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THE PRESENT STATUS OF HORMONES AND GROWTH INHIBITORS IN INSECT CONTROL

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Summary Juvenile hormone activity was discovered in insect extracts almost 20 years ago, and this year marks the first registrations of juvenile hormone mimic insecticides. In the intervening period, intense research effort has high-lighted the advantages and disadvantages of juvenile hormone mimics as insecticides.

Recently, other insect growth regulator chemicals have been discovered. These possess some of the toxicological and selectivity advantages of the juvenile hormone mimics, but, because of their different mode of action, they are applicable to a broader spectrum of insect pests. Such compounds represent a significant advance in insecticide technology.

Resume L'activité des hormones juveniles a été découverte il y a vingt ans dans des extraits d'insectes, et la première hormone imitatrice juvénile a été homologuée cette année. Un effort de recherche considérable a demontré, dans l'intervalle de ces dates, les advantages at les inconvenientes des hormones juvéniles imitatrices en temp qu'insecticides.

On a constaté que les matières actives ont une faible toxicité pour les mammifères, et peu d'effet sur l'environnement. Ces hormones se sont aussi montrées très efficaces contre les races résistantes de certains insectes. Néamoins, leur mode d'action limite leur champ d'utilisation, et on peut penser que leur usage sera surtout limité aux domaines de la santé publique et dans des utilisations vétérinaires.

Récemment, d'autres régulateurs de croissance d'insectes ont été découverts. Ces matières actives possèdent quelques-uns des avantages de toxicité et de sélectivité des hormones juveniles imitatrices, mais, à cause de leur mode d'action différent, peuvent etre utilisées sur un plus large spectre d'insectes. Ces matières actives représentent un progrès important dans la science des insecticides.

INTRODUCTION

Almost two decades have passed since an active preparation of an insect juvenile hormone was detected in extracts from male silkmoths (Hyalophora cecropia) (Williams, 1956), and the high level of physiological activity of the preparation, particularly in terms of its ability to block insect metamorphosis, suggested that the active principle, if extracted, might have potential as an insecticide.

A decade later another significant advance in the field occurred. The structure of the H. cecropia hormone was identified (Roller and Bjercke, 1965) (Fig. 1), and it was also established that certain synthetic analogues of the hormone possessed very high levels of juvenile hormone activity (Slama and Williams, 1966). These findings, together with the realisation that the biological properties of the hormone analogues might provide a number of advantages over the insecticides used at the time, provided an impetus for the initiation of a great number of research programmes in academic and commercial establishments.

These programmes were aimed particularly at the synthesis and evaluation of insect juvenile hormone mimics as a possible "third generation" of insecticides. This year we have seen a significant step forward in the realisation of these efforts, since it marks the first registrations (by the United States Environmental Protection Agency) of juvenile hormone mimic insecticides. This year methoprene (Fig. 2) was registered in the USA for control of floodwater mosquitoes (Aedes nigromaculis, A. melanimon etc) (Anon. 1975a) and horn flies (Haematobia irritans).

This is therefore an appropriate time to review the present status of these types of insecticides, to consider how far they have fulfilled their anticipated potential, and to evaluate the future utility of juvenile hormone mimics and other insect growth regulators in pest control.

JUVENILE HORMONE MIMICS

a) Advantages.

The early work with insect juvenile hormone mimics suggested that they could have a number of advantages over conventional insecticides, the following being the most significant:

Firstly, specificity to insects - i.e. low toxicity to non-target organisms, especially man and wildlife, related to the compounds' mode of action, which is on an endocrine system specific to insects. Secondly, specificity within insects - i.e. the possibility of activity against pests rather than beneficial species.

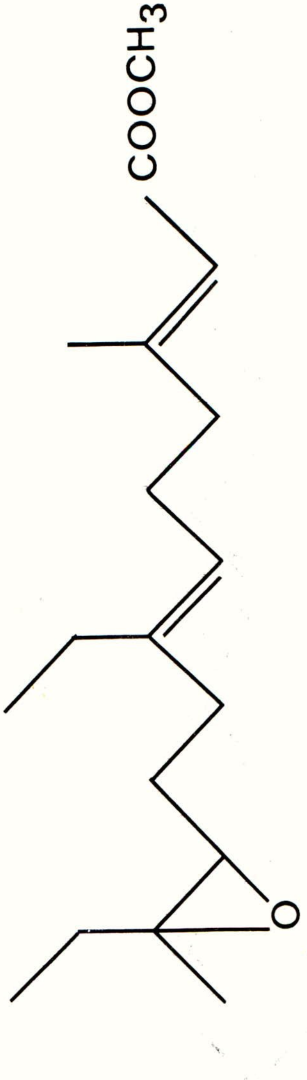
Thirdly, efficacy against insects resistant to conventional insecticides, resulting from the differing modes of action of insect juvenile hormone mimics and conventional (nerve poison) insecticides.

Our evidence to date shows that the juvenile hormone mimics have to a very large extent fulfilled expectations in these areas. With regard to mammalian effects, methoprene, for example, has been registered as exempt from residue tolerances on a number of crops, reflecting its very low mammalian toxicity. Toxicity to fish, crustacea and birds is also low, and the compound has little effect on protozoa, annelids, molluscs and amphibia (Anon, 1975 b).

Methoprene is also relatively specific to Diptera; it is of low activity on other Orders of insects, and in normal use against mosquito larvae has little effect on non-target insects such as damselflies, mayflies and water beetles (Miura & Takahashi, 1973). Similarly, a newly discovered arylterpenoid mimic (2-ethoxy-9-(p-isopropylphenyl)-2,6-dimethylnonane), though the most active juvenile hormone mimic yet tested on livestock fly pests, is relatively ineffective against representative Coleoptera and Hemiptera. (Schwarz et al, 1974).

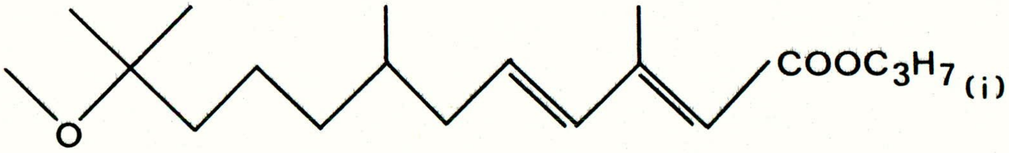
In terms of activity against resistant pest species, methoprene's effectiveness against floodwater mosquitoes is, in part, due to its activity against strains of the insect tolerant to conventional insecticides, including organophosphates and carbamates (Schaefer & Wilder, 1972 & 1973).

Fig. 1



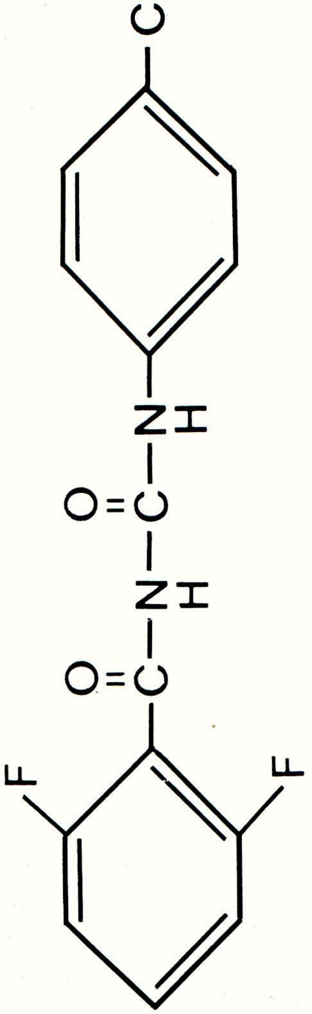
Juvenile hormone

Fig. 2



Methoprene

Fig. 3



Diflubenzuron

In general however, juvenile hormone mimics have not completely fulfilled the somewhat extravagant claims made (because of their novel mode of action) for their potential as control agents for resistant insects. A significant number of insect species tolerant to conventional insecticides have been shown to possess a degree of cross-resistance to juvenile hormone mimics. The list includes the flour beetle (Tribolium castaneum) (Dyte, 1972), the housefly (Musca domestica) (Cert and Georghiou, 1972), and the tobacco budworm (Heliothis virescens) (Benskin and Vinson, 1973).

With hindsight, it is perhaps not surprising to find such cross-resistance present in a number of insects. Despite their novel mode of action, the chemical nature of juvenile hormone mimics suggests that they would be susceptible to breakdown by the insect enzyme systems (e.g. carboxyesterases) responsible for resistance to conventional compounds. Insects have the capacity to degrade their own juvenile hormone (Slade & Zibitt, 1972), and hence probably juvenile hormone mimics applied exogenously.

Furthermore, there is no reason to suppose that insect resistance to juvenile hormone mimics will develop at a slower rate than it does to conventional compounds. For example, five-fold resistance to methoprene has been induced in the laboratory by selection of the northern house mosquito (Culex pipiens pipiens) with the compound over thirty generations. (Brown and Brown, 1974).

b) Disadvantages

The potential disadvantages of juvenile hormone mimics as insecticides were poorly emphasised in the early days, but fairly soon became apparent as work progressed.

The exploitable effects of juvenile hormone mimics are disruption of insect maturative moults (larval-pupal or larval-adult), adult sterilisation and ovicidal effects. Thus application of the mimics to final instar larvae, or to pupae, disrupts the subsequent moult, causing death of the insect, but the mimics are normally without effect on larval-larval moults. So juvenile hormone mimics do not disrupt the normal development of insect larvae - yet it is very often the larval stages of insects which are mainly responsible for crop damage.

The relatively narrow physiological window in the insect life-cycle during which the mimics need to be applied for full effect (Slama, 1971) means that the compounds have to be of relatively high environmental persistence in order to ensure their effectiveness when applied to (usually heterogeneously aged) field populations of insects. However, many of the mimics are not intrinsically stable compounds, breaking down relatively quickly in several field environments. (Pawson et al, 1972; Schaefer & Dupras, 1973).

Over the last decade it has become very apparent that these disadvantages place severe restrictions on the widespread use of juvenile hormone mimics as insecticides; in particular the lack of true larvicidal activity of the mimics means that their applicability is restricted to insects that are pests only in the adult phase.

Suitable targets are thus confined mainly to the Dipterous pests of public and animal health, and we have therefore seen the development of juvenile hormone mimic insecticides in areas such as mosquito abatement in the Western USA. Here the low toxicity of the mimics such as methoprene to mammals and non-target organisms is of course a great advantage, as is its effectiveness in the field against resistant strains.

Even in this general outlet the relatively low persistence of the compounds can be a disadvantage. Thus in order to provide good control of Californian floodwater mosquitoes (which develop synchronously), methoprene has to be used in an encapsulated slow-release formulation, but even with this, control of species which develop asynchronously has been poor - though this may also be due to differences in species susceptibility (Staal, 1975).

A related area in which juvenile hormone mimics are beginning to find a place is in the control of livestock pest and nuisance flies, such as stable flies (Stomoxys calcitrans) and face flies (Musca autumnalis), also in the USA (Miller and Uebel, 1974; Wright et al, 1974). Here again the low mammalian and bird toxicities of the compounds are a great advantage, particularly since they can be used as "feed-through" treatments, in which the mimics are consumed in the animals' diet, passing through the beasts unmetabolised and undigested, and then preventing development of flies in the manure produced (Harris et al, 1974).

The mode of action limitations inherent in juvenile hormone mimics mean, however, that we are unlikely to see significant development of these compounds in areas other than public and animal health Dipterous pest control. They are inappropriate for the control of the great majority of agricultural insect pests; and on those where the lack of larvicidal action may be less of a disadvantage (scale insects, mealybugs) activity has, in general, not been particularly high (Staal, 1975).

OTHER INSECT GROWTH REGULATORS

Although the role of juvenile hormone mimic insecticides is likely to be smaller than originally envisaged, the discovery and evaluation of these compounds has played an important part in the re-orientation of our ideas on the design of testing procedures for potential insecticides, since it has been shown that, as well as a quick kill, a relatively slow disruption of growth can be an exploitable effect.

The result has been that recently, compounds have appeared which, like the juvenile hormone mimics, act by disruption of growth, but unlike them, do not have the disadvantage of effectiveness only at maturation moults.

Such a compound is diflubenzuron (Fig. 3) which was described at this Conference two years ago (Mulder and Swennen, 1973). Diflubenzuron is not a juvenile hormone mimic; the significant difference in effect being that it disrupts growth at larval as well as maturative moults.

The biochemical basis of the activity of diflubenzuron seems to be due to inhibition of the normal process of insect cuticle formation. In general, the symptoms of poisoning with the compound are death of treated larvae due to their inability to shed the old cuticle at ecdysis, while body distortions of any newly-formed larvae suggest impairment of the mechanical properties of the new cuticle.

Electron micrographs of the newly-formed cuticle of cabbage white (Pieris brassicae) caterpillars following treatment with diflubenzuron support this interpretation. The endocuticle lacks the lamellar structure of normal endocuticle, and since this latter probably represents a strong yet suitably elastic arrangement of chitin microfibrils, the apparent weakness of the cuticle in treated insects is explained. It seems probably that the final stage in chitin synthesis - the polymerisation of n-acetyl glucosamine - is the one affected (Baldwin, 1974; Post et al, 1974).

The effectiveness of diflubenzuron on larval insects offers a great advantage over the juvenile hormone mimics, since it makes the compound applicable to the control of a much broader spectrum of insect pest species. As with the juvenile hormone mimics, the compound is of very low fish and bird toxicity, and also appears to be of low effect on crustacea, snails and amphibia (Anon, 1975 c).

The compound's intrinsic activity is very high - for example the EC_{50} on the southern house mosquito (Culex pipiens quinquifasciatus) is less than one-tenth that of juvenile hormone mimics used in the same test (Mulla et al, 1974). In contrast to many of the juvenile hormone mimics, the environmental persistence of diflubenzuron is reasonably high. (Anon, 1975 c).

An interesting property of the compound is its purely stomach poison activity. This, together with its lack of systemic activity means that diflubenzuron is effective against gross feeders such as Lepidopterous, Coleopterous and Dipterous larvae but not sucking insects such as aphids and other Heteroptera (Anon, 1975 c; Mulder and Gijswijt, 1973). As with juvenile hormone mimics, diflubenzuron also has the potential advantage of effectiveness against resistant strains of pests. (Schaefer et al, 1975).

Since treated larvae can feed and behave normally until ecdysis, some crop damage can occur if late-instar larvae are treated; "fire-brigade" applications will therefore be relatively ineffective. However early treatment against newly-hatched larvae - as normally occurs in routine insecticide applications - can give effective pest control.

Excellent kill of a number of larval crop pests, with good long term protection, has been obtained in field trials with the compound. Examples of insects controlled with low rates of application are the velvetbean caterpillar (Anticarsia gemmatalis) on soybeans, (75 g/ha) (Turnipseed et al, 1974), and gipsy moth (Porthetria dispar) on apple (40 ppm) (Granett and Dunbar, 1975). The compound has also given extended control of mosquitoes (Aedes nigromaculis and A. melanimon) (Schaefer et al, 1975), including asynchronous populations of Culex tarsalis (Mulla et al, 1974), out-performing a number of juvenile hormone mimics. The compound has also proved very effective against livestock fly pests (Wright, 1974; Miller et al, 1975).

Diflubenzuron and related phenylureas are not the only non-juvenile hormone mimics to have shown activity as insect growth regulator insecticides, though they probably have the greatest potential. At this conference four years ago the compound 2,6-di-t-butyl-4-(dimethyl-benzyl)phenol (MON 0585) was described (Sacher, 1971). This is a simple compound specific to mosquito larvae and effective in disrupting maturative moults at a relatively high rate of application (Jakob and Schoof, 1972; Steelman and Schilling, 1972; Schaefer and Wilder, 1972). More recently, 1-buten-3-yl N-(p-chlorophenyl) carbamate (H 24108) has been found to disrupt mosquito larval moults, also at relatively high rates (Mulla et al, 1974).

CONCLUSIONS

The newer insect growth regulator compounds will not, of course, be perfect insecticides. As with the juvenile hormone mimics, we must not anticipate that they will be panaceas for resistance; cross-resistance to diflubenzuron in houseflies (Musca domestica) has already been noted in the laboratory (Cerf and Georghiou, 1974). The compounds will also have a slight inherent disadvantage in that they are of little utility against late instars or adult insects.

But it has become apparent that these compounds can possess some of the toxicological and selectivity advantages of the juvenile hormone mimics, while having a much greater range of applicability as a result of their "larvicidal" effect. They thus represent a significant step forward in the pratical fulfillment of the concept of "third-generation" insecticides as envisaged a decade ago. There is no doubt that there will be further registrations of insect growth regulators as insecticides before the next Conference in this series.

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THE POTENTIAL USE OF PHEROMONES IN INSECT CONTROL

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Summary Insect sex pheromones may be used for the study of population movements, as monitors of crop infestation levels or as controlling agents by mass-trapping or direct application techniques. The pheromones of several key insect pests have been synthesised and the present status and potential of these chemicals is discussed.

Resume L'emploi de pheromones est pour etudier des movements de population, pour predire des infestations et pour des methodes directes de controle est discute.

INTRODUCTION

The main uses of pheromones in insect control are (a) for the study of population movements and distribution (b) as crop monitors or warning systems by relating catches in traps to subsequent larval infestations and (c) for controlling the insect either by mass-trapping or by direct application techniques using an attractant (pheromone) or an inhibitor.

Recent general reviews on the control of insects by sex pheromones include those by Jacobson (1972), Birch *et al*. (1974), Tette (1974) & Tamaki (1974).

The aim of this paper is to illustrate the practical uses of pheromones with particular reference to work carried out by members of the Ministry of Overseas Development.

POPULATION MOVEMENTS AND DISTRIBUTION

Insect surveys are carried out in order to estimate the changing population of the target pest insect or insects within the selected region to show whether such changes follow a predictable pattern from which a long range forecasting system might be developed. In the past such surveys have invariably been carried out using light traps, together with periodic observations in the field for the presence or absence of the target insect. A good example of this approach has been the development of a forecasting system for the East African armyworm, *Spodoptera exempta* (Brown *et al*., 1969).

The disadvantages of light traps are the lack of specificity for the target insect and hence the need to sort out the catch, and also the need for power supplies and lamps which may be difficult to provide in remote areas. Species specificity, high sensitivity and low operational cost are the main advantages of traps baited with pheromones. The sex pheromones of S. exempta have recently been isolated and synthesised (Beevor et al., 1975) and comparative studies of light trap and pheromone trap catches are in progress in East Africa (D.G. Campion et al., in prep.). Surveys using pheromone traps are being carried out in Malawi for the red bollworm, Diparopsis castanea and the pink bollworm, Pectinophora gossypiella (Marks 1975), and in Cyprus and Crete for the Egyptian cotton leafworm Spodoptera littoralis (Campion, 1974; D.G. Campion et al., in prep.). The pheromones of D. castanea and S. littoralis were identified and synthesised by Nesbitt et al. (1973, 1975) and Hall et al.(1975).

The accumulation of reliable data by an adequate number of pheromone traps when mapped by computer (see Taylor, 1974) makes it easy to follow the area occupied by, and the rate of spread of, the target insect. In Cyprus, for example, a network of 50 traps was regularly distributed throughout the island in an attempt to show whether immigrations occurred from neighbouring countries by relating daily changes in the number and distribution of moths caught within the trap network, with the meteorological situation (Campion, 1974). In order to interpret changes in trap catches, the behaviour of the insect in relation to factors such as windspeed, relative humidity and temperature must be considered. For S. littoralis this was by means of a monitoring device which recorded the exact time of arrival of individual moths at the pheromone trap. A significant relationship was established between windspeed at 1.37m above the ground and numbers caught; with a windspeed ranging from 0.8 to 5.2 m/s the higher windspeed resulted in increased catches. Increase in relative humidity was associated with a decrease in the numbers recorded; while at temperatures below 13°C, few males arrived at the trap (Campion et al., 1974b). Further studies on the orientation of S. littoralis to an odour source are in progress in Crete. This work involves measurements of the turbulence structure of the pheromone plume and of the flight tracks of the insects, in order to clarify the dominant orientation mechanisms (J. Murlis, personal communication).

POPULATION MONITORING

Phermones may be used to assist farmers in limiting the number of insecticide applications so as to achieve maximum effectiveness with reduced cost and reduced contamination of the environment. After extensive field trials in the United States using hexalure baited traps for the detection of pink bollworm, Pectinophora gossypiella, it was concluded that basing the timing of insecticide application on pheromone trap catches resulted in the same level of control and with fewer applications c.f. regular spraying by date (Toscano, et al., 1974). A similar approach has been adopted in Israel. Capture of more than five males during two consecutive nights is used as an indication to spray. Such a system is claimed to have reduced insecticide applications by half, with the same level of control. This method is also being investigated in Israel for spiny bollworm, Earias insulana using traps baited with virgin females (Vermes, 1975). Marks (1975) in Malawi, however, reports a poor correlation between moth catch of red bollworm, Diparopsis castanea with egg counts on cotton, although further investigations are in progress. In Cyprus, weekly samples of S. littoralis were collected throughout the year from lucerne by sweep-net technique, but whereas the change in larval numbers appeared to be correlated with prior light trap catches (Robinson light traps powered with mercury-tungsten lamps) on some sites, no such correlation was found using pheromone traps (W.R. Ingram in Anon., 1974). Further analysis has revealed that the positioning of the traps relative to the topography of the site is an important factor affecting the relative catches in light and pheromone traps. The highest ratios favouring pheromone traps occurred in exposed positions, while sheltered sites tended to shift the ratio of catches in favour of the light traps (Campion, 1975). Such factors will have to be considered when relating trap catches with subsequent larval infestations. Trap design and size is another factor affecting the number of insects caught. Traps range from small sticky-lined paper cylinders to large 60 x 60 cm water traps. For S. littoralis the larger the trap the greater the number of insects caught (Campion et al., 1975). Pheromones are generally dispensed in polythene vials, laminated plastic strips or rubber septa (Marks 1975). In a series of experiments in Crete for S. littoralis 2mg aliquots of pheromone, cis9, trans-11 tetradecadien-1-yl acetate plus antioxidant per polythene vial provided a continuous source of attraction for 9-10 weeks (Campion et al., in prep.).

MASS-TRAPPING

It is possible to conceive of controlling insects by mass-trapping techniques. This approach has met with some success in the control of the red-banded leafroller, Argyrotaenia velutinana (Trammel et al., 1974). Under special circumstances of small island situations or in ecologically isolated areas, mass-trapping may be effective in maintaining the pest population at tolerable levels, but this method is unlikely to be generally applicable. Simple mathematical models of mass-trapping alone or in combination with sterile insects were proposed by Knipling & McGuire (1966) and by Murdie & Campion (1971). A possibility at present being explored for S. littoralis is a bait station consisting of pheromone plus a virus preparation; attracted males are subsequently hoped to transmit the virus during the process of mating, and so rapidly spread the infection throughout the population (D.J. McKinley & T.W. Tinsley, personal communication).

COMMUNICATION DISRUPTION

The confusion principle relies on the assumption that an insect will be unsuccessful in locating a mate if the atmosphere is permeated with a number of point sources of the pheromone. However it has yet to be determined whether such confusion occurs because of reduced mate finding, attraction to artificial sources or because communication is totally disrupted as a result of habituation to the odour, which would mean that the insect would be unable to perceive any level of the pheromone.

Inhibitors may also be used to disrupt insect communication. In some cases they are easier to synthesise, and unlike attractant pheromones will not be expected to attract insects from surrounding areas. Little is known about the specific activity of the inhibitors. It has been suggested by Roelofs & Comeau (1971) that they produce their effects by interferring with a specific sex pheromone binding site in the antennal sensilla of males. However, all chemicals that inhibit the attraction of males to a point source of pheromone are not necessarily effective disruptants when evaporated in the air surrounding the source.

(a) Pheromone dispensers

Shorey *et al*. (1974) evaporated a synthetic attractant for *P. gossypiella*, hexalure, within cotton fields throughout the season. 10 mg of the attractant was applied to a knot in a short loop of cotton string; with approximately 30,000 loops distributed per week in 4.8 ha of cotton. A loop was placed around a leaf near the top of a cotton plant for every 1.6 m^2 of the field. The evaporation rate of the hexalure was approximately 400 mg/ha each night. This resulted in a reduction of larval infestation that compared favourably with commercial applications of insecticides. At the present time field trials in a plot size of 5,000 ac (2023 ha) are in progress. Although encouraging, the main problem has been the setting out of the large number of pheromone dispensers.

Small scale disruption experiments over a 19-day period were successfully carried out in Cyprus using the synthetic pheromone of *S. littoralis* (Campion *et al*., 1974a). Two hundred polythene dispensers each containing 500 µg of the pheromone, *cis*-9, *trans*-11, tetradecadien-1-yl acetate, were evenly distributed throughout plots of 0.2 ha by attaching them with string to the stems of the potato plants. Assuming a constant emission of pheromone from the polythene, this gave an approximate release rate of 1,000 µg/ha/h, a rate similar to that of Shorey *et al*. (1972), to cause 95% disruption of cabbage looper moths, *Trichoplusi ni*, in lucerne fields using the synthetic pheromone looplure, *cis*-7-dodedenyl acetate. If the number of point sources was reduced in the *S. littoralis* experiments however, no reduction in trap catch was achieved. This may have been because the pheromone dispensers were close to the ground, since McLaughlin *et al*.(1972) found that disruption of pink bollworm males decreased when pheromone dispensers were near the ground rather than just above the crown of the plants. Inadequate aerial distribution of the pheromone appeared to be the most likely reason for this decrease.

Marks (1975) in Malawi has carried out tests in a large field cage enclosing 0.2ha of cotton using the pheromone of *Diparopsis*, a mixture of 85% *trans*-9, 11-dodecadien-1-yl acetate and 15% 11-dodecen-1-yl acetate. Approximately 1mg aliquots of the pheromone mixture were applied in the first experiment to 7,100 polythene fragments and in a second experiment to 3,629 polythene vials, to give application rates of 42.3 and 21.0g/ha. This resulted in an average reduction in mating over a one month period of 72.5% and 47.9% respectively. An inhibitor *trans*-9-dodecenyl acetate used in vials at the rate of 37.9g/ha produced a reduction in mating of 71.5%.

(b) Microencapsulation

In order to dispense pheromones in a more economic manner, microencapsulated formulations have been investigated and have shown great promise for the suppression of the gypsy moth *Porthetria dispar*. The gelatin based capsules were applied at the rate of 5g/ha of the pheromone disparlure, *cis*-7, 8-epoxy-2-methyloctadecane, in test plots of 100ha or more (Cameron *et al*., 1973; Schwalbe *et al*., 1973). Smaller scale experiments on the same lines have also been carried out using microencapsulated formulations of the pheromones of the oriental fruit moth *Grapholita molesta* (Gentry *et al*., 1974).

Evidence for a mating inhibitor, *cis*-9 tetradecen-1-yl acetate as part of the pheromone complex of *S. littoralis* was presented by Campion *et al*., 1974a. Preliminary trials using microencapsulated formulations of this material have recently been completed in Crete. Plots of lucerne in the north-west of the island varying in size from 500 to 2,000 m^2 were sprayed using a Cooper Pegler CP3 knapsack sprayer with quantities of the inhibitor ranging from 1 to 10g/ha. The trials were carried out in areas of known moderate to heavy larval infestations of S.littoralis. The trials were not aimed at control, since applications were made late in the season, but were intended to show whether disruption could be achieved under typical field conditions. At the highest rate of 10g/ha almost complete disruption of communication was achieved when measured by the absence of males in traps baited with the synthetic attractant. The effect persisted until the crop was cut, a period of one to two weeks (Campion et al., in prep.). Further experiments are planned for 1976 when larger semi-isolated areas of lucerne will be treated earlier in the year with the aim of controlling the insect.

(c) Pheromone fences

The control of insect pests of lucerne can be confounded or even solved by the frequency or timing of cutting the crop (Anon, 1974). On the one hand by cropping the lucerne many insects are killed, but once regrowth has begun further insect attacks may be expected and further applications of insecticide may be necessary. With this consideration in mind the naturally occurring inhibitor of S. littoralis, cis-9-tetradecen-1-yl acetate (cis 9-TDA), was incorporated into lengths of polythene tubing and used to enclose areas of lucerne. The polythene pheromone 'fences' were held by stakes at a height of 0.5m to see whether entry to the area under treatment could be prevented as measured by the catches of males in traps baited with the synthetic attractant in the centre of the test plots. The plot sizes were 10, 100 and 1000 m^2 and in all instances the capture of males was greatly reduced when compared with captures of males in unfenced plots (Campion et al., in prep.). Whether such an approach can be of practical value needs further investigation.

(d) Multiple disruption

The compound cis-9-TDA is also part of the pheromone complex of the fall armyworm Spodoptera frugipierda. When this substance was dispensed in polythene vials together with the synthetic pheromone of T.ni, looplure, it was found that the combination not only reduced the capture of males of S. frugiperda and of beet armyworm Spodoptera exigua to traps baited with virgin females of these species but also reduced the capture of males of T.ni and the soybean looper Pseudoplusia ineludens to female baited traps (Mitchell, 1975).

It is therefore possible to concieve of an insect control programme whereby a number of disruptants are combined to match a particular pest complex situation.

CONCLUSIONS

Field trials of varying magnitude are in progress using either attractant pheromones or inhibitors for a number of economically important insects. More work needs to be done particularly with respect to formulation; the use of microencapsulation techniques seems particularly promising.

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VIRUSES AS PESTICIDAL AGENTS: POTENTIAL AND LIMITATIONS

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Summary In the past, most insect pests of agriculture, forest and public health importance could be controlled in large measure by regular application of chemical insecticides. However, the increasing resistance of many insects to insecticides, environmental problems associated with the persistence of insecticide residues and the world situation of spiralling inflation have led to a demand for cheaper and ecologically safer methods of pest control. It follows that any alternative methods that are developed must be no less effective in controlling pest populations than the chemical insecticides they are intended to replace.

The potential of insect pathogenic viruses in this context is discussed with particular reference to the limitations posed by the present lack of knowledge of the physical, chemical and biological properties of many of the viruses most frequently suggested for field use.

Insects constitute 75 per cent of the known animal species in the world and compete with man and his domestic animals for food but in addition cause considerable physical discomfort either by direct irritation or as vectors of disease-causing agents.

Previously, the only effective and long-term answer to the problem was to reduce insect numbers by applying chemical insecticides. Since World War II, both crop pests and disease vectors have been controlled in large measure by regular applications of persistent insecticides usually containing BHC and/or DDT and crop losses and incidence of pathogenic diseases carried by haemophagous insects were reduced. For example, regular application of insecticide reduced the incidence of leaf—defoliating caterpillars on cotton in Africa and U.S.A. and assisted in the eradication of malaria by the control of specific mosquito vectors in some tropical countries.

However, in recent years, serious and unpredictable events have forced a reappraisal of the wisdom of large-scale applications of insecticides. Pests both of crops and of man began to develop resistance to the pesticides and recent estimates suggest that some 300 different insect species are now in this category. The natural reactions to this situation were to increase dosage levels of the insecticides and/or to introduce other new formulations. Unfortunately, resistance to one insecticide is frequently non-specific and this results in the resistance of the insect hosts to other insecticides. This situation was aggravated further by the discovery of chemical contaminants in fish and wild birds which owed their origin to insecticides applied to arable crops. Therefore certain chemical insecticides had taken on a new but insidious role as environmental pollutants and thus a vicious circle had been created. This situation has now been aggravated by spiralling world inflation and the associated increased costs of petrochemicals and this has resulted in insecticides becoming more expensive and the costs of application have risen dramatically, and so the need to provide cheaper, alternative methods of pest control has to be regarded as a first order priority if world food supplies are to be maintained. Public anxiety over chemical pollution has naturally favoured a re-appraisal of the biological control of insect pests which is regarded as being more ecologically respectable. The concept of integrated control of pests is not new or revolutionary as it embraces systems of cultural manipulation with the breeding of resistant crop varieties and the use of the natural enemies of the insects such as parasites, predators and pathogens. Various combinations of these treatments have been practised at one time or another since the turn of the century. Unfortunately, apart from several spectacular successes arising from the introduction of exotic parasites or predators, these natural systems have frequently lost favour either because the level of control achieved was less than that obtained with chemicals or the results were quite unpredictable in that adequate control was achieved in some years and not in others.

The ability of pathogens to invade and kill their insect hosts was well known to Pasteur from his work with diseases of the silkworm and attempts have been made since then to employ fungi, bacteria, protozoa and viruses as pesticidal agents. Most authorities are now agreed that viruses offer the greatest potential in biological control systems in that highly virulent viruses can be isolated which can cause high levels of mortality when introduced into insect populations. However, it has to be remembered that viruses are replicating systems and once released it could prove difficult, if not impossible, to contain their spread, particularly if they proved to cause disease in organisms other than the target pest. Therefore, if viruses are to be used in pest control systems, then they must be used in a responsible manner for the indiscriminate release of insect viruses would be just as reprehensible as was the indiscriminate use of persistent chemical insecticides.

Insects are attacked by a large range of insect viruses and at present seven groups of viruses have been recognised. /Table 1/ In the context of this paper, it is necessary only to consider those virus types which appear to be confined to the Class Insecta, or at very least to Invertebrates, as a specific host range would imply reduced ecological hazards. Such a group is the Baculoviruses (Wildy, 1971) which are very complex viruses but appear to have no chemical, physical or biological properties in common with any known virus found in either vertebrates or plants. It was for this reason that WHO and FAO jointly recommended that, for the time being, only the Baculovirus group should be considered as possible pesticidal agents (WHO 1973).

The consideration of a virus for biological control should involve four essential stages of investigation. These are:

(1) The isolation and purification of the virus followed by detailed investigations of its physical, chemical and biological properties. An immediate practical requirement of such studies is the development of methods of unequivocal identification.

(2) The testing under laboratory conditions of the efficacy of the purified virus as a pesticidal agent and the establishment of its host range.

(3) The testing of the toxicological properties of the virus together with any associated formulative materials and the investigation of the possibility of infection and replication in non-target invertebrates and vertebrates. These would form the basis for an assessment of safety in field use.

(4) Once the virus has satisfactorily passed these safety testing procedures then field trials could be planned to confirm the results of laboratory tests on efficacy and dosage rates. In such field studies, a system of monitoring the environment must be developed and be in operation before the trials begin, during application and for a considerable period thereafter.

Unfortunately, very few investigations of this nature have been made and the principal reason for this is the serious shortage of scientific workers in this field (Tinsley and Melnick, 1973). As an illustration of an ongoing programme, The Natural Environment Research Council's Unit in Oxford has been working on four Baculoviruses (Nuclear polyhedrosis viruses) isolated from four species of the genus Spodoptera (Lepidoptera:Noctuidae). These are the East African armyworm (S.exempta); the cotton worm of the Mediterranean area (S.littoralis); the fall armyworm, United States (S.frugiperda); the beet armyworm, United States (S.exigua). These four pests are considered to be of first rank importance in the geographical areas in which they occur. This work is in collaboration with the Centre of Overseas Pest Research of the Ministry of Overseas Development. It was not known what, if any, relationships existed between the four virus isolates and so it was logical to regard them as being quite distinct entities until they proved to be related. Over the last two years, existing methods have been improved and new ones devised whereby healthy insects can be reared on artificial diets, infected with known inocula, and the virus recovered in large quantities from the subsequent moribund hosts. Chemical and physical methods of separation then led to preparations of virus in a highly purified form. The next step was to characterise the proteins and nucleic acids of the four purified viruses. These investigations are nearing completion and, using the accumulated data on physical and biochemical properties, it is possible to recognise each of these four viruses with certainty. It was soon evident that within these four isolates there were two distinct groups or serotypes and each had its own inter-related strains. Simplified serological tests using type-specific antisera are now available which are sensitive enough to detect each individual virus isolate even when occurring in mixed infections. This is the first time that such a detailed investigation has been undertaken and also the first time that it has been possible to make unequivocal identifications of any of the Baculoviruses. These identification methods are essential to undertake meaningful investigations of the pathogenicity and host range of insect viruses, for without them it is quite impossible to test whether the host insects had died from the test virus, from a second unrelated virus which occurred as inapparent infection or from a cross-contamination from other sources.

The safety tests of two of these Spodoptera viruses are being undertaken by the Microbiological Research Establishment, Porton, under a contract from the Ministry of Overseas Development. Once these tests are completed, an assessment of potential ecological hazard will have to be made, and then field tests in the areas in which the pests occur can be considered.

Many Baculoviruses of insects have been used in field programmes at one time or another, e.g. pine sawfly (Neodiprion sertifer) in Canada and Great Britain, the cotton ear worm (Heliothis zea) and the cabbage looper (Trichoplusia ni) in the United States. The scale of these applications has varied from small scale local releases to aerial spraying. The ability of certain viruses to create extensive epizootics which become effective controlling-factors need not be questioned as there is ample evidence of such efficacy. This would apply equally to artificial epizootics as well as those that frequently occur under natural conditions. Considerations of safety and ecological hazard apart, there are still problems of costs of preparation and application, the retention of infectivity under field conditions as well as the time factor. Viruses which take a long time to kill the insect pests would have reduced effectiveness as death may only result after the pest had already created severe economic damage. It is known that the older the insect larva becomes, the longer the time taken to die from virus infections.

Solar radiation can have deleterious effects on infectivity of these viruses so that the time of application and the site of deposition of the virus on the food plant become key factors in determining efficacy of the viruses. The association of the virus particles with different leaf surfaces may also be important in the availability or virulence of effective inoculum. It is very probable that certain field applications of virus in past years failed not because the virus was ineffective or the host not susceptible, but that the inoculum was rendered non-infectious by some or all of these unknown variables soon after application.

A detailed knowledge of how insect viruses are disseminated under natural conditions and what factors control such movements are essential before commencing control programmes. Unfortunately, there is a serious lack of information in this area and it is for these reasons that my colleagues in Oxford are devoting half of our research effort in trying to answer these ecological problems.

In conclusion, it is pertinent to speculate what could be the future developments in this particular field? Meaningful field trials with the Spodoptera viruses are likely to take place before too long. This, however, represents only two viruses and results from the trials may or may not be applicable to others. It could be predicted that the emphasis will be on application systems involving very low dosages of virus, protected against environmental inactivation, and very probably applied in the form of a prophylactive high volume spray to young crops before the insect eggs hatch. If one can kill 1st instar larvae over an extended period, then it follows that little damage will result. The very low dosages required to kill the hatching larvae with consequent reduction of aerosols would considerably reduce the possibility of ecological hazards.

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Table 1

Groups of insect pathogenic viruses

Group	Nucleic Acid	Inclusion Body	Symmetry of Particle	Similarities to viruses of vertebrates and plants based on morphological and biochemical criteria	
				Vertebrate	Plant
Baculoviruses	DNA	+	Helical	Non - apparently restricted to invertebrates	None
Cytoplasmic Polyhedrosis Viruses	RNA(DS)	+	Spherical	Reoviruses, Blue-tongue Virus	Wound tumour, Rice Dwarf, Viruses of Fungi
Entomopox Viruses	DNA	+	"Brick"	Poxviruses	None
Icosahedral Cytoplasmic Deoxyriboviruses	DNA	-	Spherical	Viruses isolated from Fish, Frogs, Lizards, African Swine Fever	Viruses in Fungi and Algae
Parvoviruses	DNA(SS)	-	Spherical	Parvoviruses	None
Picornaviruses	RNA	-	Spherical	Enteroviruses	Small RNA Viruses
Rhabdoviruses	RNA	-	Bacilliform, Bullet shape	Rabies, Viruses of Fish	Potato Yellow Dwarf, Lettuce Necrotic Yellows Etc.