The strategy of controlling insect pests with growth regulators

MITTEILUNGEN DER SCHWEIZERISCHEN ENTOMOLOGISCHEN GESELLSCHAFT
BULLETIN DE LA SOCIÉTÉ ENTOMOLOGIQUE SUISSE
Band 44 Heft 1 u. 2 15. September 1971

The strategy of controlling insect pests with growth regulators

H. A. SCHNEIDERMAN

The fact that half of the world is hungry most of the time testifies that man cannot afford to sharecrop with insects. New approaches to insect control are clearly needed. Most of us at this symposium believe that analogues, mimics and antagonists of insect growth regulators such as the juvenile hormones and the ecdysones may provide the basis for a new generation of pesticides. Hopefully these agents will replace many of the compounds now used for pest control which have unwanted ecological side effects or high mammalian toxicity and will become key parts of the network of integrated controls we are establishing to aid us in our never-ending competition with other species for food. However, because insect growth regulators are so different from conventional insecticides, their application requires special strategy. This report outlines key features of this strategy.

Overall strategy of insect control — evolutionary considerations

Evolution has set two boundary conditions which govern the strategy of insect control. First, the historical fact of a common evolutionary ancestry for living organisms means that the basic biochemistry of all organisms is the same. They probably have the same genetic code which appeared two billion years ago. This means that any pesticides which affect basic metabolic processes and damage specific cell structures will affect organisms other than insects. The second boundary condition is set by the process of evolution by natural selection which guarantees the appearance eventually of organisms resistant to any pesticide. There has been a tendency to overlook this problem of resistance in connection with insect growth regulators and it will be considered at the end of this report. Although, there is no way to bypass these boundary conditions, recognizing them can help in planning strategy.

Fossil remains of the ancestors of the insects and the vertebrates, the trilobites and protochordates, are found in Cambrian rocks. Hence,

arthropods and vertebrates must have diverged from their common ancestry before that time, at least six hundred million years ago. During the six hundred million years in which these two groups of organisms have undergone divergent evolution, there have been numerous opportunities for biochemical innovation. Many processes such as protein and nucleic acid synthesis, mechanisms of cellular respiration, etc. have not changed. Other processes such as the mechanism of gas exchange and the hormonal control of reproduction are very different in vertebrates and arthropods. One process which is peculiar to arthropods is cuticle synthesis and molting. Hence, interfering with cuticle synthesis and molting is, *a priori*, a reasonable approach to arthropod control. Cuticle synthesis and molting are controlled by three groups of growth regulators — the brain hormones (which may be polypeptides), the ecdysones (which are steroids) and the juvenile hormones (which are epoxides of homosesquiterpenoic esters). These growth regulators also control other developmental processes in insects including metamorphosis, sexual maturation and reproduction.

Insect hormones

Let us begin our analysis of insect growth regulators by briefly examining these hormones. The brain hormone, secreted by neurosecretory cells in the protocerebrum, activates the prothoracic glands. These glands in turn secrete one or several closely related steroids, the ecdysones. These ecdysones themselves or their metabolic products cause the insect to molt. The kind of cuticle secreted by the epidermal cells at each molt is affected by a third group of hormones, the juvenile hormones, which are secreted by the corpora allata. In the presence of the juvenile hormones, larvae molt into larvae. However, in their absence, in hemimetabolous insects, larvae metamorphose into adults, and in holometabolous insects, larvae molt into pupae and then into adults. Molting is controlled by regulating the release of brain hormone. Maturation and metamorphosis are controlled by regulating the release of juvenile hormone. In simplest terms, the ecdysones stimulate the synthetic activities necessary for molting, whereas juvenile hormones influence the kinds of synthetic activities that occur in response to the ecdysones.

From this analysis it becomes clear that juvenile hormones are handmaidens to the ecdysones. In their effects on morphogenesis, juvenile hormones only act in concert with the ecdysones. There is a common misconception that juvenile hormones and ecdysones act antagonistically. This idea probably arose from the fact that metamorphosis requires ecdysones and can be blocked by juvenile hormones. However larval molting requires both juvenile hormones and ecdysones. There is no evidence that these two groups of hormones act antagonistically to one another.

Ecdysones — toxic effects

Since it is not possible to understand the effects of juvenile hormones on development without considering the ecdysones, let us examine the ecdysones first. Insects use alpha ecdysone, beta ecdysone and perhaps other ecdysones as molting hormones. Recent experiments by Moriyama *et al.* and by Oberlander suggest that beta ecdysone may be the active material and that alpha ecdysone is rapidly converted to it by certain tissues such as the fat body. However the situation in other arthropods is less clear. In the Spring of 1968 no one had demonstrated that ecdysones or any other chemicals caused molting in any arthropods other than insects. About two years ago my colleague Krishnakumaran and I made a detailed examination of the effects of several ecdysones on various arthropods other than insects. We discovered that we could cause essentially any arthropod to molt by either topical application or injection of ecdysone. Animals induced to molt included crustaceans, arachnids and members of the most primitive of living arthropods, the horseshoe crabs. Hence ecdysones appear to be the molting hormone of all of the arthropods. Whether the juvenile hormones affect arthropods other than insects has not yet been determined.

Another feature of ecdysones which is of special interest is that by themselves they can sometimes produce juvenile hormone-like effects. During a normal molt cycle, the prothoracic glands release ecdysones at a precise rate, and normal development ensues. If insects are experimentally exposed to large amounts of ecdysones or exposed to ecdysones at an abnormal time, they may be toxic and cause various developmental abnormalities including juvenile hormone-like effects.

Alpha ecdysone appears to be less toxic than the other ecdysones. Perhaps the insect regulates its rate of conversion to the active form, beta ecdysone. Exogenous ecdysones interfere with wax deposition in larvae. If a larva is treated with an ecdysone soon after a molt, it often fails to secrete wax and the insect starts to make a new cuticule before the old one is completed. Ecdysones are also toxic to larvae about to metamorphose, and to pupae where they may cause juvenile hormone-like effects. Thus, pupae treated with large amounts of ecdysones may molt into non-viable second pupae or into non-viable intermediates between pupae and adults. For example, if a pupa of *Tenebrio* is injected with several micrograms of beta ecdysone, it molts into a second pupa instead of into an adult.

The reasons for this paradoxical juvenilizing effect were made clear by a number of recent experiments performed in collaboration with my colleague Krishnakumaran. An autoradiographic study of *Tenebrio* pupae injected with tritiated thymidine revealed that the epidermal cells of normal pupae engaged in extensive DNA replication before molting, whereas the epidermal cells of pupae given large doses of beta ecdysone

engaged in almost no DNA replication before molting. These data suggest that the large dose of ecdysone stimulated the epidermal cells to secrete a new cuticle before the cells had time to "reprogram" for adult syntheses. Normally, low concentrations of ecdysone stimulate DNA replication and cell division, whereas larger amounts stimulate the immediate synthesis of a new cuticle. Apparently, reprogramming of epidermal cells requires DNA replication. A cell that is making larval or pupal cuticle must "clean its genes" before it can make pupal or adult cuticle. This reprogramming apparently occurs only at times of DNA replication. If high concentration of ecdysones force epidermal cells to make a new cuticle before they have undergone DNA replication, they make the same old kind of cuticle again. These observations have a bearing on the time of action of juvenile hormone and the pracal application of hormonally-based insecticides which are considered next.

Juvenile hormones — time of action and toxic effects

Growth in higher vertebrates and in higher insects is associated with the non-reproductive juvenile stage and in both vertebrates and insects, maturation is under hormonal control. But the control mechanisms for maturation are very different for both groups. Maturation in man and other higher vertebrates is promoted by the secretion of maturation hormones, the gonadotropins of the pituitary. The juvenile condition in man hinges upon the absence of these maturing hormones. In insects it is just the opposite. The juvenile condition in insects depends upon the continued presence of the juvenile hormones, which act on the cells themselves, and prevent them from maturing.

Other reports in this symposium discussed various bioassays for juvenile hormones. We have learned that when juvenile hormones act on epidermal cells, they influence the kind of cuticle the epidermal cells secrete. The juvenile hormones also effect the development of internal organs of the animal including the brain, the gonads, and the midgut, where they prevent maturation and metamorphosis. They also exert a gonadotropic effect and promote the synthesis of yolk proteins by the fat body and the accumulation of these proteins by the developing oocytes. Juvenile hormones also may activate the prothoracic glands of Lepidoptera and break adult diapause in insects such as the alfalfa weevil. Juvenile hormones may also have conspicuous effects on behavior. They also interfere with embryonic development, a matter which will be considered by Lynn Riddiford subsequently. It is of some interest and practical importance that, so far as we can tell, all of these activities are shared by all of the compounds that show juvenile hormone activity in morphogenetic tests. Apparently, the active sites in target tissues for all of these juvenile hormone effects are similar. Perhaps during evolution, various target organs have

"captured" juvenile hormones and used them to coordinate various activities. Such endocrinological opportunism is common in nature and there are many examples in vertebrates.

Although we know almost everything about the structure of the juvenile hormones excepting their absolute configuration, their mode of action remains the subject of much controversy. However, there is considerable evidence relating to the timing of hormonal stimuli, and to the changing sensitivity to hormonal stimuli, which provide clues as to the mode of action of the juvenile hormones.

For example, in one experiment, pupae of the Polyphemus silkworm were injected with juvenile hormone at different times after the initiation of the pupal-adult transformation and simultaneously injected with tritiated thymidine. The appearance of pupal patches in the resulting adults coincided with patches of cells that showed maximum incorporation of the isotope, a fact which suggests that only those cells which had not completed DNA replication had responded to juvenile hormone. Invariably we find that juvenile hormones affect only epidermal cells which have not replicated their DNA. Cells which have replicated their DNA are insensitive to juvenile hormone except at extremely high doses. These and other results indicate that juvenile hormone affects cells only during a limited part of the cell cycle, probably the end of the G_1-period or during the S-period. Many studies pointing to this conclusion have been conducted by my colleague Frantisek Sehnal over several years both in my laboratory and at the Entomological Institute at Prague. It appears that juvenile hormone acts on cells before transcription occurs and, in some way determines what part of the genome will be used. This idea is now being investigated in a number of laboratories using various DNA/RNA hybridization techniques.

The principal cause of juvenile hormone toxicity is the production of intermediate between larva and pupa, pupa and adult, or larva and adult. These invariably fail to reproduce and often fail to undergo normal ecdysis. Such intermediates are not the result of excessive amounts of juvenile hormone. Rather, they occur when cells are exposed to juvenile hormone at abnormal times in their developmental programs. Application of juvenile hormone to larvae at a time when some cells have lost their sensitivity to juvenile hormone, but others have not, leads to the production of intermediates. Other toxic effects of juvenile hormone appear during embryonic life and are considered elsewhere by Lynn Riddiford.

The precise mechanism of juvenile hormone action in both embryonic and post-embryonic development remains to be elucidated. Carroll Williams presents one hypothesis elsewhere in this symposium. (I am rather glad I do not have to present one now, because it is so much easier to shoot down someone else's ideas than to try to present your own !) However, I think that most people who have thought about

it seriously, agree that juvenile hormone in some way controls the reading of genetic information needed for new syntheses. It seems to permit current genes to operate but it prevents the activation of other groups of genes. For our present purposes, particularly in connection with the practical questions with which many of the people at this symposium are concerned, it is important to note that juvenile hormone primarily affects cells which are replicating DNA. This fact influences the practical application of juvenile hormone analogues as control agents as I should now like to demonstrate.

Juvenile hormone analogues and mimics as insecticides

In order for juvenile hormone analogues and mimics to affect insect development, they must act during the last larval instar or during metamorphosis by causing the production of intermediates. Alternatively, juvenile hormone analogues must act upon adults where they function as chemosterilants or upon embryos. Juvenile hormone analogues must be applied at the proper time and must persist for several days to be effective in producing non-viable intermediates. This matter is analyzed in some detail by Frantisek Sehnal and myself in a forthcoming paper. Our main argument is this : to produce maximum kill with minimum dose, juvenile hormone analogues must be applied when some parts of the insect's body are sensitive to these substances whereas other parts are not. Since juvenile hormones appear to act only at certain times in the cell cycle, one can predict these hormone-sensitive stages from studying the timing of DNA replication in various parts of the integument of developing insects.

For example, bugs, locusts, and other hemimetabolic insects usually become insensitive to juvenile hormone analogues a few days after the last larva ecdysis. Consequently, one must apply juvenile hormone analogues shortly after the last larval ecdysis, generally during the first third of the last larval instar or, much later, after adult emergence, where juvenile hormone analogues will have an ovicidal effect. Larvae of most holometabolic insects such as Lepidoptera and Coleoptera can only be deranged at the end of the last larval instar. Pupae of holometabolic insects are sensitive to juvenile hormone analogues only for several hours or at most a few days after the last larval ecdysis. These represent the periods of DNA replication. To be effective in the field, juvenile hormone analogues must be applied at the right time and must persist long enough so that all members of the population are exposed to them for an adequate time when they enter their periods of sensitivity to juvenile hormone. Thus, juvenile hormone analogues must persist in the environment for several weeks if they are to be effective. This requirement for field stability of juvenile hormone analogues is extremely important if these agents are to be of any practical use.

Resistance to insect growth regulators

My last remarks concern the development of resistance to juvenile hormone analogues and other insect growth regulators. A. W. A. Brown has emphasized at this symposium that insects develop resistance to conventional insecticides and often cross-resistance to several insecticides. Already some 250 pests have become so resistant that the pesticides formerly used to control them are no longer effective. It has been suggested on several occasions that hormonally-based insecticides, that is analogues and mimics of insect growth regulators, would have the supreme advantage in that it would not be possible for pests to develop resistance to them. However, I believe one can advance strong arguments that resistance is likely to develop. Consider for example juvenile hormone analogues. At certain stages in their development, insects normally inactivate, sequester or excrete juvenile hormone and many juvenile hormone analogues. Thus, nature has endowed insects with a built-in mechanism to resist the artificial application of juvenile hormones and their analogues at specific stages. John Siddall pointed out earlier in this symposium that the half life of the C^{18} juvenile hormone in larvae of the tobacco horn worm, *Manduca sexta*, is only twenty minutes. Now, to be sure, mechanisms which inactivate juvenile hormone and juvenile hormone analogues normally function only at specific times in development. However, the existence of such mechanisms guarantees that natural selection could produce populations of insects which would be resistant to exogenous juvenile hormone analogues. I think one can make similar arguments in connection with almost any insecticide one can imagine.

Another circumstance which insures the appearance of insects resistant to juvenile hormone analogues is the fact that there are great differences in the sensitivity of different families of insects to specific juvenile hormone analogues. The evidence at hand indicates that this specificity may extend to the family level. Thus, some juvenile hormone analogues affect bugs of the family Pyrrhocoridae but are much less active on bugs of the family Lygaeidae. Other juvenile hormone analogues affect Tenebrionid beetles but not Curculionid beetles, and so forth. Now this group-specificity in sensitivity to juvenile hormone analogues does not mean that we should expect to discover a different natural juvenile hormone for each family of insects. (Indeed, it appears likely that there may be only a very few different naturally-occurring juvenile hormones.) It appears more likely that the differences in the sensitivity to juvenile hormones we see among different families stems from differences in rates of penetration, breakdown, excretion, storage and so forth of these agents or from differences in behavior, feeding habits, etc. of the insects tested. For purposes of the present arguments, the fact that insects of one genetic constitution may respond to a particular juvenile hormone analogue, whereas rather closely related insects

of a different genetic constitution fail to respond, tells us that resistance to juvenile hormone analogues could be selected for in nature.

Now it is possible that resistance to juvenile hormone analogues will develop slowly in insect populations, perhaps at the negligible rate that plants have developed resistance to 2,4-D, 2,4,5-T, naphthalene acetic acid, and other synthetic plant growth regulators. But I would direct your attention to the fact that most insects have many more generations per year than do most weeds. Any insecticide, hormonally-based or not, is bound to act as a powerful sieve for concentrating resistant mutants that are present in low frequencies in the original population. In the Center for Pathobiology at the University of California at Irvine, my colleagues Peter Bryant, Robert Arking, Ernest Vyse and I have begun to select mutants of *Drosophila* resistant to juvenile hormone analogues and mimics and to ecdysones. Hopefully by identifying the mechanisms of resistance to these agents, we can anticipate resistance before it occurs in the field and learn how to minimize it. Advance knowledge of the possible counter measures which insects may adopt in response to synthetic insect growth regulators should permit us to stay several steps ahead of them.

Conclusion

I remain optimistic and confident that insect growth regulators like juvenile hormone analogues and mimics will become parts of our overall programs of integrated control of arthropod pests. Hopefully, through the efforts of scientists at this symposium from industry, universities and governments, we will use these tools and others to battle effectively against insects so that we may continue to practice mass outdoor agriculture. But in the event we start to lose the battle, let me assure you the war is not really lost. For recall that insects themselves are an excellent source of protein. As an enlightened entomologist I find the prospect of bread fortified with insect meal no different from eating bread fortified with fish meal. So if we can't beat them, we'll eat them !

REFERENCES

KRISHNAKUMARAN, A., S. J. BERRY, H. OBERLANDER, and H. A. SCHNEIDERMAN. 1967. Nucleic acid synthesis during insect development. II. Control of DNA synthesis in the cecropia silkworm and other saturniid moths. *J. Insect Physiol.* **13**: 1–57.

KRISHNAKUMARAN, A. and H. A. SCHNEIDERMAN. 1968. Chemical control of moulting in Arthropods. *Nature* **220**: 601–603.

MEYER, A. S., E. HANZMANN, H. A. SCHNEIDERMAN, L. I. GILBERT and M. BOYETTE. 1970. The Isolation and Identification of the Two Juvenile Hormones from the *Cecropia* Silk Moth. *Arch. Biochem. Biophys.* **137**: 190–213.

MORIYAMA, H., K. NAKANISHI, D. S. KING, T. OKAUCHI, J. B. SIDDALL and W. HAFFERL. 1970. On the Origin and Metabolic Fate of α-Ecdysone in Insects. *Gen. Comp. Endocrinol.* **15**: 80–87.

RICHMAN, K. and H. OBERLANDER. 1971. Effects of fat body α-Ecdysone induced morphogenesis in cultured wing disks of the wax moth *Galleria mellonella*. *J. Insect Physiol.* In press.

RIDDIFORD, L. M. and C. M. WILLIAMS. 1967. The effects of juvenile hormone analogues on the embryonic development of silkworms. *Proc. Nat. Acad. Sci. U.S.* **57**: 595–601.

ROBBINS, W. E., J. H. KAPLANIS, M. J. THOMPSON, T. J. SHORTINO, C. F. COHEN, and S. C. JOYNER. 1968. Ecdysones and analogues: Effects on development and reproduction of insects. *Science* **161**: 1158.

RÖLLER, H. and K. H. DAHM. 1968. The chemistry and biology of juvenile hormone. *Recent Progr. Hormone Res.* **24**: 651–680.

SCHNEIDERMAN, H. A. 1969. Control systems in insect development, pp. 186–208. In "Biology and the Physical Sciences. Symposium in Biology in Relation to the Physical Sciences". Ed. S. Devons. Columbia University Press.

SCHNEIDERMAN, H. A. and L. I. GILBERT. 1964. Control of growth and development in insects. *Science* **143**: 325–333.

SCHNEIDERMAN, H. A., A. KRISHNAKUMARAN, P. J. BRYANT, and F. SEHNAL. 1969. Endocrinological and Genetic Strategies in Insect Control. Proc. Symposium on Potentials in Crop Protection. *N. Y. St. Agric. Exp. Sta.*, Geneva, New York.

SEHNAL, F. and V. J. A. NOVAK. 1969. Morphogenesis of the pupal integument in the wax moth *Galleria mellonella* and its analysis by means of the juvenile hormone. *Acta Entomol. Bohemoslov.* **66**: 137–145.

SEHNAL, F. and H. A. SCHNEIDERMAN. 1971. How to use a third generation pesticide. Submitted for publication.

SEHNAL, F. and H. A. SCHNEIDERMAN. 1971. Action of the corpora allata and of the juvenile hormone and its analogues on the larval-pupal transformation. Wilhelm Roux' Arch. Entwicklungsmech. Organismen. In press.

WILLIAMS, C. M. 1968. Ecdysone and ecdysone-analogues: Their assay and action on diapausing pupae of the cynthia silkworm. *Biol. Bull.* **134**: 344–355.

WILLIAMS, C. M. 1970. Hormonal Interactions between Plants and Insects. In "Chemical Ecology". Ed. E. Sondheimer. *Academic Press, Inc.*, New York.

WILLIAMS, C. M. and W. E. ROBBINS. 1968. Conference on insect-plant interactions. *Bioscience* **18**: 791–792, 797–799.

Prof. H. A. SCHNEIDERMAN
School of Biological Sciences
University of California
Irvine, California 92664
USA