.

2. Mechanisms of action of those agents affecting insect development and behavior, including host plant resistance and specificity to insects.

3. Biosynthesis and metabolism of agents affecting insect development and behavior.

4. Organic synthesis of natural products, their analogues and antagonists; correlation of structure and biological activity.

5. Evaluation of the most promising agents that affect insect behavior and development in both laboratory and model field tests aimed at practical application, including methods of use.

   a. Biological potency, selectivity, and stability;

   b. Biological effect on other organisms; ecological impact.

To facilitate the development of a program as proposed, it was recommended that IBP establish an office with a competent individual and an advisory panel to facilitate, evaluate, and coordinate actions relating to the advancement of research and the practical application of agents of natural origin that affect insect development and behavior.

The participants at the conference recommended that IBP sponsor similar international work conferences in the future to permit scientists to continue the exchange of new information obtained in the important and rapidly developing field of insect-plant interactions. Such conferences should include discussions of plans for ongoing research and the identification of appropriate research projects for submittal to IBP and other research-funding agencies.

II REPORT SUMMARIES

It was not feasible to include a detailed report of all the information presented or of all the discussions by participants in the conference on insect-plant interactions. However, each participant submitted a summary describing the nature of the research, indicating the principal investigators, and presenting the research findings.

Biology and Biochemistry of Insect-Plant Relationships

Stanley D. Beck

A wide variety of problems pertaining to insect-plant relationships have been investigated in past years. Recently our work has emphasized problems mainly associated with the development of satisfactory meridic and holidic culturing media for a number of phytophagous species, with the investigation of specific nutritional requirements and some of the associated metabolic systems, and with the larval feeding behavior as it relates to both nutritional requirements and host-plant specificity.

Laboratory investigations of the pea aphid, *Acyrthosiphon pisum* (Harris), have included aspects of mineral requirements, amino acid requirements, and amino acid metabolism. Calcium and phosphate ionic ratios were found to influence reproduction, which was totally suppressed in the presence of relatively high dietary levels of calcium. Magnesium:potassium ratios were shown to influence nymphal growth rates. Optimum growth was promoted by Mg:K ratios approximating 1:5.

The pea aphid was shown to require a dietary source of cysteine as well as the classical ten essential amino acids. Cysteine and methionine were independently required, with no mutual sparing effects detectable. A metabolic study showed that the cystathionine synthetic pathway was apparently absent. The insect's cysteine requirement could be partially met by dietary compounds containing free sulfhydryl groups, e.g., homocysteine and dithiothreitol. Inorganic sulfate spared both methionine and cysteine, suggesting the involvement of the aphid's intracellular symbiotes. Transamination was also studied, using an aspartate aminotransferase system in which the amino transfer was from aspartate to alpha-ketoglutarate to yield glutamate and oxalacetate. High aminotransferase activity was found in a soluble form; no mitochondrial isozyme could be detected. The titer of aspartate aminotransferase activity declined sharply between the fifth and sixth days of the insect's 12-day nymphal growth period.

The effects of different dietary components on the feeding behavior of first-instar larvae of the cabbage looper, *Trichoplusia ni* (Hübner), were investigated. Larval feeding was found to be stimulated by proteins, sugars, wheat-germ oil, and inorganic salts. Ascorbic acid and cellulose deterred feeding. The phospholipid fraction of wheat germ oil stimulated feeding, and of the purified phospholipids tested, only the plant lecithins were found to be feeding stimulants. The dietary inorganic salt mixture stimulated feeding when incorporated in the complex meridic diet, but not in the absence of other nutrients. The salt mixture was found to synergize the stimulatory effect of wheat-germ oil. The synergism was shown to be attributable to the feeding stimulation produced by the combination of potassium ions and the neutral lipid fraction of wheat-germ oil. The optimal concentration of potassium was found to be 0.1 M, and the synergistic effect was reduced when the anion was dihydrogen phosphate.

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Natural and Synthetic Compounds with Juvenile Hormone Activity

William S. Bowers

A wide variety of compounds have been found to duplicate the biochemical and physiological action of the hormones of the insect corpora allata. We are attempting to study the structure-function relationships of these compounds in as many physiological systems as possible for morphogenetic, prothoracotropic, and gonadotropic effects and their involvement in lipid metabolism and diapause.

Although we have shown that several open-chain sesquiterpene epoxides and straight-chain saturated aliphatic ethers have strong morphogenetic and gonadotropic activity, we have also demonstrated that certain cyclic sesquiterpenes have morphogenetic effects on insects through our isolation and identification of juvabione from balsam-fir wood.

We have been able to terminate diapause in several species of adult Hemiptera, such as Pyrrhocoris apterus and the boxelder bug, Leptocoris trivittatus (Say), with our synthetic juvenile hormone analogue trans, trans 10,11-epoxyfarnesenic acid methyl ester in the same manner as we have reported for certain Coleoptera.

We have found that a number of common insecticide synergists have juvenile hormone activity in the Tenebrio genitalia test and on the large milkweed bug. Of these, sesoxane, piperonyl butoxide, and propyl 2-propynyl phenylphosphonate were active at submicrogram levels, whereas MGK 264, tropital, and Bucorpolate were active at microgram levels. Synthesis of farnesyl piperonyl ether and 10,11-epoxy farnesyl piperonyl ether resulted in compounds with very high juvenile hormone activity in the same assays. By contrast, the naturally occurring synergists sesamin and sesamolin were only moderately active.

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Electrophysiological Studies of Food-Plant Relationships of Lepidopterous Larvae

Vincent G. Dethier

One of the fundamental relationships between insects and plants involves the feeding behavior of phytophagous insects. The initial reaction in this relationship involves the response of the chemical sense organs. In lepidopterous larvae both olfaction and taste play an important role in food-plant preference and in the initiation of feeding in general. Our laboratories have been concerned primarily with monitoring by electrophysiological techniques the input of sense organs in response to various plant odors and tastes in attempting to understand the sensory code. The action spectra and specificities of the receptor cells are partially understood.

Temperature, relative humidity, and olfactory and gustatory receptors have been studied in several species of lepidopterous larvae. Evidence suggests that T and RH senses together enable caterpillars to assess the water balance status of leaves. Olfactory receptors code information about odors of a plant in terms of frequency and temporal patterns of firing of receptors with overlapping but not congruent action spectra. Analyses of maxillary taste receptors still reveal no comprehensible code.

Caterpillars can "learn" to modify plant preferences, but it is not know whether the neural modification occurs in the sense organs themselves or in integrating mechanisms in the CNS.

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Insect Exocrines

Thomas Eisner

Jerrold Meinwald

Our chief concern has been with the defensive secretions and other exocrines of terrestrial arthropods. Attempts to isolate and identify the active components and, in some cases, to elucidate their biosynthesis are being coupled with biological studies of their function, mode of action, and means of production and distribution.

A variety of substances have so far been categorized. They include hydrocarbons, carboxylic acids, aldehydes, and ketones, phenols, benzoquinones, as well as more complex structures such as a cyclopentanoid monoterpene, an allenic sesquiterpene, a polyacetylenic acid, and quinazolinones.

Biosynthetic studies have shown that the compounds are produced by the arthropods themselves (certainly in the case of quinones and monoterpenes). In other cases, the active constituents bear close similarity to compounds available in the diet and may be exogenous in origin.

Studies on the anatomy of the defensive glands have revealed some highly refined mechanisms of toxicant production and have shed light on the means by which living cells produce poisons without poisoning themselves. The secretions are generally effective against arthropod and vertebrate predators and, as a rule, act as topical irritants. Some are neurotoxic or otherwise systemically effective.

Parallel studies are being conducted of sex pheromones and of the "aphrodisiacs" of danaid butterflies, in particular. The characterized components include long-chain acetate esters, a sesquiterpenoid diol, and a methyl pyrrolizidinone. Behavioral studies on the specific function of the components are being done in conjunction with investigations (in collaboration with D. Schneider, Seewiesen) of their neurophysiological mode of action.

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Effect of Juvenile Hormone and Ecdysterone on Rectal Pad Development during Adult Development of *Hyalophora cecropia*

Lawrence I. Gilbert

K. Judy

When up to 5 μg/g of ecdysterone was injected into brainless cecropia pupae, they molted into normal adults and had normal rectal development. Injection of about 10 μg/g resulted in adult development with incomplete pigmentation of the scales. Massive injections of up to 50 μg/g resulted in the development of "monsters" with pupal-like antennae and few or no scales. The rectal pads were completely abnormal, with six longitudinal folds of the pupal rectal pouch as opposed to about 300 rectal pads in the normal adult. This overdose of ecdysterone prevented the normal segregation of cells and resulted in a condition characteristic of the primitive insects.

When juvenile hormone was injected into cecropia pupae, the amount of juvenilization was reflected in the differentiation of the rectal glands. The mitotic activity of the epithelial cells appeared to be inhibited by juvenile hormone and the radial cells were most sensitive. With large doses, the cortical cells of the rectal pads failed to differentiate. Lower doses of juvenile hormone allowed segregation of these cells, which then became polymorphic and likely polyploid.

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The bibliography that follows the next summary applies to the above summary also.

Effects of Juvenile Hormone on *Sarcophaga bullata*

Lawrence I. Gilbert

U. S. Srivastava

There have been no reports dealing with the effects of juvenile hormone or analogues on the higher flies. This has led to informal speculation that they possess a different juvenile hormone, which is of great interest to developmental biologists, since *Drosophila* is the only animal about which enough genetics is known to conduct modern studies on the molecular genetics of differentiation. We have studied the effects of *dl* juvenile hormone and the C_{17} methyl ester on *Sarcophaga*. Injection of 2,000 to 2,500 Tenebrio Units (TU) into young third-instar larvae resulted in an average delay of three days in puparium formation, and those that did pupate did not assume the typical shortened form. Many of these were arrested in their development at the third day of pupal-adult development. True juvenile hormone effects were obtained when juvenile hormone was applied to pupae within the puparium. Application of about 2,000 TU to the abdomens of young pupae resulted in 75 percent of the animals developing into pupal-adult intermediates. Usually the head and thorax were completely adult and the abdomen was pupal. The fat body in the abdominal region was also pupal. Histological studies showed that the epidermal cells of the pupa secreted a second pupal cuticle and was without doubt a true juvenile hormone effect. The age of the test pupa is critical and one can obtain some effects on the thorax and head if juvenile hormone is applied early enough. Similar effects were obtained with the mixture of chlorinated farnesenic acid reported by Law *et al.* (1966). Good effects can be obtained with 0.04 μl of this crude material. Thus, the higher Diptera do respond to juvenile hormone, and we now have a unique tool for the study of differentiation in flies.

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Chemical Components in the Cotton Plant and Their Effect on Boll Weevil Behavior

Paul A. Hedin

This laboratory has been working on the identification of components in the cotton plant that attract the boll weevil and elicit its feeding activity. In conjunction with this, the essential oils of several alternate host plants, all in the family Malvaceae, are being examined to determine whether they contain the same or related attractive components as cotton. Studies are also under way to identify the sex attractant biosynthesized by the male. This work is noteworthy in that the frass is attractive and a dietary requirement for cotton plant material has been demonstrated.

With respect to the feeding stimulant, isolational studies led to the conclusion that no single plant compound evokes a full response. Feeding activity could be elicited with each of a series of successive solvent extracts of increasing polarity. Subsequent fractionation implicated several compound classes, but activity was decreased or even disappeared with the isolation of pure components. Nevertheless, at least 18 compounds identified as present in cotton and representing several classes were shown to elicit appreciable responses.

Since recombination of fractions or fortification of fractions by sugars and buffers often rejuvenated part of the activity, efforts were directed to formulate feeding active mixtures from known cotton constituents, common metabolites, and primary mammalian odor and flavor compounds. Synthetic mixtures were formulated that elicited greater puncturing than did cottonseed oil and equivalent puncturing with that of aqueous extracts.

Since cotton distillates, expressed juices, and freeze-dehydrated vapor concentrates had previously been demonstrated to attract the boll weevil, large-scale collections were accomplished by distilling 3 tons of cotton squares. One pound of essential oils was harvested for chemical work. Column chromatographic separation of the oil yielded hydrocarbon, midpolar, and polar fractions. Twelve terpene hydrocarbons and eight sesquiterpene hydrocarbons were identified and comprised nearly all of the nonpolar fraction. At least three sesquiterpene hydrocarbons gave full laboratory bioassay responses while two monoterpene hydrocarbons gave partial responses. Fourteen carbonyl compounds, comprising 1.4 percent of the total oil, were not attractive. Work is still in progress on the most polar fraction. To date, a new sesquiterpene alcohol comprising 6 percent of the total oil, beta-bisabol, has been isolated that gives a 60-80 percent bioassay response. Five other compounds, all of which appear to be sesquiterpene alcohols or esters and that give responses as good as or better than beta-bisabolol have been isolated. In addition, ten C_5-C_9 alcohols have been isolated, but they do not show appreciable activity.

Studies on the attractivity of several alternate host plants of the family Malvaceae are in the initial phase. Comparison of gas chromatographic profiles with that of cotton shows the presence of some attractive components and the absence of others. Differences in the relative concentrations of individuals and classes are also apparent and may explain differences in insect behavior to these hosts.

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Investigation of Insect Hormones

Peter Hocks

Our work in this field is concerned with the insect molting hormone ecdysone, the 20-hydroxyecdysone, and some further synthetic substances active in the *Calliphora* assay. We are also actively working on the synthesis of *juvenile* hormone and analogues.

The following research work was done, is in progress, or is planned:

A. Ecdysones

1. Isolations

a. Ecdysone was isolated from pupae of the silkworm *Bombyx mori* (L.) according to Karlson *et al*. (1963).

b. From the same origin the 20-hydroxyecdysone (crustecdysone) was isolated and its structure elucidated (Hocks and Wiechert, 1966). This substance proved to be identical with β-ecdysone (Hocks *et al*., 1967) and with the 20-hydroxyecdysone isolated from plants (Galbraith *et al*., 1967).

c. Extracts from various plants were submitted to the *Calliphora* assay and were found to be highly active. The plants were as follows: *Dryopteris* sp. and *Polypodium vulgare* L. (Polypodiaceae); *Vitex megapotamica* (Spreng.) Moldenke (Verbenaceae); *Achyranthes aspera* L. (Amaranthaceae); *Solanum dulcamara* L. (Solanaceae); *Agave lophantha* Schiede (Agavaceae); and *Digitalis lanata* Ehrh. (Scrophulariaceae). Only *Vitex megapotamica* was further investigated and 20-hydroxyecdysone was isolated (Rimpler and Schulz, 1967).

2. Synthetic work

a. Starting from readily available steroids, workers synthesized ecdysone by two routes (Schering AG, Berlin, Germany; Hoffmann-LaRoche u. Co. AG, Basle, Switzerland) (Kerb *et al*., 1966; Furlenmeier *et al*., 1967).

b. Some further ecdysone-homologues highly active in insect metamorphosis were synthesized (Hocks *et al*., 1966).

3. Biological evaluation

Synthetic ecdysone is under investigation in various pharmacological tests for possible biological effects.

B. Juvenile hormone

1. Synthetic work

a. The dihydrochloride of methylfarnesoate was synthesized according to the methods described by Romaňuk *et al*. (1967).

b. Juvenile hormone is synthesized according to Dahm et al. (1967).

c. It is planned to prepare some analogous substances by variation of the synthesis of juvenile hormone.

2. Biological evaluation

Juvenile hormone active substances are being screened for their possible use in pest control.

a. The dihydrochloride of methylfarnesoate was tested by topical or oral application to a series of insect species and their different instars: Diptera (*Musca domestica* L., *Calliphora vicina* Robineau-Desvoidy), *Drosophila melanogaster* Meigen, Lepidoptera (*Porthetria dispar* (L.), *Plutella xylostella* (L.)), Coleoptera (*Sitophilus granarius* (L.), *Tenebrio molitor* (L.)), and Orthoptera (*Carausrus morosus* Brunner, *Periplaneta americana* (L.), *Blatella germanica* (L.)).

b. It is planned to examine the biological effects of further synthetic substances with juvenile hormone activity on the test animals previously mentioned and some additional ones.

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Natural Products Chemistry

D. H. S. Horn

During the past 15-20 years, our laboratory has been engaged in an extensive program of research on the chemistry of natural products. There have been two main interests, one concerned with the constituents of plants toxic to grazing animals and the other with a survey of Australian and New Guinea plants for substances of potential value to the pharmaceutical industry. In addition, a number of projects of biological significance in such diverse fields as plant-leaf waxes, wool wax, beeswax, root exudates, plant oestrogens, and antibiotics have been undertaken.

More recently, because of the growing problem of insect and tick control in Australia, we have started a program of research on synthetic insecticides and on natural products that play an important role in regulating insect growth and development.

Our main interest in the latter field has been in the elucidation of the structure of molting hormones of insects and crustaceans and in the determination of their mode of biosynthesis. Initial studies provided a structure for crustecdysone, the main molting hormone of crayfish and the silkworm, Antheraea pernyi Guérin-Méneville. Surprisingly, ecdysone was not detected in these extracts. A tentative structure (2-deoxycrustecdysone) has been proposed for a second hormone, deoxycrustecdysone, that is also present in extracts of crayfish but in much smaller amount. A good deal of effort was devoted to the working out of the structure of this second molting hormone because it was expected to provide a further clue to the biosynthesis of the molting hormones. Further studies are in progress to isolate less polar hormone precursors, particularly from crayfish extracts.

The discovery by K. Nakanishi of compounds with molting hormone activity in Podocarpus nakaii led us to investigate the steroids of Podocarpus species indigenous to Australia. We hope these studies will lead to the isolation and identification of substances that are precursors of ecdysone in plants and insects. This work has so far provided a good source of crustecdysone, a number of substances of mainly undetermined structure, and podecdysone, considered to be 24-ethylcrustecdysone, which is probably biosynthesized from β-sitosterol. Podecdysone is as active as crustecdysone in the Calliphora bioassay. It is thus of particular interest because Calliphora are reported to be unable to develop to maturity on a diet containing β-sitosterol as the only sterol.

Crustecdysone is also being used as a starting point for the synthesis of ecdysone analogues, and recently the synthesis of an isomer of ecdysone (22-deoxy-20R-hydroxyecdysone) was accomplished.

Some work is also in progress to elucidate the structure of ecdysones of insects (e.g., Calliphora) and crustaceans (e.g., Callinectes sapidus) at different stages of development. Recent studies have unexpectedly shown that crustecdysone is the only hormone present in considerable amount in _Calliphora stygia_ (F.) during pupation and imaginal development.

A study is being made of the biological functions of the ecdysones. A preliminary study of the short-term effects of crustecdysone in _Calliphora_ has shown that this compound at certain times stimulates protein and ribonucleic acid synthesis.

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Some Population Aspects of Plants in Insect Suppression

Carl B. Huffaker

This conference has concerned itself with the chemistry involved physiologically in suppression of plants and of insects by plants. Plants, however, are also important aspects of interaction in the suppression of insects at the population level, and although this interaction is rather foreign to the main theme, we might give some thought to it.

1. Alternate plants serve as essential hosts of insects attacking major economic plants or ones serving as vectors of disease. Removal or control of key alternate plant reservoirs, however far away, may thus alleviate the problem. Use of competitive displacement of the weed hosts of the beet leafhopper (*Circulifer tenellus* (Baker) was shown to have merit by Piemeisel and Chamberlin (1936) and Piemeisel (1945). Flushing, alternate drying, and impounding of reservoir shorelines has been shown to be important in suppression of vegetation essential to mosquitoes, snails, and other vectors of disease. Related vegetation control is a key to control of tsetse flies in Africa. Some kinds of vegetation are inimical to such vectors and others are conducive to them. The nature of the inimical features may or may not be chemical. Study on this point is needed. Displacement of one sort by another is an obvious means of control and may often be achieved by management practices.

2. Essential alternate hosts of the predatory and parasitic enemies of many crop pests are found on alternate vegetation--often on certain weedy species. Maintenance of such essential plants close to the crops concerned at critical times may permit good control of the crop pests by the respective enemy species. Doutt and Nakata (1965) showed that the planting of small patches of evergreen blackberries near grape vineyards (that have no wild blackberry hosts at hand) in California supports the essential overwintering host leafhopper (*Dikrella*) of the specific grape leafhopper parasite (of *Erythroneura*) and control by the egg parasite is thus made possible.

3. Nectar plants, interplanted or adjacent to a crop threatened by a serious pest, may also furnish foods essential to key species of parasites or predators of the pest species. These materials or their substitutes also may be artificially dispersed in the crop (K. S. Hagen, unpublished data).

4. There are interrelations between plant chemistry and the interaction population genetics structure. Populations of insects may need both "pioneers" and "followers." The total ecology may be such as to maintain a necessary balance between types tending in these alternative directions, and the reactions of such individuals to plant chemicals, or other key behavioral factors may be quite different. In any event, there may be a strong interplay between the shifting genetic structure of a population and the susceptibility or concurrent availability of different populations of utilizable host plants.

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Behavioral and Chemosensory Background of Host Specificity in Phytophagous Insects

Tibor Jermy

At the Hungarian Research Institute for Plant Protection, Budapest, research on insect-plant relationships for the past ten years has been directed toward the nature of chemical stimuli determining the host range in phytophagous insects.

From the results obtained so far the following conclusions on food specificity can be drawn:

1. Narrower food specialization is connected with higher sensitivity of chemoreceptors (at least in chewing insects) to inhibitory stimuli present in the nonhost plants.

2. The behavioral effect of specific phagostimulants that are supposed to be present in the host plants is usually much weaker than the effect of inhibitors. Therefore, feeding of a highly specialized insect can be inhibited even on its most preferred host plants by applying the specific substances of most nonhost plants. It was also found that organic and inorganic compounds of very different molecular structures can inhibit feeding of a given insect.

3. The botanical distribution of strong feeding inhibitors is very uneven among the plant families. The species of some families are strongly rejected by a series of insects tested, while the plants of other families are generally much less rejective.

4. The sensitivity of the chemoreceptors to inhibitory stimuli makes it possible to use antifeeding substances (both natural and synthetic) for plant protection purposes. This has also been proved in field experiments. It has been suggested that an ideal antifeedant for practical use should be persistent enough to protect the plant for a reasonable period of time, should be able to penetrate and distribute in the plant (systemic compound), and should be harmless to all organisms except the insect pest.

Experiments carried out by F. E. Hanson, V. G. Dethier, and the author at the University of Pennsylvania have shown that the potential host range of an oligophagous or polyphagous insect can be restricted by induced preference, i.e., preference to previously eaten food. This signifies that even changes of food quality within the innate limits of acceptability can influence feeding rate.

In connection with the oviposition behavior, it has been found that the supposedly specific oviposition stimuli can be masked by inhibitory stimuli, e.g., as in bruchid beetles.

The following projects are in progress:

1. Food selection behavior in chewing and sucking insects (receptors and nature of stimuli involved),

2. Oviposition behavior of insect pests showing specific oviposition habit (receptors and nature of stimuli involved),

3. Bioassays with compounds for possible use as antifeedants or oviposition inhibitors, and

4. Electrophysiological studies on chemoreceptors involved in host selection (in collaboration with the Biological Research Institute of the Hungarian Academy of Sciences, Tihany).

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Effects of Ecdysones and Analogues on Insect Development and Reproduction

John N. Kaplanis

William E. Robbins

The insect ecdysones (a-ecdysone and 20-hydroxyecdysone) and about 10 synthetic analogues were examined for growth inhibition in a number of insects. Results in growth inhibition in an insect species would serve as a basis for developing and standardizing assays that would be used (1) to search, isolate, and identify other growth inhibitors; (2) to correlate structure with biological activity of naturally occurring and synthetic compounds; and (3) to study the physiological action and metabolism of the ecdysones and related steroids.

The insect systems currently used for measuring growth inhibition are the tobacco hornworm, Manduca sexta (Johannson); confused flour beetle, Tribolium confusum Jacquelin duVal; house fly, Musca domestica L.; German cockroach, Blattella germanica (L.); and firebrat, Thermobia domestica (Packard). These were selected because they are readily reared on diets that provide for optimum growth, development, and reproduction, and because of their diversity in relation to orders, types of metamorphosis, and general patterns of sterol utilization and metabolism.

Certain synthetic analogues, including Δ^7-5β-cholestene-2β,3β,14α-triol-6-one, were found to disrupt larval growth and metamorphosis when ingested. In some insects, the analogues gave almost complete mortality or inhibition of development when present in the diets in the ppm range. The triol mentioned above was also found to inhibit ovarian development and reproduction when fed to the house fly or to the confused flour beetle, suggesting these compounds as potential insect chemosterilants. The insect ecdysones were found to have little if any inhibitory activity in the larval growth tests. The deleterious effects of the synthetic analogues were often found to be associated with molting and morphogenesis. This, taken with the finding that the corresponding 5α-analogues (which were hormonally inactive in the house fly assay) were not inhibitors of growth and development, indicates the effects of these 5β-analogues were related to their hormonal activity.

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Host Plant Resistance to the European Corn Borer

Jerome A. Klun

The compound 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (DIMBOA) is a chemical factor in the resistance of dent corn to the European corn borer. Quantitative analysis of dried whorl corn tissue for the benzoxazolinone degradation product of DIMBOA can be used as a chemical indicator of level of host plant resistance in single cross strains and inbred strains of corn.

A study of the concentration changes of DIMBOA in five inbred strains of corn from germination to pollen shedding showed that highest concentrations of DIMBOA occur in the plumule and radical of the germinating seed. Biosynthesis takes place throughout plant development; however, over all whole plant concentrations decrease as the plant matures. Concentrations were generally highest in plant roots followed in decreasing order of concentration by the stalk, whorl, and leaves. Concentrations in these plant parts were in turn different for each inbred studied. The high concentration of DIMBOA found in seedling corn can account for the apparent resistance of young corn plants to the European corn borer. Those strains of corn that characteristically maintain high concentrations of the compound at later whorl stages of plant development are resistant to the borer, while those that do not become susceptible to borer attack. The resistance of inbred B49 to the second-brood borer at the pollen-shedding stage of plant development can be attributed to DIMBOA, whereas the resistance of inbred B52 is due to unidentified antibiotic factors.

Research is in progress to isolate and characterize the chemical factor (or factors) responsible for the resistance of B52 to the second-brood borer. In addition, studies are underway to determine the mechanism of first-brood resistance in terms of larval behavior on resistant and susceptible plants and the association of this behavior with concentration distribution of DIMBOA in the two types of plants.

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Development of Cotton Plants Resistant to *Heliothis* spp. and Other Cotton Insects

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Basic and applied research at the Cotton Insects Research Laboratory, Brownsville, Texas, is directed toward utilizing morphological plant characters and naturally occurring plant substances as sources of resistance to Heliothis spp. attacking cotton.

Morphological Characters

The extrafloral nectaries from Gossypium hirsutum strains were removed by transference of the nectariless character from G. tomentosum. The elimination of the nectaries, thereby limiting the adult food, resulted in a significant decrease in longevity and fecundity of Heliothis spp. The absence of trichomes from the vegetative portions of G. hirsutum resulted in at least 50 percent decrease in oviposition by the bollworm, Heliothis zea (Boddie), and the tobacco budworm, H. virescens (F.). The glabrous surfaces provide an unfavorable oviposition site and, in addition, the eggs do not readily adhere to these surfaces. The effectiveness of this character in reducing Heliothis populations has been demonstrated under field conditions.

Plant Substances

Gossypol and related compounds have been found to inhibit larval growth. Experimental lines have been developed that effectively limit populations of H. zea and H. virescens. Several flavonoid compounds have been shown to inhibit larval growth whereas other closely related compounds have been found to act as growth stimulants. Fruiting forms from wild races of G. hirsutum are bioassayed to determine if any antibiotic compounds are present. Extracts from the promising lines are then incorporated into the wheat-germ-casein diet and their effects on larval development are studied. The active ingredients are then characterized and isolated by various analytical methods. Tests are conducted to determine the presence of compounds in various plant parts and different species of host plants.

Olfactometer tests are conducted on extracts from various plant parts and assayed against adults of Heliothis spp. and pink bollworm, Pectinophora gossypiella (Saunders). An ethanol extract from cotton has been found that is attractive to pink bollworm females only.

Water extracts from cotton squares and ethanol extracts of cotton leaves have been found to act as oviposition stimulants on the pink bollworm female. A water-soluble extract from crude cottonseed oil and cottonseed meats acts as a feeding stimulant on Heliothis larvae.

Field observations and laboratory studies have indicated that the boll weevil, Anthonomus grandis Boheman, may have been associated with Hampea rovirosae since antiquity. This dioecious plant may have developed a unique resistance mechanism, since only the buds produced on male trees are susceptible to attack by the boll weevil.

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Biologically Active Substances in Cotton and Related Plants That Affect Boll Weevil Behavior and Development

Fowden G. Maxwell

Since the official opening in early 1962 of the U.S. Department of Agriculture Boll Weevil Research Laboratory, State College, Mississippi, the following substances that affect the boll weevil, *Anthonomus grandis* Boheman, have been found and characterized in cotton and certain related plants:

Arrestant and Feeding Stimulant

Extracted from cotton squares, with the main activity associated with water extract, this substance stimulates weevils to feed on foreign material such as beans, filter paper, cork, and meat. It is present in highest concentrations in buds, cottonseed, and cottonseed oil, but is found in decreasing amounts in leaves, stems, and roots of the cotton plant. Cottonseed and cottonseed oil serve as major crude sources for feeding stimulant for bait formulations. Feeding stimulant and arrestant varies in concentrations between species and between some varieties of cotton, but screening for reduced feeding stimulant has not been fruitful because a large reduction is needed to be significant under field conditions. Chemical identification work has indicated that a number of chemicals are probably involved, which has also discouraged this approach.

Boll Weevil Plant Attractant

This substance was first found in chloroform extract of defrosted water from lyophilizer utilized in drying cotton buds for antibiosis studies. Because of problems in bioassay, very little work has been done toward screening or utilization. The chemistry section is engaged in identification work, and there are possibilities that a screening technique using gas chromatography can be worked out for determining concentration differences between different lines and species of cotton.

Boll Weevil Repellent

This was found to be present in plant attractant extract. Removal improves effectiveness of plant attractant. Initial work suggests, but has not proved, that an attractant:repellent ratio may exist in cotton and other hosts of the boll weevil that helps determine the initial degree of preference exhibited by the insect in the selection process. No chemical work has been initiated to identify the substance (or substances).

Oviposition Suppression Factor

This factor was originally found through screening of cotton lines for reduced oviposition by the boll weevil. Sources were two *Gossypium barbadense* stocks: Russian Sea Island and S. I. Seaberry. The factor has not been extracted nor has it been determined positively whether the reduced oviposition results from a suppressor or whether the effect may be due to a deficiency or lack of oviposition stimulant(s). Nutrition is apparently not involved. The factor is inherited and original crosses were made to commercial types in 1963. A breeding program is currently in an advanced stage with recovery of resistance of 40-50 percent in a hirsutum type. Thus, we have been able to utilize source effectively in our breeding program without knowing the chemical basis of the reduction in oviposition.

We are combining oviposition suppression factor with frego bract, a morphological source of resistance, in the hope of increasing resistance over that expressed by either source alone.

Feeding Deterrent

A water-soluble substance was found in the calyx of Rose of Sharon, Hibiscus syriacus L., that is primarily responsible for nonpreference exhibited by the boll weevil for this plant in nature. Upon physical removal of the calyx from buds, boll weevils feed and oviposit normally. Weevils fail to locate the plant in nature as readily as they locate cotton because of reduced amounts of plant attractant and the presence of repellent factor(s) in volatiles.

A strong water-soluble feeding deterrent has also been demonstrated in tung by Hardee et al., of this laboratory.

No chemical identification work is under way with the feeding deterrents.

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Ecdysones in Plants

Koji Nakanishi

Great interest has been focused on the molting hormone, ecdysone, since it was first isolated in 1954. The structure was finally elucidated in 1965 and was synthesized subsequently by two groups, Syntex and Schering/Hoffman-LaRoche; more recently, Teikoku Hormone Company has also synthesized it. β-ecdysone and two other ecdysones have since been isolated in minute quantities from animals.

Since our isolation and structure determination of ponasterone A from *Podocarpus nakaii* and the independent isolation by Takemoto and co-workers of ecdysterone and inokosterone from *Achyranthes fauriei*--the first ecdysones from plant sources--the number of phytoecdysones has been increasing rapidly. Thus far, 14 have been characterized. The structural features common to all of these steroids are an A/B ring *cis* oriented, a 14α-hydroxyl, a Δ^7-double bond, and a 6-keto group. In *P. nakaii*, as in other plants, the yields of phytoecdysones are *very high*. The screening and isolation of active compounds from plants has been greatly facilitated by a rapid bioassay and a combination of preparative TLC (boric acid) and liquid chromatography.

The Chilo dipping test, developed by the Takeda Company, affords a rapid and easy method of assaying molting activity. Final-instar insects are dipped for 10 sec in a methanol solution containing the compound to be tested. This is the first instance in which dipping has been effective in causing molting.

The various polyhydroxy steroids can be effectively separated by liquid chromatography in which a resin and the linear gradient technique are used. This technique, developed by M. Hori, Takeda Company, can in certain cases be applied directly to a crude plant extract.

A potential application of the ecdysones is illustrated as follows: When an ecdysone dosage of 0.5-1 μg is given to *Bombyx mori* after the fourth day of the fourth molting, 100 percent of the insects are induced to initiate spinning of cocoon after 12 hr. In certain cases, ecdysones also exhibit insecticidal activity. For example, when *Culex pipiens molestus* Forskal (mosquito, final-instar) is immersed in aqueous solution of 10 ppm of ponasterone A or 50 ppm of ecdysterone, 100 percent mortality is induced.

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Biological Basis of Insect Resistance in Crop Plants

Reginald H. Painter

At Kansas State University Experiment Station, 21 crop-plant varieties, more resistant to at least one insect than those previously grown, have been recommended and distributed to Kansas farmers. These include five different crops and seven different insects. Recommended varieties must be as good as those previously grown in most respects and superior in at least one. The effect of using resistant varieties as insect control is illustrated by the Hessian fly, once the worst pest of wheat, which was eliminated for more than 15 years over large areas in Kansas by the use of Hessian fly resistant wheat varieties.

Parallel to these practical studies, research has been carried out recently on the biological (as contrasted with biochemical) basis of resistance to the spotted alfalfa aphid and pea aphid in alfalfa. These concern the effect of the plant on the insect in regard to growth, length of life, and nymph production per female (antibiosis) or in regard to nonpreference or repellence, and the effect of the insect on the plant damage and tolerance to aphid attack. It is possible to select alfalfa clones with a high level of tolerance but little or no antibiosis or nonpreference. Such clones show the same degree of tolerance to spotted alfalfa aphid under either high or low temperatures, but the differences in speed of response between tolerant and nontolerant is increased at high temperatures. With most clones, tolerance is less when testing detached branches; with a few others there is greater tolerance in detached branches.

Alfalfa clones resistant to one aphid species usually are not resistant to the others unless the two kinds of resistance have been intentionally combined genetically and the population of plants reselected for resistance to both aphids. In some alfalfa clones the basis for resistance to the pea aphid is able to pass downward through the graft union into a susceptible clone, but in at least one example, this appears not to happen with a clone resistant to the spotted alfalfa aphid.

Clones differ in the kind of response the aphid gives after forced feeding on a particular plant. When confined on some alfalfa clones, aphids will starve to death rather than feed enough to sustain life, but when confined on other clones there appears to be an adverse nutritional effect on the aphid as measured by weight or by length of life.

Some alfalfa clones are resistant to the pea aphid or spotted alfalfa aphid at high temperatures but not at low; fewer clones are resistant at both high and low temperatures. Plants also differ in the susceptibility of parts; flowering parts and stems near flowering parts are often susceptible on a clone in which vegetative parts are highly resistant. Leaf petioles are less preferred by the pea aphid even on highly susceptible clones but are a quite satisfactory plant part of the same clones for rearing the same aphid.

Some clones are resistant to several biotypes of the pea aphid; others are susceptible, but one clone may be resistant to the Kansas population but susceptible to a biotype in Quebec, while the reverse is true for a different alfalfa clone.

As in other studies of insect resistance, there are a number of separate bases in the alfalfa crop for resistance to each aphid, all of which contribute (usually in combination) to give a variety resistance to one of the aphids. Each biological basis probably has a different genetic and biochemical mechanism so that no single biochemical test, however useful in understanding resistance, is likely to be of practical value in a search for resistance. Furthermore, those varieties with the greatest multiplicity of bases have the greatest practical value since such varieties are least likely to favor the selection of biotypes able to feed on them.

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Bark Beetle Manipulation with Natural and Synthetic Attractants

Gary B. Pitman

The basic objective of the Boyce Thompson Institute in Grass Valley for the last nine years has been the elucidation of mechanisms whereby certain *Dendroctonus* and *Ips* select and mass aggregate on their coniferous host. It is hoped that natural attractants or man-made compounds will be applied in a forest management scheme to manipulate and therefore regulate populations of our more serious scolytid pests.

The research thus far has consisted of three distinct but interrelated phases. They are identification and synthesis of attractant materials, investigation of host-insect interactions in relation to pheromone biosynthesis, and implementation of natural or synthetic attractants under actual field conditions.

Work in the first phase has resulted in the identification of *trans*-verbenol, a terpene alcohol, as the major attractive component of *Dendroctonus frontalis* Zimmerman, *D. ponderosae* Hopkins, and *D. brevicomis* LeConte. A tentative identification has been made in *D. jeffreyi* Hopkins. The ketone verbenone has been identified from the nonattractant-producing male of *D. frontalis* and *D. brevicomis*. Synthetic *trans*-verbenol has been bioassayed in the field and found capable of increasing a response by a factor of 10 to 12 times in *D. ponderosae*.

In studying the bark beetle-host plant interactions, it was found that the amount of moisture in the host plant material affects the insect's capacity to synthesize *trans*-verbenol. Log sections that had been permitted to desiccate for a short time were highly unsuitable to *D. ponderosae*. Standing living trees were the most suitable host material as a substrate for production of the beetle's aggregating pheromone. The capacity of the trees to produce oleoresin in response to the feeding activity of the invading female has been tentatively implicated in a cause-effect relationship.

Deployment of attractive material that had been manually infested with emergent females of *D. ponderosae* and *D. frontalis* has resulted in the establishment of principles important in population manipulation efforts. Through the deployment of crude attractants, *D. ponderosae* has been induced to concentrate in large numbers in predetermined and closely grouped host trees. Similarly, *D. frontalis* has been induced to attack highly resistant hosts, thus causing excessive mortality in a predefined population.

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Artificial Diet for Saturniid Silkworms

Lynn M. Riddiford

An artificial diet containing no leaf material has been developed for the rearing of *Hyalophora cecropia* (L.). It consists basically of Vander Zant-Adnisson wheat-germ diet with the following additions: the fungal inhibitors--formaldehyde, sorbic acid, and methyl-p-hydroxybenzoate; the antibiotics--aureomycin and kanamycin; and either cholesterol or β-sitosterol. One of these two sterols is necessary for 65-75 percent viability as compared with 10 percent or less on diets without these sterols. Although *H. cecropia* apparently do not require inositol, *Antheraea polyphemus* (Cramer) do. Also, the addition of linolenic acid or of linseed oil is essential for proper wing development in *A. polyphemus* but not in *H. cecropia*. The basic diet with minor variations also can be used for rearing *Samia cynthia* (Drury) and *Hyalophora gloveri* (Strecker). *Antheraea pernyi* Guérin-Méneville will not feed on the diet unless *cis*-3-hexenol is added.

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Oak Leaves and the Mating and Ovipositional Behavior of the Polyphemus Moth

Lynn M. Riddiford

A female polyphemus moth will not release her sex pheromone except in the presence of oak leaves, which emit *trans*-2-hexenal. In low concentration the vapors of *trans*-2-hexenal elicit the "calling" behavior, but in high concentration they elicit ovipositional behavior, even from virgin female moths. The vapors are received by the female antennae, possibly by a specific receptor protein. The internal relay system leading to the release of the pheromone requires an intact connection of the corpora cardiaca to the brain but not of the corpora allata.

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See bibliography on page 61.

Effects of Juvenile Hormone on Embryonic Determination

Lynn M. Riddiford

When juvenile hormone is applied to adult female insects (H. cecropia, Oncopeltus, Pyrrhocoris, Tenebrio), embryonic development is completely blocked at the blastoderm stage. However, once the eggs are oviposited, partial larval differentiation cannot be prevented. Until the early germ-band stage, hatching can be blocked, but application later in embryonic development elicits effects only in larval life or at the time of metamorphosis. By applying juvenile hormone at specific times during the latter half of embryonic development, one can mimic the ability of juvenile hormone when applied to last-instar larvae to inhibit metamorphosis. In H. cecropia, the turning-on of the pupal gene set can be affected by juvenile hormone until the time of spinning. After this time, juvenile hormone can no longer interfere with the formation of a pupa but can prevent the obligatory diapause so characteristic of the cecropia silkworm. Apparently, the presence of juvenile hormone prevents the inactivation of the freshly pupated brain. In the bugs Oncopeltus and Pyrrhocoris, one can obtain sixth-instar nymphs either by juvenile hormone to the embryo or to the fifth instar. A preliminary autoradiographic study of the site of action of tritiated juvabione in two species, the insensitive Oncopeltus and the sensitive Pyrrhocoris, indicates a concentration of the hormone around the nuclear membrane only in the sensitive species.

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Research on Insect Hormones and Pheromones

Herbert A. Röller

Our research program on the juvenile hormone (JH) of insects was begun in September 1962. By 1965 we had developed procedures for isolating JH on a preparative scale from *Hyalophora cecropia*. In 1966, we identified the structure of the hormone, and the following year we synthesized its racemic form and initiated a broad program to determine the spectrum of biological effects it induces in different species. These accomplishments have made it possible to extend our studies on JH to a biochemical basis.

We propose to study the metabolism of JH in different developmental stages of various species of insects using tracer methods in *in vivo* and *in vitro* systems. Two aspects of the action of JH will be investigated in detail: its gonadotropic effect and its morphogenetic effect on a suitable epidermal system. From these studies we hope to be able to determine the primary site of action of JH and to identify discrete biochemical steps that lead to specific developmental processes. We will synthesize the labeled JH required for these studies as well as labeled precursors for investigation of the biosynthesis of JH. An attempt to solve the question of the *dl* isomerism of natural JH will be made. Our study of the range of biological properties of JH and JH-analogues will be continued. We also plan to investigate the question of possible structural diversification of JH during evolution.

The results obtained will be significant for our understanding of the mechanisms controlling the developmental processes of insects. In addition, they will contribute to our basic knowledge of growth, differentiation, and hormone action in general. There is also widespread interest in using JH-active compounds in a new approach to the control of insect pests.

Although the sex attractants of five species of Lepidoptera have been identified, a comparative study on the sex pheromones of a group of closely related species has never been reported. We have undertaken a broad comparative program of integrated chemical, biochemical, morphological, and behavioral studies on the sex pheromones of eight species of pyraloid moths: *Plodia interpunctella* (Hübner), *Ephestia elutella* (Hübner), *Anagasta kuehniella* (Zeller), *Cadra figulilella* (Gregson), *Cadra cautella* (Walker), *Galleria mellonella* (L.), *Achroia grisella* (F.), and *Aphomia gularis* (Zeller). To date, we have completed the purification, isolation, and identification of the sex attractant of *Galleria mellonella*, the greater wax moth, as *n*-undecanal.

For each of the species considered, we plan to determine the type of sex-pheromone-producing structures present. We will describe the events in the courtship and mating behavior of each species. We will elucidate the role of each of the sex pheromones involved by identifying the behavioral responses they release. On the basis of our behavioral studies, a quantitative bioassay for each pheromone will be devised. We will then collect, purify, isolate, and identify each of the sex pheromones and study the degree of their species-specificity. We will describe the structure of the pheromone-producing organs at the gross anatomical, histological, and ultrastructural levels and attempt to elucidate the mechanisms involved in pheromone production and secretion by histochemical and other techniques. Finally, we will attempt to study the biosynthetic pathways utilized for pheromone production in the different species.

In addition to their potential significance for evaluation of the evolutionary relationships within the family Pyraloidea, the results of this comparative study could shed light on the evolution of chemical communication systems in general. Knowledge gained about the sex attractants of these pest species may also be of value in developing effective control measures.

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Studies on Timber Beetles

Julius A. Rudinsky

At the Forest Insect Laboratory, Oregon State University, Corvallis, which lies in the center of the Douglas-fir region, research on insect-plant interactions is centered on the bark and timber beetles (Scolytidae) associated with the Douglas-fir beetle. Several genera are not only invading various parts of this important tree but are also affecting different physiological conditions. Among the timber or ambrosia beetles, three species may occur at high population levels: Trypodendron lineatum (Olivier), Gnathotrichus sulcatus (LeConte), and G. retusus (LeConte). Among the phloem-feeding beetles, Dendroctonus pseudotsugae Hopkins and Dryocoetes autographus (Ratzeburg) invade the trunk, and Pseudohylesinus nebulosus (LeConte) and Scolytus unispinotus LeConte invade the tops and larger branches of mature trees, whereas the two species of the genus Hylastes [nigrinus (Mannerheim) and ruber Swaine] invade the roots of Douglas fir. Opportunity is thus present for comparative behavioral studies of responses to the primary attractants as related to the host's physiological condition and to beetle-produced substances after a suitable host has been successfully invaded. Response appears to be related to the physiological stages of the adult beetles (sexually mature, before or after maturation feeding, virgin, mated, re-emerged, etc.) and corresponding anatomical and histological changes in internal organs (digestive system, reproductive system, Malpighian tubules, salivary glands, mycetangia in ambrosia beetles, etc.) on the one hand and to the host condition and environmental factors (light intensity, temperature, wind, etc.) on the other.

At present, we have a continuing National Science Foundation project, "Flight and Attraction of the Douglas-fir and Other Beetles Associated with the Douglas-fir Forests," and a National Institutes of Health project, "Chemical Basis for Attraction of Insects (Ambrosia Beetles)."

We have found that the Douglas-fir beetle first aggregates in response to host substances (i.e., oleoresin and terpenes: camphene, limonene, and alpha-pinene). Under endemic conditions, the beetles are attracted to physiologically weakened trees characterized by low oleoresin pressure and high internal water stress. Secondary attraction occurs to a pheromone found in the hindgut of the virgin female; this pheromone was extracted in solvent and chemical identification studies have been started. Similar primary and secondary attraction exists in both Pseudohylesinus nebulosus and Scolytus unispinosus and is related to different levels of water stress of the host as measured by a pressure bomb. Also, terpenes were found to be the substances aggregating the predators (Cleridae) of these scolytids. In contrast, Hylastes nigrinus, a root insect, is attracted to deteriorating phloem and, unlike the other scolytids, responds in numbers also to beta-pinene. Among ambrosia beetles, Trypodendron lineatum produces its pheromone only after mating and feeding on wood particles. Chemical studies of the pheromone have been started and the site of production is being studied by I. Schneider. Gnathotrichus species respond to geraniol and various fatty acids, and attraction is increased after beetles enter the host. In laboratory tests, most of the terpenes found in the Douglas fir act as repellent at concentrations above 1 percent, but are attractive at 0.1 percent and below to most of the insect species associated with Douglas fir.

For future research in Oregon, we plan further measurements of internal water stress, especially as related to attraction of the Douglas-fir beetle and associated scolytids, and further studies of response mechanisms in relation to the biology of the beetles. In addition, a comparative study of indigenous Central European bark beetles in introduced Douglas-fir forests is planned through cooperation with forest research institutes in West Germany and Czechoslovakia on my projected leave of absence.

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Factors Controlling Behavioral Responses of Insects to Plant Constituents

K. N. Saxena

An insect may show differences in its behavioral responses to a given plant constituent at different times. A plant constituent in a given concentration may serve as an attractant or feeding stimulant, or both, at one time but not at another. Factors controlling such differences in the behavioral responses of insects to plant constituents are being investigated.

These factors may be arranged in two categories: physiological condition of the insects and interaction of environmental stimuli with the stimuli emanating from plants. The role of these factors is being studied with reference to two types of insects: predominantly seed-feeding Heteroptera, e.g., *Dysdercus* and *Oxycarenus*, and sap-feeding Homoptera, e.g., *Empoasca* spp. Maximum information has been obtained for *Dysdercus*.

Dysdercus koenigii (F.) infests mainly malvaceous plants, particularly cotton and okra. On these plants, it feeds mostly on seeds in dehisced and dry fruits, and occasionally on the succulent parts like leaves and unripe fruits. The insect's preference for feeding on cotton plants is mainly due to the following types of substances: (1) A "leaf factor" in the steam distillate of the leaves, which serves as an olfactory attractant and incitant for the insect, and (2) the oil in the seeds, which is an olfactory as well as gustatory stimulant serving to attract, incite, and stimulate continuation of feeding. The water content of various parts of the plants and sugars also serve as stimuli for the insect.

The behavioral responses of the insect to the previously mentioned olfactory and gustatory stimuli are controlled by a number of its physiological conditions, of which the following have been examined: stage of postembryonic development, period of starvation, stage of molting cycle, ovarian development, rearing medium, and water content of the insect.

Of the various nonolfactory and nongustatory stimuli, water content of the plants also determines the insect's responses to even the olfactory and gustatory stimuli.

The interaction of the above factors determines the type of response the insect will show toward the constituents of plants at a given time.

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Some Research Activities Dealing with Insect Hormones

Howard A. Schneiderman

Our research group includes the following investigators working on insect hormones: André S. Meyer, Frantisek Sehnal, Kornath Madhavan, A. Krishnakumaran, Narayan G. Patel, Peter Bryant, Elisabeth Gateff, and Eugene Granger. Principal interests of our laboratory related to insect hormones are the following: (1) mode of action of insect hormones, (2) causal analysis of insect development and developmental genetics, (3) isolation and identification of juvenile hormones. Two matters will be discussed briefly.

First, we wish to report the isolation and structural identification of a second natural juvenile hormone from insects. In 1965 in collaboration with André Meyer and Lawrence Gilbert nearly pure preparations of two biologically active juvenile hormones were isolated from the cecropia silkworm. Purification was conducted using the mildest methods available and gas liquid chromatography was employed only as an analytical tool after purification. To identify the compounds Meyer employed nuclear magnetic resonance, paramagnetic resonance, and infrared spectroscopy. High-resolution mass spectra were provided by K. Biemann and his associates at Massachusetts Institute of Technology. The data revealed that the two juvenile hormones were homologues. One is identical to the compound recently isolated by H. Röller and accounts for about 80 percent of the juvenile hormone content of cecropia. The second juvenile hormone is a new compound, accounting for about 20 percent of the juvenile hormone content of cecropia. The two compounds have similar biological activity. The second juvenile hormone is the lower homologue of the first and has a methyl instead of an ethyl group at C_7, i.e., methyl 10-epoxy-7-methyl-3,11-dimethyl-2,6-tridecanoate. These results indicate that the juvenile hormone is not a single substance but, like the ecdysones, it consists of a class of related substances. They also provide independent confirmation of the structure of the juvenile hormone isolated by Röller.

Second, we wish to mention the importance of the cell cycle in hormonal sensitivity. Studies performed in collaboration with F. Sehnal, K. Madhavan, and E. Granger demonstrated that cells are most sensitive to ecdysone and to juvenile hormone at specific times in the cell cycle. At other times the cells are only slightly sensitive. These differences in sensitivity must be taken into account in practical programs employing hormone-based insecticides.

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Synthetic Studies on Insect Hormones

J. B. Siddall

Work on the synthesis of insect hormones began with the synthesis (Siddall et al., 1966) of the steroidal molting hormone α-ecdysone (I) and has continued with synthesis of possible biological precursors of the molting hormones (Figure 1). A study of the biological properties of α-ecdysone in mammals showed no significant classical activities besides sodium retention in ♂ rat by subcutaneous injection at 100 μg. Improvements in the chemical synthesis allowed distribution of requested samples (some 70 to date), making possible a considerably wider study of this hormone.

Synthetic methods were developed and applied to synthesis (Hüppi and Siddall, 1967) of the more complex β-ecdysone (II) (20-hydroxy-ecdysone) and recently (Hüppi and Siddall, 1968) to Ponasterone A (III). The latter plant extractive bears a remarkable resemblance to the arthropod hormones I and II. Proof of stereochemical identity of II with III at all 10 asymmetric centers was obtained by synthesis (Hüppi and Siddall, 1968) of II and III from a common immediate precursor.

Preparation of various analogues of the natural hormones I and II has not improved biological activity significantly. A joint synthesis (Siddall et al., 1967) and careful search for the 20-ketone (IV) in crustacean extracts by D. H. S. Horn suggests that IV is not a significant metabolite of II in crustaceans.

In more recent work, two novel routes to the possible biological precursors of ecdysis hormones were found. Reduction of the acetylenic alcohol (V) afforded the allene (VI), convertible to tritium labeled 22-desoxyecdysone. Hydrogenolysis of the intermediate VII afforded 25-desoxy-ecdysone in addition to natural α-ecdysone (I), both heavily labeled in the side chain only.

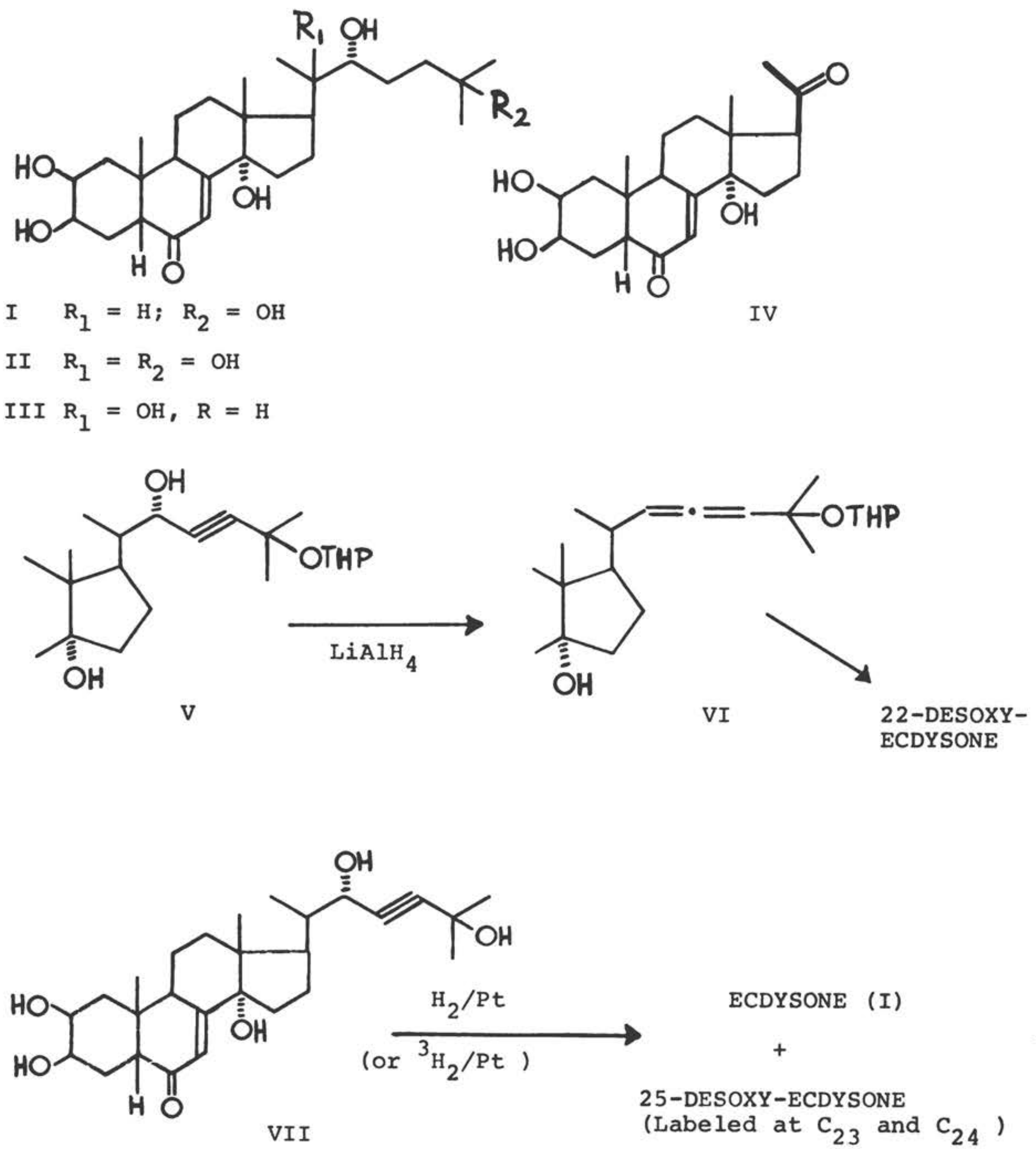

FIGURE 1 Synthesis of insect hormones and possible biological precursors.

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Terpenes and Insect Behavior

Robert M. Silverstein

Terpenes are ubiquitous in nature. Even at the early stage of development of insect chemistry, a number of terpenes have been identified and their functions elucidated. They have been implicated as alarm substances, defensive secretions, trail markers, feeding stimulants, feeding deterrents, growth hormones, and sex attractants.

The same terpenes found in insects are very often also found in plants. One would guess offhand that insects would derive their terpenes from plant terpenes; however, in the few cases studied, the insects make the terpenes from acetate.

In our work, we have isolated, identified, and synthesized three terpene alcohols that collectively comprise the attractant pheromone of a bark beetle, *Ips confusus*. In the field, *Ips confusus* beetles were attracted to a synthetic mixture of 3 mg of one compound and 1 mg of each of the other two compounds over a 6-hour period. *Ips latidens* (LeConte) was attracted when one of the compounds was omitted. This compound thus creates an attractant to *I. confusus*, but masks the attractant for *I. latidens*. Two bark beetle predators, *Enoclerus lecontei* (Wolcott) and *Temnochila virescens* (F.), also responded to the synthetic compounds.

Pheromone response in the genus *Ips* is strongest within the species, but interspecies responses occur; they parallel the natural species groups based on external anatomy.

A recent paper reports that *trans*-verbenol and verbenone are the main components of the female and male hindgut respectively of three species of *Dendroctonus*. *Trans*-verbenol was found to increase the attractiveness of a beetle-infested log to *D. ponderosae* in the field.

We found that the major attractant component in the frass of *D. brevicomis* was not a terpene but a novel bicyclic compound.

There is a continuum of host materials and insect-produced compounds that bark beetles use as attractants. At one extreme is a beetle, such as *Dendroctonus valens* LeConte, which is attracted to the oleoresin of a freshly cut tree and does not seem to produce a secondary attractant. At the other extreme is *Ips confusus*, which does not seem to respond to host material but relies completely on secondary attractant.

Specific Projects and Summary of Results

1. Bark beetles and defoliators of western forests. Supported by the U.S. Forest Service. Joint program with David L. Wood, Department of Entomology, University of California, Berkeley. We have isolated, identified, and synthesized the attractant pheromones of the bark beetles, *Ips confusus* and *Dendroctonus brevicomis*. Successful field tests have been carried out with

I. confusus, and field tests with D. brevicomis are scheduled for next month. We intend to isolate, identify, synthesize, and field test the pheromones of D. ponderosae (ponderosa pine), D. pseudotsugae (Douglas-fir) and Trypodendron lineatum (Douglas-fir), a tussock moth, and several other defoliators of economic importance. Preliminary studies on these insects have been carried out.

2. Dermestid beetles. Supported by the U.S. Department of Agriculture. Joint project with Wendell E. Burkholder, Department of Entomology, University of Wisconsin. We have isolated, identified, and synthesized the sex attractant of the black carpet beetle, Attagenus megatoma (F.). This compound has been successfully field tested. We are investigating Trogoderma inclusum LeConte and T. glabrum (Herbst).

3. Investigations to determine the mechanism of host preference in Agasicles. Supported by the U.S. Department of Agriculture. Joint study with John Simons, Stanford Research Institute. This study is in its early stages. Simons has developed a laboratory bioassay, and we hope to contribute to studies on the chemical basis of feeding stimulants and deterrents.

4. Pheromones of the leaf-cutting ant, Atta texana (Buckley). Support requested from the National Institutes of Health for joint study with John Moser, U.S. Forest Service, Pineville, Louisiana. Preliminary studies resulted in the identification of two alarm pheromones in the mandibular glands, and elucidation of the responses of a laboratory colony to these compounds. The trail-marking pheromone was found to contain at least two pheromones, one of which is volatile, the other nonvolatile.

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Natural and Synthetic Materials with Insect Hormone Activity

Karel Sláma

Most of the work is organized in cooperation with the Institute of Organic Chemistry and Biochemistry of the Czechoslovak Academy of Sciences. The general research project is based on isolation from natural sources of chemical substances with insect hormone activity or the synthesis of these substances. The aim is to find materials with potential pesticide effects that could be used in insect control.

Our studies were initiated after discovery of the presence of juvenile hormone analogues in American paper products (Sláma and Williams, 1965; 1966a; 1966b; Williams and Sláma, 1966). We succeeded in identifying one of the active principles responsible for juvenile hormone activity of balsam fir extracts and paper products, dehydrojuvabione (Černý et al., 1967a; 1967b). We have also isolated the most active principle with juvenile hormone activity from the preparation described by Law et al. (1966) and identified it as a methyl ester of 3,7,11-trimethyl-7,11-dichloro-2-dodecenic acid (Romaňuk et al., 1967). Further, we have prepared various other chlorinated derivatives of farnesenic acid (M. Romaňuk, K. Sláma, and F. Šorm, unpublished data), some of which appeared to be very potent juvenile hormone analogues with potential pesticide properties. In our search for new juvenile hormone analogues, we have discovered a group of related compounds derived from p-(1,5-dimethylhexyl) benzoic acid (Sláma et al., 1968), which appeared to be selective pesticides with juvenile hormone effects against cotton stainers (*Dysdercus*). We have found and described the juvenile hormone activity of some alkylethers derived from aliphatic β-hydroxy acids (Ratuský et al., 1968). During the period of juvenile hormone research, we have found many different terpenic materials with juvenile activity with completely different selective actions only on certain groups of insect species. The results will be published in the near future.

We have found that certain juvenile hormone analogues appear to be ideal sterilants of adult females of insects (Masner et al., in press). Using the system *Pyrrhocoris apterus*-methylfarnesoate dihydrochloride, we have demonstrated that doses of about 1 μg per spec. cause permanent female sterility. A new method has been elaborated in which the females are permanently sterilized by mating with males that had previously been contaminated by larger doses of the juvenile hormone analogue (Masner et al., 1968). We conclude that the sterility effects caused by these highly effective, nontoxic, selective analogues of juvenile hormone are most promising as a new approach to insect control.

Jizba et al. (1967a) discovered large amounts of ecdysterone in the roots of a firm *Polypodium* where they also found a new ecdysone analogue containing one hydroxyl group more than ecdysterone contains--Polypodin B. This compound is more active in biological assays than ecdysterone (Jizba et al., 1967b). There has been found a series of steroid compounds that inhibit the postecdysial hardening and sclerotization of the cuticle in some insects (Hora et al., 1966; Velgová et al., 1967). These compounds also inhibit development of insect larvae and cause sterility effects in adult Diptera.

In order to throw more light on the physiology of hormone action in insects, we have undertaken a thorough physiological analysis of the effects caused by synthetic juvenile hormone analogues and compared them with those caused by the hormone released from the corpora allata. We have also analyzed the principles of specificity of juvenile hormone analogues (Sláma, 1967), developed and improved the assays for juvenile hormone activity, and determined physiological effects of juvabione and dehydrojuvabione on various insects.

Further research will be directed toward the following projects: (1) finding the new juvenile hormone analogues, natural or synthetic, with potential pesticide use; (2) finding the physiological background and mode of action of juvenile hormone analogues; (3) finding the physiological conditions for insect sterility caused by juvenile hormone analogues; (4) grouping and classifying insect species according to their sensitivity to particular juvenile hormone analogues; and (5) conducting further research on physiology of action of ecdysone analogues.

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Host Factors in the Resistance or Susceptibility of Pines to Bark Beetles

Richard H. Smith

Properties of pines that cause them to attract or repel bark beetles, to kill or immobilize them, and to inhibit their feeding, oviposition, or brood development are being determined, and the genetic and ecologic control in the variations of these properties is being ascertained.

Laboratory studies suggested that the host specificity of bark beetles might be determined by their inability to tolerate xylem resin vapors of nonhost pine species; both mortality rate and sublethal feeding inhibition were used to measure this inability. In other tests, beetles did not respond equally to the various individual monoterpene constituents of a pine that they normally attack. Circumstantial evidence has been obtained in field tests to support this differential effect by comparing the monoterpenes of attacked and unattacked trees, by attracting natural populations of beetles to groups of trees with varying resin composition, and by various types of forced-attack experiments on pines and pine hybrids with different monoterpene composition of the xylem resin.

This work is being continued in an effort to determine the factors modifying the expression of resin quality.

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Investigations of the Mechanism of Dealkylation of Phytosterols in Insects and Inhibition of This Mechanism

James A. Svoboda

The utilization of phytosterols in insects through the process of dealkylation is being examined in depth. This biochemical conversion of plant sterols is being investigated in an attempt to chart the pathways of dealkylation. Desmosterol (24-dehydrocholesterol) has been positively shown by isolation and identification to be an intermediate in the biochemical pathway of the dealkylation of phytosterols such as β-sitosterol to cholesterol. This is the first known intermediate in this conversion. Other intermediates in this pathway are being examined, and positive identification of at least one other compound is nearly complete.

The effects of certain synthetic and naturally occurring compounds that inhibit the conversion of β-sitosterol to cholesterol are being investigated. Several compounds (including diazasterols and triparanol) known to inhibit the Δ^{24}-sterol reductase system in vertebrates were found to be quite active in the tobacco hornworm. We have also isolated and identified two other compounds (steroids) from natural sources that block the conversion of phytosterols to cholesterol. In a large percentage of insects fed certain hypocholesterolemic diazasterols (e.g., 20,25-diazacholesterol) plus phytosterol in the diet, a significant disruption of growth and development results. Hormonally mediated processes are adversely affected. As a result, fourth-instar prepupae and prepupal-pupal intermediates are formed in the hornworm, perhaps because of a reduced supply of hormone precursor, an accumulation of unusual metabolites, or involvement of the steroidal inhibitors themselves in normal sterol metabolic pathways.

Investigations of the enzyme systems and the subcellular sites of dealkylation are integral parts of our study of dealkylation in insects. These systems provide valuable tools for studying the effects of inhibitors and provide additional confirmation of *in vivo* studies.

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Synthesis of Structural Analogues of Ecdysone

Malcolm J. Thompson

A number of structural analogues of ecdysone that contained the 5β- or 5α-configuration have been prepared from cholesterol. The compounds are being examined in several insects to correlate the relationship of structure to molting hormone activity, to determine the minimum structural requirement for activity, and to serve as model compounds for developing biological assays. They are also being used as candidate compounds for evaluating physiological activities that affect growth, metamorphosis, or reproduction. Derivatives that make these ecdysone analogues more organosoluble are being examined for their effectiveness in penetrating the insect cuticle.

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Olfactory Influences of Plants on Behavior and Physiology of Phytophagous Insects

A. J. Thorsteinson

The effects of odorous food plant constituents (N-propyl disulfide and N-propyl mercaptan) on the behavior of larvae and adult females were investigated in our laboratory recently by Y. Matsumoto. He found that the larvae aggregate at the odor source and that the adult females are stimulated to probe with the ovipositor and to lay eggs in sand treated with the chemicals. Traps baited with the same compounds attracted the adult females in the field.

Some years ago we showed that adult female diamondback moths lay eggs much more densely on rugose plastic surfaces coated with allyl isothiocyanate, a characteristic constituent of its food plants. In current investigations we find that the influence of mustard oil is not limited to the oviposition behavior but actually stimulates ovarian development in adult moths that are in a prereproductive state, as many are several days after eclosion. This effect of the mustard oil simulates an influence of the host plant that was discovered in our laboratory a few months ago by R. Hillyer, who had previously observed a similar phenomenon in *Oscinella frit* (L.) in England. We have observed that food plants devoid of mustard oils have a distinctly weaker effect, if any, on ovarian development.

The novelty of these observations lies in the fact that the diamondback moth does not itself feed on the plant on which it lays its eggs, and it appears that we have demonstrated a physiological response to sensory inputs of a type usually associated only with behavioral responses. The adaptive significance of this phenomenon is clear when it is recalled that the diamondback moth is a prominent member of the list of insects observed to disperse great distances in large numbers.

If we rear larvae individually on high-quality food *ad libitum* we can produce adult females that are already in the reproductive state at eclosion. These moths lay their eggs rather readily even when there is no host-plant odor, seemingly contradicting our earlier results. This apparent anomaly can be explained, however, if we think of the olfactory stimulation of oviposition as the result of reduced kinesis of the moths whenever they encounter food-plant odor. More eggs would then be laid near the odor source because the moths spend more time there and, by a similar argument, the eggs would not be dispersed as they would if the moths moved about more. This same hypothesis was invoked some years ago to account for food-plant finding in the field and forest. Computer simulation of such goal seeking behavior without directional information is being investigated in our laboratory.

One of the early problems of plant-insect relations--the rationale of specificity and diversity of host plant species--is now ripe for renewed interest because of greatly increased interest in chemical plant taxonomy. We propose to take advantage of the resurgent activity in this field in our studies of insect-food plant relations.

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Biochemistry of 1, 4-Benzoxazinones Related to European Corn Borer Resistance in Maize

Carl L. Tipton

Several years ago A. I. Virtanen and co-workers, in a series of papers, described a cyclid hydroxamic acid that occurred in maize and wheat (Wahlroos and Virtanen, 1959), mostly as the glucoside, and a related compound that occurred in rye (Virtanen and Kietala, 1960). They also showed that injury of the plant tissues released a glucosidase that catalyzed the hydrolysis of the glucosides (Wahlroos and Virtanen, 1959; Virtanen and Kietala, 1960). The resulting ether-soluble aglucons are readily isolated from aqueous extracts of plant material. They showed further that the aglucons are converted quantitatively to benzoxazolinones upon heating in aqueous solution (Honkanen and Virtanen, 1961; Bredenberg et al., 1962). Because of a postulated relationship of cyclic hydroxamic acid content in maize to European corn borer resistance, we undertook the isolation of the compound from maize for bioassay purposes. In the course of this work some related compounds have been identified. We are also investigating the pathway of biosynthesis of these compounds in maize.

The aglucon isolated by Virtanen from maize is 2,4-dihydroxy-7-methoxy-1,4(2H)-benzoxazin-3-one (DIMBOA) (Wahlroos and Virtanen, 1959). This compound has been isolated in sufficient quantity for bioassay and has been found to be a powerful feeding deterrent for larvae of the European corn borer.

A homologous compound that lacks the 7-methoxy substituent was reported by Virtanen to occur in rye (Virtanen and Kietala, 1960). We have isolated this material in small quantities from maize seedlings. Evidence is accumulating that a third hydroxamic acid, found in some strains of maize, is a homologue with two methoxy substituents on the aromatic ring.

Isolation and purification of the glucosides has been more difficult than of the aglucons. An attempt to isolate the 2-0-glucosyl derivative of DIMBOA from field-grown plants resulted in the isolation, instead, of the closely related lactam, 2-0-glucosyl-7-methoxy-1,4(2H)-benzoxazin-3-one. Repetition of the isolation procedure reported by Wahlroos and Virtanen (1959) has yielded a mixture of DIMBOA glucoside and the lactam glucoside.

Riemann and Byerrum (1964) have shown that the carbon skeleton of DIMBOA can be derived from quinic acid, ribose, and the methyl group of methionine. We have shown that ^{15}N- and ^{14}C-labeled anthranilic acid are incorporated into DIMBOA and the corresponding lactam by seedlings. Preliminary data on the kinetics of incorporation of ^{14}C-anthranilic acid into these two compounds indicates the lactam may be the precursor of the hydroxamic acid. There is also evidence, from the feeding of ^{14}C-shikimic acid, that 3-hydroxyanthranilic acid may be an intermediate following anthranilic acid. Studies of the biosynthesis and reactions of these compounds are continuing, with the eventual goal of correlating biochemical information with data on the genetic control of benzoxazinone content in maize.

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Perspectives in Insect-Plant Interactions

Carroll M. Williams

The lesson of juvabione encourages the belief that some and perhaps all of the thousands of terpenoid esters that plants synthesize for no apparent reason may, in fact, constitute analogues of the juvenile hormones of specific kinds of herbivorous or wood-boring insects. In short, the plants, and more particularly the ferns and evergreen trees, may have gone in for this incredibly sophisticated mechanism as self-defense against insect predation. Evidently, this is the place to look for a whole battery of selective hormonally active pesticides.

The potential of this approach was documented during the summer of 1968 when I served as chief scientist of Phase C of the research vessel Alpha Helix expedition to the upper Amazon. I was amazed to note the absence of aquatic insects in the Rio Negro--a black-water tributary of the Amazon draining no less than 0.5 million sqnare miles of inundated jungle. This was in marked contrast to the abundance of aquatic insects in such white-water tributaries as the Rio Branco, Rio Madeira, and Solimões.

The Rio Negro proved to be a river of plant-derived pesticides. Crude extracts prepared in toluene have demonstrated substantial juvenile hormone activity when subjected to biological assay on Pyrrhocoris apterus. Assays for the presence of phytoecdysones have been uniformly negative. The waters of the Rio Negro, therefore, appear to be an almost infinite source of pesticidal materials of the juvabione type.

Recent discoveries, principally in Japan and Czechoslovakia, indicate the presence of incredible amounts of ecdysone and ecdysone analogues in certain ferns, evergreen trees, and even in a few angiosperms. To date, a total of 15 phytoecdysones have been isolated and characterized, including authentic α- and β-ecdysones. Certain of these phytoecdysones are far more active than α- or β-ecdysone; for example, cyasterone and ponasterones A and B are up to 20 times as active.

At Harvard University, tests have shown that the ecdysones can penetrate the unbroken skin of insects when dissolved in certain nonvolatile lipid solvents. The most effective of these are α-tocopherol, undecylenic acid, and caprylic acid. Presumably, in their native dissolved state within the plant, the phytoecdysones can do likewise. Therefore, any contact of overwintering insects with these materials can cause a termination of diapause and culminate in what might be termed "ecological suicide."

The phytoecdysones are further characterized by their ability to provoke abnormal and lethal metamorphosis. When administered in doses twice the physiological level, metamorphosis is speeded up in a pathological way so that, in the case of diapausing pupae of the cynthia moth, 11 days of normal development are telescoped into 4 days of abnormal development. The net result is a lethal derangement of metamorphosis. Presumably, this is another cogent reason why certain plants synthesize these highly active phytoecdysones.

Unpublished studies carried out in collaboration with T. Ohtaki have demonstrated that the half-life of α-ecdysone is only 1 hr after its injection into mature larvae of Eoettcherisca peregrina (Robineau-Desvoidy). For diapausing pupae of Samia cynthia, the half-life is 8 hr.

These findings call for a fresh evaluation of one of the dogmas of insect endocrinology--that ecdysone accumulates to a threshold titer at a certain critical period to discharge a certain morphogenetic event such as molting or metamorphosis.

Now it appears that ecdysone undergoes only trivial accumulation. What accumulates is not the hormone itself, but the covert biochemical effects of the hormone. It is as if a molecule of hormone provokes a quantum of covert effect and is then "erased." What accumulates are these covert effects that undergo, as it were, spatial and temporal summation in the target tissues to attain discharge of overt endocrine action. If the process is interrupted prior to the discharge of the overt action, then the latent covert effects are subject to decay.

For these several reasons, it appears that the insect hormones constitute "weak spots" in their physiological armor and open them to attack by specific materials from which they have little defense or prospect of evolving defense. Meanwhile, from the standpoint of pure science, we are encouraged to seek in these simplified systems the Rosetta stone to the mechanism of hormonal action.

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Bark Beetle Pheromones

David L. Wood

Isolation, Identification and Synthesis of the Pheromones Produced by Destructive Bark Beetle Species

We will complete this work for Dendroctonus brevicomis and D. ponderosae. We also will attempt to identify the compounds produced during the mass attack that elicit aggregation by certain parasites and predators. (This study is in collaboration with R. M. Silverstein, Stanford Research Institute.)

Manipulation of Bark Beetle Populations with Synthetic Pheromones

1. Effects of concentration, combination, trap size and design, and location of trapping efficiency will be determined.

2. Competition of synthetic pheromones with naturally occurring sources will be assessed.

3. Potentialities of confusing the orientation of populations by increasing the background attractant levels in order to interrupt or delay the concentration flight will also be assessed.

Population Dynamics of *Dendroctonus brevicomis*

Population studies have been in progress for 6 years over a 10,000-acre area so that estimates can be made of the number of beetles per acre and the total input and the total output from infested trees. We will attempt to relate the numbers trapped by synthetic pheromones to population fluctuations in order to predict damage trends and to estimate sources of in-flight mortality for the partial life tables now available. (This study is in collaboration with R. W. Stark, University of California, Berkeley, and W. D. Bedard, U.S. Forest Service.)

Specificity of Pheromones in the Genus *Ips*

Closely related species of Ips in subgeneric groups I and IX with largely allopatric distributions are cross-attractive in both the laboratory and nature. Species from other subgeneric groups are sympatric and generally not cross-responsive. Three species of Ips from group IX respond to the three synthetic I. confusus attractants at lower concentrations and to different combinations. In order to elucidate the mechanisms of specificity, the pheromones produced by male I. pini (Say), I. latidens, and a new species will be isolated, identified, synthesized, and field tested. (This study is in collaboration with R. M. Silverstein, Stanford Research Institute.)

Developmental Studies of *Temnochila virescens chlorodia* (Mannerheim)(Ostomidae)

Temnochila fed continuously on a parboiled Galleria larval diet will develop normally, but they continue to add supernumerary molts. These larvae do not pupate after removal from the diet. Larvae removed from the diet as early as the second instar are able to pupate and transform to the adult. Adults reared on this diet do not achieve sexual maturity until they receive an adult bark-beetle meal. Adults fed *Galleria* larvae, *Tenebrio* adults, and *Bruchus* adults are also unable to reproduce. Further studies on this phenomenon are being carried out by C. W. Wilson, Department of Entomology and Parasitology, University of California, Berkeley. (This study is in collaboration with T. E. O'Connell, ARS, USDA, Mexico City.)

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Oviposition Behavior of the Tobacco Hornworm, *Manduca sexta*

Robert T. Yamamoto

It was shown previously that an oviposition stimulant was present in solanaceous plants that could be extracted and applied on artificial or nonhost plants and still induce oviposition. Assays of many different families of plants indicated that only solanaceous plants possessed this stimulant, thereby restricting the host-plant range of the tobacco hornworm. Another factor, distillable with steam from tomato leaves, was thought to provide the necessary olfactory cues in guiding the moths to the plant.

With the development of an artificial diet for the larvae, sufficient moths are now available throughout the year to continue and extend this work. At the present time we are concerned with (1) the isolation and identification of the oviposition stimulant, (2) preimaginal conditioning and natural preferences of moths, and (3) receptor systems of the moths.

Statements on current findings follow.

Orientation

Visual and olfactory cues presumably guide moths to host plants in nature. In large cages, moths can locate an isolated 3-in. long tobacco leaf placed at ground level and oviposit on it. Moths with eyes painted or with antennae coated with petroleum jelly do not locate the leaf. Moths treated as above but confined with tobacco leaves in small cages (1 ft^3) laid full complements of eggs.

Natural plant preferences and preimaginal conditioning

A survey taken in North Carolina in 1967 showed that tomato and tobacco were the only plants naturally infested with larvae although six wild solanaceous plants were available in fair abundance. In Illinois, during a field survey taken a number of years ago, these two species of plants were also the only ones infested with larvae. Tobacco and tomato therefore appear to be the naturally preferred host plants of the tobacco hornworm.

Oviposition preference for tobacco over wild solanaceous plants was confirmed in the laboratory. Adult preference for tobacco, however, is not always related to larval feeding preferences. For example, newly hatched larvae preferred potato and Petunia, a toxic plant, over tobacco by a significant margin.

Assuming that response to the oviposition stimulant is genetically fixed in the tobacco hornworm, the natural predilection for tobacco in nature may reside in other factors since all solanaceous plants palatable to the larvae possess the stimulant. Preimaginal conditioning to odorous components (attractants) of tobacco is one possibility and was tested. Larval conditioning, from instar to instar, to various species of solanaceous plants and even to the artificial diet was readily demonstrated; however, F_1 generation of Jimsonweed-reared moths still preferred tobacco for oviposition.

Responses of diet-reared moths

Diet-reared moths showed a higher threshold requirement for the oviposition stimulant than did plant-reared moths. The difference was about 15-fold. It appears that some reinforcement of response to the stimulant may be needed in the larval stages.

Chemical study of the oviposition stimulant

The material is water soluble. When applied on artificial leaves constructed out of sponge rubber, moistüre is necessary for its activity. Its chemical identity is unknown.

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