TOPIC 2B

NON-CONVENTIONAL CHEMICAL AGENTS

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NON FUNGICIDAL COMPOUNDS, WHICH PREVENT DISEASE DEVELOPMENT

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ABSTRACT

Compounds without fungicidal or fungistatic activity in vitro, may provide disease control by interfering with pathogencity or by increasing the resistance of the host plant. The mechanism of action of a few commercial disease control agents, which belong to this category, and some experimental compounds, are discussed. Attention is paid also to resistance due to 'sensitization' of host tissue at a distance. The possible advantages of the application of non-fungitoxic compounds, and the prospects for use of them in the future are discussed.

INTRODUCTION

The study of disease control by non-fungitoxic compounds may be of scientific as well as practical interest. Compounds which in a subtle way interfere with the relation between host plant and parasite may be helpful tools to broaden our knowledge about the physiological-biochemical background of parasitism. With respect to disease control in practice they might offer, at least theoretically, certain advantages above intrinsically fungitoxic compounds:

- less hazard to man, non-target organisms and the environment in view of highly selective, non-biocidal action
- induction of resistance in hosts which lack specific genes for resistance may enable agriculture to make better and greater use of existing agronomically superior plants (Kuč, 1981).
- depending on the mechanism of action, chance for development of 'fungicide-resistance' may be less than for systemics which are direct fungitoxicants
- disease control by 'sensitization' of host tissue, without distribution of fungicides in the plant or elicitation of fungitoxic compounds before the fungus attacks
- the possibility exists that protection by induced resistance, perhaps at hormonal level, will be longer lasting than that of fungicides and will require only extremely low concentrations of a specific chemical
- chemical control of diseases, which hitherto can not be controlled effectively by pesticides, e.g. wilt-, bacterial- and virus diseases

Although also some fungicides may exert indirect action, via the host plant, emphasis in the discussion will be on compounds, which provide disease control exclusively or mainly by other than direct fungitoxic action. No attempt, however, has been made to present an exhaustive review of all chemicals, which have been reported to act in this way. Reading of reviews by Dimond and Rich (1977) and Langcake (1981) can be recommended.

The aims of this treatise are to discuss the principles, upon which disease control by non-fungicidal compounds is based, to review the results obtained until the present, and to consider the prospects for the use of such compounds in agriculture. Although induction of disease resistance by biotic agents falls outside the scope of this treatise, some attention will be paid to it, as also here non-fungicidal chemicals, produced or induced by these agents, might be involved.

MECHANISMS OF ACTION

Compounds without fungitoxic action in vitro and which are not converted into a fungicide after uptake, may influence the relation between plant and parasite in favour of the host in various ways. They may act on the parasite, interfering with pathogenic processes or sporulation, on the host, increasing its resistance to the pathogen, or they may influence both host and parasite. This may impair recognition between host and parasite, penetration or colonization.

Effects via the plant

Non-fungitoxic chemicals, which provide disease control, may do this by induction or stimulation of specific resistance mechanisms, or by changing the plant metabolism in a non-specific way, rendering it less suitable as a host. Such compounds are said to 'sensitize' the host tissue, when the changes in treated plants only occur upon contact with the parasite. Systemic sensitization, at a distance from the inciting agent, will be discussed separately. Disease control by induced resistance of the plant, may involve various processes, as illustrated by the following examples.

Lignification

In cucumber plants, naturally resistant to *Cladosporium cucumerinum*, lignification was observed around infection sites. This did not or less readily occur upon infection of susceptible plants (Hijwegen, 1963). By lignification a physical barrier may be formed, which stops or retards growth of the pathogen. Chemicals which stimulate the host to such physical barriers upon infection, may increase disease resistance. Even if these barriers do not completely stop the pathogen, fungal growth may be retarded, so that more time will be available for accumulation of phytoalexins or build-up of other defence reactions. Phenylthiourea, a weak fungicide, which provides good control of cucumber scab, has been reported to act in this way (Kaars Sijpesteijn and Sisler, 1968).

Phytoalexin formation

Upon inoculation of plants with pathogenic or non-pathogenic micro-organisms, fungal elicitors may induce the production of fungitoxic chemicals, which may inhibit or retard growth of the pathogen (Kuć, 1977). Such compounds were called phytoalexins, as they were considered to play a role in disease resistance. It has been found that the formation of phytoalexins may also be induced by non-biotic agents, among which fungicides (Reilly and Klarman, 1972) as well as non-fungitoxic compounds (Erwin, 1977).

Changes in polyphenol metabolism

In studies on natural resistance of plants to pathogens, it has been observed frequently that, upon infection, the metabolism of polyphenols in resistant plants differs from that in susceptible plants. These metabolic changes may influence susceptibility in two ways. There may be an accumulation of fungitoxic polyphenol derivatives at the infection sites, and an enhancement of lignification may be made possible by the availability of phenolic precursors for lignin. Compounds which stimulate such changes in polyphenol metabolism may contribute to resistance. Evidence has been obtained that phosétyl-Al (Aliette) (Bompeix et al., 1981) and probenazole (Oryzemate) (Sekizawa and Mase, 1981), might act, at least partially, along these lines.

Strengthening of cell walls

The composition of the cell wall may play a role in natural disease resistance. In cucumber plants of a cultivar resistant to anthracnose, resistance appeared due to a chemical change in the middle layer of the radial wall of the epidermis cells, so that the fungal pectic enzymes could not digest the material (Yasumori, 1964). It seems that such a type of resistance of the cell wall to fungal attack can be influenced by chemicals. Certain growth substances, based on naphthalene acetic acid, reduced *Fusarium* wilt of tomato.

It has been reported that compounds with auxin activity may reduce the degree of methoxylation of pectates in the middle lamella, which allow an increased formation of calcium pectate bridges. The latter strengthen the middle lamella against attack by pectolytis enzymes, which might explain reduced susceptibility of the treated plants (Corden and Edgington, 1960).

Anatomical changes, closure of stomata

Disease progress of vascular diseases may be influenced by the anatomy of the xylem vessels. Some growth substances have been reported to contribute to blockage of these vessels, among others by the formation of gums and tyloses, thus restricting the spread of the pathogen propagules (Dimond and Rich, 1977). These authors also suggest that compounds, which cause stomata to close, might temporarily reduce infection.

Other changes in the plant

Natural disease resistance may be due to insensitivity to fungal toxins and enzymes, or to the ability of the plant to inactivate these fungal products. Compounds which stimulate these capacities of the plant may contribute to resistance. It was observed by Tamari et al. (1966), that chlorogenic acid or ferulic acid which are naturally present in the plant, detoxify the rice blast toxin pyricularin by forming an inactive complex with this compound. Chemicals which stimulate the formation of chlorogenic or ferulic acid in the plant might therefore contribute to resistance. Pretreatment of oat plants with sulphhydryl containing compounds made the tissue more resistant to victorin, a toxin produced by *Helminthosporium victoriae*, probably because these compounds attach to the toxin receptor in the membrane (Gardner and Scheffer, 1973). α-Galactosides reduced sugar cane eye spot caused by *Helminthosporium sacchari*, presumably by competing with the toxin helminthosporoside for the membrane receptor sites (Strobel, 1973).

Effects via the pathogen

It has been shown that compounds, which do not inhibit fungal growth or spore germination, may interfere with those capacities of the pathogen, which it needs for infection or build-up of an epidemic.

Inhibition of pathogen enzymes, which degrade cutin or cell walls

Non-fungicidal compounds may reduce disease by inhibition of the production or the activity of fungal enzymes, necessary for infection. Inhibition of pea epicotyls by *Fusarium solani* was prevented by the cutinase inhibitor diisopropylfluorophosphate and by specific antisera prepared against fungal cutinases (Maiti and Kolattukudy, 1979). Also some commercial fungicides as Hinosan and Kitazin P and some insecticides were reported to protect pea plants against *Fusarium solani* at non-fungicidal concentrations, presumably due to cutinase inhibition (Köller et al., 1982).

The formation of pectolytic or cellulolytic enzymes by *Fusarium* or *Verticillium* spp. is inhibited by various chemicals, among which rufianic acid. This compound gave also reduction of tomato wilt, caused by fungi (Grossmann, 1968). Also compounds which stimulate polyphenol metabolism might act in this way. Glucose, monogalacturonic acid and 2-deoxyglucose decreased wilt symptoms in tomato stems, infected with *Fusarium oxysporum* f. sp. *lycopersici*, presumably due to repression of polygalacturonase synthesis in the pathogen (Patil and Dimond, 1968).

Effect against fungal toxins

Many fungi produce compounds, which are toxic to plant cells. Some of these do not contribute to disease symptoms, but others are wholly or partially responsible for disease development in the plant. Chemicals which inhibit the biosynthesis of the latter toxins, or reduce their activity, may provide disease control, as has been suggested already by Horsfall and Zentmeyer (1942). There are only few cases where reduction of disease has been obtained by compounds which specifically counteract fungal toxins. Application of ferulic acid protected rice plants against Pyricularia oryzae, presumably by complexing with the toxin pyricularin (Tamari et al., 1966). Kinetin has been reported to counteract the toxic effect of Pseudomonas tabaci (Farkas and Lovrekovich, 1963).

Counteraction of other fungal products

In addition to enzymes and toxins, other fungal products may be important for infection. Inhibition of the biosynthesis of melanins by tricyclazole (Woloshuk and Sisler, 1982) and of myo-inositol by validamycin A (Wakae and Matsuura, 1975) have been claimed to be primarily responsible for control of rice blast and sheath blight, respectively, by these chemicals.

Reduction of sporulation

On the basis of studies on fungal differentiation, with the use of a number of metabolic inhibitors, Lukens and Horsfall (1973) suggest that metabolic pathways exist, which are specific for sporulation. Inhibitors, which act specifically at these sites, may effect sporulation without being fungitoxic. Although various fungicides reduce sporulation already at low concentrations, non-fungitoxic compounds which interfere specifically with sporulation seem to be rare.

Systemic sensitization of plant tissue

It has been observed that inoculation of certain plant parts with biotic agents, for example a pathogen, an avirulent form of a pathogen or a non-pathogen, may 'sensitize' tissue in other plant parts in such a way that a resistance response follows upon challenge inoculation with the pathogen. Kuć et al. (1975) obtained systemic protection against the fungal pathogen Colletotrichum lindemuthianum in the upper leaves of bean plants, when 4-5 days in advance the first leaf had been inoculated with a non-pathogenic race of this fungus or with C. lagenarium, which is not pathogenic on beans. No fungitoxic compounds or other changes could be detected in these upper leaves before inoculation. Only after a challenge inoculation fungitoxic substances or physical barriers were formed together with a restricted collapse and browning of host cells. Apparently a signal is produced, which enables cells in other leaves to respond in a resistant way upon a challenge inoculation. Systemically induced resistance appeared not to be limited to plant parts above the site of the first inoculation. It was postulated that a 'resistance inducing substance' moves upwards as well as downwards in the plant. Cohen and Kuć (1981), who observed systemically induced resistance to blue mould in tobacco leaves by prior stem inoculation with Peronospora hyoscyami f. sp. tabacina, suggest a shift in the hormonal balance.

The systemic induction of resistance resembles the systemic elicitation of proteinase inhibitors in response to infection. Inoculation of the lower leaves of tomato plants with Phytophthora infestans increased inhibition of proteinases in non-inoculated upper leaves. The increase was about two times greater after inoculation with an incompatible than with a compatible race of the pathogen and was associated with hypersensitive resistance (Peng and Black, 1976). Inhibition of certain extracellular pathogen enzymes, which are important for virulence, might be involved. The factor, which induces systemic increase of proteinase inhibitors at a distance from the site of inoculation, is called proteinase inhibitor inducing factor, PIIF. The activity of this factor appears to reside in oligosaccharides, which are enzymatically released from cell walls (Bishop et al., 1981). They are suggested to play hormone like roles in regulating plant defense responses away from their site of release (Ryan et al., 1981). When young excised tomato leaves were treated with PIIF, mRNA could be detected, coding for precursors of proteinase inhibitors I and II (Nelson et al., 1981).

Systemic changes in tomato plants upon inoculation with Cladosporium fulvum have also been observed by De Wit and Bakker (1980). A new protein was formed in a non-inoculated leaf, opposite to the inoculated leaf. The production of this protein appeared to be associated with the hypersensitive response. Its induction at a distance from the inoculated site, indicates the formation of a signal, the nature of which is still obscure.

The above mentioned observations show that biotic agents, without causing the direct formation of physical or chemical barriers in the inoculated host, may sensitize the host tissue even at distant sites in such a way that a resistance reaction follows upon challenge with the pathogen.

There are indications that also certain chemicals may 'sensitize' host plant tissue, so that it reacts with resistance upon inoculation. This seems to be the case after treatment of rice seedlings with dichlorocyclopropanes (Cartwright et al., 1980). It should be investigated whether, apart from transport of such compounds, also signals might be involved, which act upwards as well as downwards from sites where such chemicals are present.

NON-FUNGICIDAL DISEASE CONTROL AGENTS

Phenylthiourea

Although phenylthiourea is a very weak fungicide, it gave, when administered to roots of cucumber seedlings, a remarkable protection against Cladosporium cucumerinum. Upon infection of PTU treated plants a lignification reaction was observed at the infection sites (Kaars Sijpesteijn and Sisler, 1968). Inactivation of polyphenoloxidases and an increased peroxidase activity occurred in PTU treated plants, which suggested that lignification was enhanced by the availability of larger amounts of phenolics. Induced resistance in PTU treated plants might be attributed to lignification.

Procaine hydrochloride

Procaine hydrochloride provides a high degree of systemic protection against powdery mildew when administered to the roots or to the leaves of various plants (Dekker, 1961; Dekker and Van der Hoek-Scheuer, 1964). As it is not toxic to fungi in vitro, and does not inhibit germination of powdery mildew conidia in vitro, an indirect action via the host plant is suggested. It is further known that local anesthetics like procaine-HCl act on membranes by interaction with phospholipids (Papahadjopoulos, 1972). These changes might render the cell membrane more resistant to attack by fungal phospholipases. However, a direct effect on the penetration phase of the powdery mildew pathogen can not be ruled out.

Validamycin A

Validamycin A, produced by Streptomyces hygroscopicus var. limoneus, was introduced in 1972 under the trade marks Validacin and Valimon for control of rice sheath blight, caused by Pellicularia sasakii. This antibiotic is only active against diseases, caused by Rhizoctonia type fungi (Wakae and Matsuura, 1975). In tests with 3000 species of fungi and bacteria no antimicrobial activity was found of validamycin A, when sufficient nutrients were available, but on poor agar media radial growth of R. solani was reduced. Although the antibiotic altered the morphology of the fungus, it did not reduce the total mass of mycelium (Nioh and Mizushima, 1974). On treated rice plants, the pathogenicity of Pellicularia sasakii appeared remarkably reduced. Myo-inositol, which shows some structural resemblance to validamycin A, was able to restore pathogenicity to a certain extent. It also counteracted the morphological effect of the antibiotic on R. solani in vitro. It was suggested that the antibiotic interferes with biosynthesis of myo-inositol and that the latter compound is indispensable for pathogenicity, at least in this type of fungi (Wakae and Matsuura, 1975).

Dichlorocyclopropanes

The compound 2,2-dichloro-3,3-dimethylcyclopropane carboxylic acid (WL 28325) is a highly specific agent for control of rice blast, although its toxicity for Pyricularia oryzae in vitro is low and not sufficient to explain disease control. The ability of various dichlorocyclopropanes of similar structure to inhibit fungal growth in vitro did not correlate with their ability to prevent rice blast (Langcake et al., 1977). WL 28325 is not converted into a more fungitoxic compound by the plant. These observations indicate that disease control might be obtained primarily by increased resistance of the host plant. After administration of WL 28325 to healthy plants, no induction of new fungitoxic compounds could be detected. Upon inoculation of treated plants, however, a hypersensitive type of browning reaction was observed, and formation of fungitoxic compounds occurred. These compounds have been indentified as momilactobes A and B (Cartwright et al., 1980). It seems therefore that dichlorocyclopropanes 'sensitize' the rice plant in one way or another, so that on subsequent challenge with the pathogen a resistant type of response occurs. Although with these systemic compounds a significant reduction of rice blast could be obtained in laboratory experiments, the results were not such that it has been decided to develop them for application in practice.

Probenazole

Probenazole, 3-alloxy-1,2-benzisothiazole-1,1-dioxide, has been introduced in 1977 for control of rice blast, under the brand name Oryzemate. Although it showed only weak antifungal activity in vitro, it provided effective disease control when applied to the paddy water. Probenazole is converted in the plant into several other compounds, non of which is fungitoxic. It has therefore been investigated whether the defence reactions of the host are stimulated. When rice plants were treated with probenazole and subsequently inoculated with Pyricularia oryzae, there was an increased peroxidase activity and formation of anticonidial factors in the leaves (Watanabe et al., 1979). Four antimicrobial compounds, among which α-linolenic acid, could be isolated. It was further found that, in addition to peroxidase, also the activity of phenylalanine ammonia lyase and catechol-o-methyltransferase in rice leaves was increased, contributing to the formation of lignoid barriers at the infection site (Sekizawa and Mase, 1981). The same authors found that also indol acetic acid (IAA) and ethylene, when applied to rice leaves, increased peroxidase activity and induced production of antimicrobial factors. These effects were antagonized by abscissic acid, and also by inhibitors of protein synthesis, such as cycloheximide or blasticidin-S. Probenazole nor its degradation products showed growth regulating activity, so that it can not act in the same way as IAA or ethylene. It was further found that extracts of conidia or mycelium of P. oryzae contained factors, responsible for the induction of peroxidase, which apparently was stimulated in probenazole treated rice plants.

Taking the above mentioned observations into account, Sekizawa et al. (1982) proposed a reaction chain, starting with recognition between host and parasite and ending in the production of chemical and physical barriers, which they named 'signal transmission hypothesis'. They presume that probenazole acts at the first part of this chain, namely the recognition system.

Phosétyl Al

Phosétyl Al, aluminium tris (ethylphosphonate), was introduced in 1978 under the brand name Aliette. It is active against diseases, caused by Oomycetes, particularly downy mildews and Phytophthora's. Although fungitoxicity of Aliette in vitro is low, it provides remarkable disease control, even of some root diseases after administration to the leaves. In view of this it has been suggested that Aliette stimulates the defence mechanisms of the host. This subject is being studied extensively by Bompeix and coworkers (1981). Phosétyl Al stops extension of Phytophthora capsici in tomato leaves rapidly after administration. This effect, however, is counteracted by simultaneous addition of one of the following compounds: glyphosate, α-amino acetic acid and α-amino-β-phenylpropionic acid. These are enzyme inhibitors which interfere with the shikimate pathway and with the biosynthesis of phenyl propane units, necessary for formation of lignin. It is thought therefore that phosétyl Al stimulates the defence reactions of the plant by interference with polyphenol metabolism. In leaves of plants, treated with the fungicide and inoculated with the pathogen, a necrotic zone appears, which probably blocks colonization of the plant tissue by the pathogen or at least retards, the fungal development, leaving the plant more time to develop other defence mechanisms, e.g. the accumulation of phytopalexins. Bompeix et al. (1981) suggest that fungicides with such an indirect type of action might have less chance to encounter problems with fungicide resistance.

If the hypothesis about the mechanism of action of phosétyl Al proves to be true, it still has to be elucidated why the physical and chemical barriers act specifically against parasitism by Oomycetes.

Tricyclazole, tetrachlorophthalide and compounds with related mode of action

Tricyclazole was introduced in 1975 for control of rice blast under the names Beam, Bim and Blascide. It provides disease control at concentrations, which do not inhibit growth of *Pyricularia oryzae* in vitro. At very low concentrations it inhibits biosynthesis of melanins, the primary site being between scytalone and vermelone. A mutant of *P. oryzae*, deficient in melanin biosynthesis, appeared non-pathogenic (Woloshuk et al., 1980). Tricyclazole does not interfere with conidial germination and appressorial formation on isolated epidermal strips of *Bryophyllum pinnatum*, but it does prevent penetration of the epidermal wall. Appressoria do not melanize on strips of treated plants. The observation that successful penetration only occurs from melanized appressoria indicates a relation between melanin biosynthesis and pathogenicity (Woloshuk and Sisler, 1982). Also 4,5,6,7-tetrachlorophthalide, introduced somewhat later for control of rice blast, under the name Rabcide, provides rice blast control at concentrations, which are not fungitoxic in vitro. Although not structurally related, it blocks melanin biosynthesis at the same site as tricyclazole. On treated rice plants haustoria were formed, but no penetration pegs (Chida et al., 1982).

Other compounds which have been found to possess the same mechanism of action are pyriquilon, coumarin, 2,3,4,5,6-pentachlorobenzyl alcohol (PCBA), 4,5-dihydro-4-methyltetrazolo[1,5-α]quinazolin-5-one (PP 389), s-triazolo [4,3-α]quinoline, N-methyl-2-quinolone (Woloshuk and Sisler, 1982) and chlobenthiazone (Inoue et al., 1982). It has not yet been elucidated why these compounds selectively control only rice blast. Although tricyclazole inhibits melanization also in *Verticillium dahliae*, it does not control diseases caused by this pathogen (Chrysayi Tokousbalides and Sisler, 1979). It is not known whether the silicium content in rice leaves requires extra penetrating power by the pathogen.

Miscellaneous compounds

A vast literature exists on the role which growth regulators, such as cytokinins, auxins, ethylene and gibberellins, may play in pathogenesis (Heitefuss and Williams, 1977). It is not surprising therefore that administration of growth regulators or growth retardants to the plant may influence disease development. Most studies on the effect of growth regulators concern wilt diseases, e.g. *Fusarium* or *Verticillium* wilt in various crops. Erwin (1977), who reviewed this field, mentions also disease control by some growth retardants, namely of *Verticillium* wilt of cotton. It has been postulated that in control of wilt diseases, the blockage of xylem vessels, restricting the spread of propagules might play a role. In only few instances growth substances were active against other types of diseases, for example kinetin against powdery mildew of cucumber (Dekker, 1963) and tobacco (Cole and

Fernandes, 1970). In view of limited disease reduction and side effects on plants, prospects for growth regulators as disease control agents in practice seem limited. Promising results, however, have recently been obtained with the non-bactericidal growth retardant 3,5-dichlorophenoxy acetic acid against potato scab, caused by Streptomyces scabies (McIntosh et al., 1981).

A number of amino acids or derivatives have been found to reduce fungal plant diseases. In some cases this can be explained by direct action on the pathogen, e.g. N-lauroyl-L valine (Shida et al., 1981). The activity of this compound is attributed to surfactant action, causing leakage and cell death. In other cases disease control could not be explained by direct fungitoxic activity. D- and DL phenylalanine reduced apple scab (Kuč et al., 1959), L--threo-β phenylserine was active against cucumber scab (Van Andel, 1966). The latter compound increased oxidative breakdown of IAA in extracts from cucumber seedlings, and a relation between chemotherapeutic effect and influence on growth regulator metabolism has been suggested. Disease reduction seems restricted to those amino acids, that are not normally involved in the nitrogen metabolism of plants. An exception seems L.methionine, which was active against powdery mildew on cucumber (Dekker, 1969) and barley (Akutsu, 1977). The former author reports reversal of methionine activity by folic acid, suggesting interference of this amino acid with folic acid metabolism. One report concerns the induction of resistance in rice to Pyricularia oryzae by seed treatment with DL-alaninate hydrochloride (Arimoto et al., 1976)., but the mechanism of action is obscure. Methionine and serine, which control bean rust, counteract the pathogen stimulated incorporation of amino acids into protein (Pozsar et al., 1966). Serine, which is also active against cucumber scab, might act as a methyl donor, influencing methylation of cell wall pectins (Van Andel, 1966). It might be concluded that explanations for the various mode of actions of amino acids are still highly speculative. Non of these compounds is commercially used for disease control.

Soybean lecithin, a compound without fungicidal or fungistatic activity in vitro, appears active against strawberry and cucumber powdery mildew. Indirect action via the host plant has been suggested (Misato et al., 1977).

CONCLUSIONS, PROSPECTS

Although in laboratory studies quite often non-fungitoxic compounds have been found, which influence the host-parasite relation, the results are mostly not sufficient or rather variable due to environmental changes. In spite of the extensive screening in vivo of many thousands of new synthetic compounds each year, only relatively few compounds have become available for use in practice, which provide disease control by other than direct fungitoxic action. Due to lack of knowledge about the physiological-biochemical background of this relation, a rational design of non-fungitoxic disease control agents has not yet become possible. Discovery of such compounds has so far been a matter of trial and error. It should be mentioned that also some compounds which primarily acti by direct fungitoxicity, in addition may exert indirect action, via the metabolism of the host plant.

A second line of approach is the use of natural compounds, which are thought to play a role in disease resistance. Could phytoalexins or elicitors be applied to plants for disease control? Practical application of phytoalexins is, however, full of problems and pitfalls. Synthesis of these usually complex structures is difficult and expensive. Moreover phytoalexins are not all innocuous chemicals, some of them being membrane antagonists and others potent toxicants to mammals. Although local production of such compounds in invaded plant cells may not harm the plant, overall application might be phytotoxic, and even cause residue problems in food crops. The same problems might arise with application of elicitors, apart from the fact that it would be difficult and expensive to obtain elicitors, which are high molecular weight polysaccharides, glycoproteins or unsaturated fatty acids, in sufficient quantity for practical application. Many other natural, non-fungitoxic compounds have been found to influence disease development, for example growth regulators, amino acids and derivatives of these compounds. Their activity is mostly insufficient for disease control in practice, and undesirable side effects often occur. Elucidation of their mechanisms of action is still elusive.

A third line of approach could be the use of chemicals which sensitize the host tissue in such a way that it shows a resistance response upon challenge inoculation with a pathogen. Studies on induced resistance by microbiotic agents indicate the formation of signals, which move upwards and downwards, and sensitize the host tissue also in plant parts at a distance. The nature of these signals is still elusive, and the application of chemicals, which induce such signals, is music of the future.

Much more information about and insight in the intricate relation between host plant and parasite will be needed to design chemicals which in a subtle, specific way interfere with one of the steps of the infection process. On the other hand non-fungitoxic compounds, found by trial and error, may prove important tools towards elucidation of this relation. It still remains to be seen to what extent the possible advantages of non-fungitoxic compounds for plant disease control can be realized, e.g. a lower chance for development of 'fungicide-resistance, as suggested for Aliette by Bompeix et al. (1981) and for Rabcide by Uesugi (1982). Probably this will depend upon the nature and the number of factors, involved in the induction of resistance or reduction of pathogenicity.

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CONTROL OF WEEDS USING CHEMICAL AGENTS OTHER THAN CONVENTIONAL HERBICIDES

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ABSTRACT

Among the non-conventional agents for chemical weed control herbicide safeners are the most widely used, but there is potential for further development to cover more crops and more herbicides. Fumigants continue to be used only in very high value situations, but it is suggested they have potential for more extensive use with modest improvements in economy. The possibility of their use for dormancy breaking rather than seed killing is emphasised. Natural toxins are of particular interest from the environmental safety viewpoint and for the possibility of their production by bio-technology. A few antibiotics have already found use as herbicides and it seems likely there will be increased commercial interest in this approach. Among growth regulators, the retardants will have an increasing role in integrated weed management systems. Ethylene is already in practical use for breaking dormancy of Striga seed and has potential on some other species. There are many other possible uses for growth regulators in weed control but high costs have so far precluded commercialization. A common factor in most of these approaches is the need for non-commercial research and initiative to support and encourage further developments.

INTRODUCTION

The range of conventional synthetic herbicides is now large but weed problems continue to change and present new challenges. The rate of introduction of new compounds to cater for these problems is declining, partly due to the problems of toxicological testing, and all alternative approaches need to be appraised. In the "non-conventional" chemical category I intend to discuss fumigants (synthetic in origin but different in the way they act) natural toxins (conventional in their use but different in their origin), growth regulators acting either alone or to enhance the effectiveness of herbicides, and finally herbicide safeners, used to modify the selectivity of conventional herbicides. I shall not discuss other modifiers of herbicide action such as surfactants, activators or 'extenders'.

FUMIGANTS

Treatments with methyl bromide, chloropicrin, metham-sodium, dazomet and 1,3-dichloropropene are too expensive to be seriously considered for general weed control. There have been no major new developments in recent years but White (1978) and Bond & White (1982) have pointed out that the use of polythene sheeting with metham-sodium and 1,3-dichloropropene makes them as efficient in weed control as methylbromide and cheaper to use. They also point out that the less mobile dazomet is relatively effective in the upper soil layer and might usefully be combined with metham-sodium without the need for sheeting.

Under the right conditions these treatments are capable of killing the seeds of most weeds to plough depth and in the absence of re-infestation a single treatment could provide weed control for several years. With current high cost of repeated use of selective herbicide it is worth re-considering the more extensive use of fumigants. Costs are high because doses are usually at least 100 kg a.i./ha and sheeting is also expensive in material and labour but there would seem to be no obvious reason why more highly active compounds should not be discovered, and there is scope for development of a spray-on film to provide a temporary impermeable barrier which would then degrade naturally or be incorporated into the soil. There would still be the question of the environmental acceptibility of the wider scale use of fumigants, and the problem of some species surviving the treatment and others being blown or carried in to re-infest the area. The technique is therefore not without problems, but I suggest that it deserves more attention than it has received in recent years, perhaps starting with fundamental work to understand the mode of action of the existing fumigants. Attention is drawn particularly to the possibility of a link between the ability of fumigants to kill dormant seeds and an observed tendency for some of them to break seed dormancy (see Growth Regulator section below).

NATURAL TOXINS

Although there may be no difference between natural toxins and conventional herbicides in the way they are used they are considered here because of the quite different way in which they might be produced, whether by isolation from natural products or by bio-technology. Their natural origin may also be important in terms of reduced requirements for environmental safety testing. Natural toxins that may be considered as herbicides include extracts or exudates from higher plants, especially allelopathic substances, mycotoxins (products of pathogenic fungi) and antibiotics.

It has already been proposed that the phenomenon of allelopathy might be exploited in agriculture, by the selection of crop varieties which produce allelochemics (eg Putnam & Duke, 1978), but it seems doubtful that seedling crop plants can produce the quantity of allelochemic required for practical weed control. A system could, however, be envisaged for the production of allelochemic and its concentration, or indeed its synthesis. Those few papers reporting quantitative studies with pure preparations of allelochemic suggest relatively low levels of activity. Williams & Hoagland (1982) found coumarin, juglone, hydrocinnamic acid and pyrocatechol to be the most active of those tested, but even these required 10^{-3}M (eg 146 mg/l in the case of coumarin) to inhibit germination of Amaranthus retroflexus. Shettel and Balke (1980) found that caffeine, p-hydroxy coumarin, salicylic acid and p-hydroxybenzoic acid mixed into soil before sowing controlled a few weed species at 56 kg a.i./ha but not at 11.2 kg/ha. Khozla et al (1980) refer to the 'interesting' activity of parthenin on Cyperus rotundus but the dose required was 600 kg/ha. Reynolds (1978) tested a wide range of aromatic compounds on germination of lettuce and confirmed the relatively low activity of many of the commoner allelochemics. However, dihydroactinidiolide, isolated from Eleocharis sp by Stevens & Merrill (1980) caused 50% inhibition of radish germination at 5 mg/l, and there is the remarkable activity of agrostemmin affecting grass growth at 1.2 g/ha reported by Gajic (1974), suggesting that further studies could reveal compounds of genuine value.

Mycoherbicides, particularly those based on suspensions of fungal spores are of great current interest and will no doubt be covered in detail in a different section of this Congress. They are normally indigenous fungi whose host range is already known. The chance of unforseen spread and attack of crop species is therefore small. The organisms so far have had narrow specificity to the target species only but others under consideration such as *Pyrenophora avenae* for *Avena fatua* can also attack the oat crop (*Avena sativa*). Assuming that at least in some cases the pathogenic effect of a fungus is the result of a toxin, it could be safer to use the mycotoxin rather than the organism producing it. There is also the advantage of being able to concentrate the toxic factor to produce a quicker and more reliable effect on the weeds. Although many fungi have narrow host specificity, the toxins involved may not have the same specificity (Yoder 1980). One of the few mycotoxins to have been considered as a herbicide, bostrycin, from the fungus *Alternaria eichhorniae*, is virtually specific to *Eichhornia crassipes*, but the isolated toxin damaged wheat, maize and several other species, as much as *E.crassipes* (Charudattan & Rao 1982). As bostrycin is also known from several other fungi this result is not unexpected, but it does not eliminate the possibility of toxins with a useful degree of selectivity against a range of weeds. The 14 host-specific toxins listed by Yoder (1980) are all specific to crops but weed-specific toxins could also occur.

The mycotoxin, tentoxin, from *Alternaria alternata* is active on a range of broad-leaved species but not on tomato or Cruciferae nor on any monocotyledons (*Templeton et al* 1967). However, its selectivity has not been thoroughly evaluated. Walker & Templeton (1978) studied the specificity of toxic metabolites from the host-specific fungus *Colletotrichum gloeosporioides* ssp. *aeschynomene* which is now registered in U.S.A. as a mycoherbicide for the rice weed *Aeschynomene virginica*. The results failed to confirm specifity (*Sida spinosa* was even more sensitive than *A.virginica*) but maize, sorghum, wheat and rice were all unaffected and the authors are continuing to explore the potential of these metabolites as herbicidal preparations.

Several antibiotics have already been exploited as herbicides and fungicides in Japan. One of these, bialaphos, is obtained from the fermentation broth of *Streptomyces hygroscopicus* (Wakabayashi 1982). It is chemically related to the synthetic herbicide glufosinate (Hoe-661). Both have very similar spectrum and type of activity, intermediate between paraquat and glyphosate, and Wakabayashi and Matsunaka (1982) conclude that bialaphos is acting as a "masked" herbicide releasing the toxic molecule only within sensitive plants.

Methoxyphenone (3,3'-dimethyl-4-methoxybenzophenone) is derived from the antibiotic anisomycin and is used as a herbicide for selective control of *Echinochloa crus-galli* in rice (Wakabayashi, 1982). This compound also has an application as a herbicide safener (Wakabayashi & Matsunaka, 1982).

The antibiotics, blasticidin-S (from *Streptomyces griseochromogenes*), tricothecin (from *Tricothecium roseum*) and cycloheximide (a synthetic analogue of streptomycin) each have some problems of phytotoxicity when used as fungicides and this activity has been shown to be potentially useful in the range of 1-10 mg/l on the parasitic Cuscuta spp (Muromtsev & Agnistikova 1970). Fedorinchik and Panchenko (1973) also controlled *Orobanche aegyptiaca* on melons with a salt of blasticidin-S at 5-10 g/ha. Rhizobitoxine (from *Rhizobium japonicum*) is active as a herbicide at about 0.2 kg/ha (Rice 1979). Some of these compounds, especially blasticidin have high mammalian toxicity and care is also needed to avoid promoting resistance to medically important compounds, but the potential of antibiotics, especially for *Cuscuta* spp appears to deserve further study.

GROWTH REGULATORS

Inhibitors or retardants

There can be no clear distinction between conventional herbicides and growth retardants but the latter may loosely be distinguished by the purpose of their development, for suppressing the growth of crops (including turf) rather than of weeds. Mefluidide is borderline as it has been developed both as a turf retardant and as a herbicide for use in soyabean. In this latter case the sub-lethal retardant effect on weeds is merely acceptable rather than deliberately sought. Thomas (1976) however, has proposed the use of growth retardant to suppress weeds in crop interrows as an alternative to complete kill, to reduce risks of soil erosion. He used ethephon (at 3 kg/ha) and chlorflurecol in various combinations with dalapon and MCPA. Another proposed use of retardant is for the suppression of aquatic weed growth, where complete kill may be undesirable (Robson, 1972). The growth regulator paclobutrazol (PP333) has proved promising for this use but is not yet fully developed and cleared (T O Robson & P R F Barrett, personal communication). Another aquatic use proposed by Center et al (1982), is for a retardant to check the growth of Eichhornia crassipes while it is controlled biologically by the insect Neochetina eichhorniae. Other uses for retardant in integrated control systems can be envisaged.

Dormancy breakers

It has long been recognised that breaking the dormancy of seeds or buds could be an important key to the control of difficult weed species. Auxins, cytokinins and gibberellins, and some conventional herbicides have increased germination of various species (reviewed by Parker 1976) but effects are usually incomplete or require high doses, and no field uses have yet been developed.

As noted above under fumigants there does appear to be some connection between fumigant action and dormancy breaking. There are no records of increased weed emergence with sub-lethal doses of methyl bromide or metham-sodium but ethylene dibromide, ethylene chlorhydrin and 1.1-dibromo-3-chloropropane (DBCP) have all shown stimulation of weed emergence in the field (reviewed by Parker 1976). More recently, Wilson & Courtney (1981) have reported increased weed emergence following "low" doses of dazomet (200 kg/ha) and Unterladstater and Fuentes (1978) found the germination of Rottboellia exaltata to be increased from zero to over 90% by a mixture of methyl isothiocyanate, dichloropropane and dichloropropene (Di-Trapex E.C.) at 320 kg/ha. Fay & Gorecki (1978) showed that sodium azide incorporated into the soil at 11.2 kg/ha increased emergence of A.fatua from 12% to approximately 50% of the total seed in the soil. Unfortunately the mammalian toxicity of azide makes full development unlikely. Similarly the observation by Cairns and Villiers (1980) of the effects of aluminium phosphide in breaking dormancy of A.fatua may only be of academic interest. Taylorson & Hendricks (1979) have shown that the dormancy of several grass weeds (but not A.fatua) could be broken by ethanol (at about 1000 l/ha) and chloroform, and have suggested a general hypothesis for the action of anaesthetics in dormancy breaking, associated with their action on membranes.

The one substance to have been used on a field scale for breaking weed seed dormancy is ethylene. Egley & Dale (1970) showed that both ethylene and ethephon stimulated germination of the parasitic weed Striga asiatica. In recent years some thousands of hectares in U.S.A. have been treated annually with ethylene gas at 1.5 kg/ha as part of a programme to eradicate S.asiatica (Eplee & Langston, 1976). Ethephon has been shown to stimulate the main African species S.hermonthica (Babiker & Hamdoun, 1983) but it has yet to be demonstrated that ethylene has an equivalent potential on this species in the field. An alternative class of chemical for germination of Striga is based on the natural germination stimulant strigol. Johnson et al (1976) reported on the synthesis of simpler strigol-analogues which had high activity on Striga spp and on Orobanche spp. There have been further studies with these compounds, but as yet no commercial development.

Ethylene has generally been found to be inactive on Orobanche spp but Chun et al (1979) report some stimulation of O.ramosa in U.S.A. Both ethylene and ethephon have stimulated germination of a range of other important weeds (reviewed by Chancellor, 1982), but the results have generally been less complete than for Striga.

The possibility of preventing seed dormancy before seeds are shed from the parent plant has been demonstrated by Peters et al (1975) using GA_3 (0.06 mg/plant) on A.fatua. Cytokinins might be expected to act in this way also, at least on some species, but no results have been noted.

The breaking of dormancy of buds on rhizomes, tubers or other propagules of perennial weeds has received research attention, but even where substantial sprouting has been induced eg by cytokinin treatment of Cyperus rotundus, it has not resulted in increased susceptibility to herbicides (Parker & Dean 1972). Ethylene and ethephon break the apical dominance of rhizomes in Elymus repens but the laterals grow out as healthy branch rhizomes and no advantage is achieved (Chancellor, 1970). Chlorflurecol-methyl has more seriously disruptive effects preventing the onset of a new dominance system and causing secondary laterals to develop (ibid), but again no practical exploitation has been reported.

Other approaches with growth regulators

The ability to control abscission could be advantageous in two ways. Stimulating early shedding of seed before full maturity could result in seed with less dormancy, or reduced viability or longevity, while preventing abcission could permit the removal of the weed plant complete with seeds at the time of crop harvest. This could create extra problems of separation or cleaning the crop seed but prevent build up of weed population in the field. Good basic research has been done on this and other aspects of plant growth, but no practical applications have yet been developed.

HERBICIDE SAFENERS

In a previous review (Parker, 1983) I used the term 'antidote' because of doubts about the validity of the term safener but the latest supplement to the Oxford English Dictionary (Burchfield, 1982) records that the term was used as early as 1941 for zinc protecting peach against damage from lead arsenate insecticide. The verb, to safen, is still not listed but can be regarded as a valid term (A.M.Hughes, personal communication).

Briefly, herbicide safeners are used to protect crops against herbicide damage and so allow improved crop safety and/or improved control of weeds. Their development has received increased attention in recent years and here at Brighton a year ago Gray et al (1982) reviewed the evolution of the three safeners already available commercially, NA (1,8-naphthalic anhydride), R25788 (N,N-diallyl-2, 2-dichloroacetamide) and cyometrinil (CGA 43089) and the two new experimental compounds CGA 92194 (N-1,3-dioxolan-2-yl-methoxy)-imino-benzeneacetoni-trile) and MON 4606 (2-chloro-4-trifluoromethyl-5-thiazole carboxylic acid, benzyl ester).

NA was the first safener to be discovered and developed commercially, as a seed dressing at 0.5 to 1% w/w to protect maize against the thiolcarbamate herbicides. This use was quickly superseded by Stauffer' Chemical Company's R25788, but NA has continued to be of interest for improving herbicide selectivity in maize (for herbicides not safened by R25788) and in other crops on which R25788 is generally ineffective. Best results have been with chlorsulfuron and diclofop-methyl on maize, alachlor and metolachlor on sorghum, and alachlor and diclofop-methyl on rice, (4-fold protection often observed) Wheat, barley and ryegrass are also protected but to a lesser extent. In spite of these promising results there is no evidence of current commercial use on any of these crops.

R25788 was discovered within 2 years of NA and marketed as a mixed formulation with EPTC for the control of shattercane, a wild form of *Sorghum bicolor* - hence the trade name "Eradicane". Use for more general weed control in maize has also been possible because R25788 is almost specific to maize in its protective action and no weeds are sufficiently protected for weed control to be reduced. Other crop species are not generally protected, although sorghum, rice, oat, wheat, barley and *Vicia faba* have each shown up to 2-fold protection against various herbicides. R25788 safens less herbicides than NA, the main compounds other than thiolcarbamates being barban and chlorsulfuron.

Cyometrinil was introduced in 1980 by Ciba Geigy Corporation as a seed treatment at 0.15% w/w to protect sorghum and has allowed the use of metolachlor for the control of grass weeds in place of more expensive alternatives. As a result of some problems of reduced germination of sorghum the analogue CGA 92194 is now being introduced in its place. Sprayed overall, both compounds have a protective effect on certain grass weeds and they cannot therefore be used as tankmix formulations.

MON4606 is being developed by Monsanto for a similar purpose as cyometrinil, i.e. to protect sorghum so that the company's own amide herbicide, alachlor, can be safely used. Monsanto's propachlor can be used in sorghum without safener, but is too expensive for general use.

Apart from these developed compounds, many hundreds of others of diverse chemistry have been listed in patents, all showing considerable activity against EPTC. A few groups have been studied in detail and some structure/ activity relations have been established (Stephenson & Ezra 1982). No compounds have yet been developed for protecting crops against any substituted urea or triazine herbicides nor has there been any practical development of safeners for any broad-leaved crop.

The mode of action of R25788 has been well reviewed by Stevenson & Ezra (1982). There is evidence for both enhanced de-toxification of EPTC and for interference at the site of action but the relative importance of each of these is still not fully resolved. Interference with herbicide uptake into the plant is generally considered unimportant for R25788 but may play some part in the action of cyometrinil (Ebert, 1982). Further understanding of the modes of action of safeners should greatly improve the possibilities for the discovery and development of new safeners. The objectives of such development can be various. The most obvious to the non-commercial researcher is the opportunity to achieve selectivity against weeds closely related to crops: *Rotboellia exaltata* in maize has been one such case, wild *Oryza* spp and 'red rice' in rice another. *Avena fatua* in oat has received attention in Canada and is the subject of a current 3 year programme in U.K. Control of wild beet in sugarbeet would be another target. These objectives are only likely to be achieved by seed-applied safeners. Control of 'shatter-cane' in maize was the original spur to development of both NA and R25788 but the latter has also allowed the use of EPTC in maize for a control of other weeds, more cheaply than was possible with the related herbicide butylate. Broadening the use of a less expensive herbicide is an objective which non-commercial researchers would also welcome but for industry there is the danger of the safener being used to broaden the use of a competitor's product. Hence the restricted availability of both R25788 and cyometrinil, the first only being available as a formulated herbicide mixture and the second in the form of treated seed sold through the company's own seed distributors. This need for restricted availability could be inhibiting some companies from releasing potentially promising new safeners. It is to be hoped, however, that safeners may eventually become available to broaden the use of inexpensive herbicides in the substituted urea and triazine groups and also perhaps of glyphosate or paraquat. One of the particular benefits from an increased range of safeners should be improved options for selectivity in "minor" crops.

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PHEROMONES IN INSECT PEST MANAGEMENT - A PERSPECTIVE

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ABSTRACT

Chemicals that affect insect behavior are becoming increasingly valuable in insect pest management. Their applications in insect traps for detection of infestations, population study, and population suppression are constantly being extended.

Progress in structural elucidation and synthesis of pheromones is rapid. Although the goal of increased utilization of pheromones for insect pest management has been aggressively pursued, economic and technical factors have compelled a reappraisal of over-optimistic forecasts. There is now a clearer realization that the technology of insect pheromone use has little in common with that of directly lethal insecticides and that specific insect problems cannot be approached without an understanding of the ethology of the target species in its habitat.

There has been considerable progress in application technology, and versatile controlled-release formulations that can deliver pheromones satisfactorily are becoming available. Government regulatory agencies have recognized the special problems entailed in pheromone technology, and generally the use of such pest control techniques is not overly constrained by regulatory requirements.

Naturally occurring behavior-modifying chemicals would appear to offer a range of environmentally and ecologically sound pest management techniques (Lewis *et al.*, 1980). However, their application to pest management depends on a greater depth of understanding than was initially anticipated. In the last decade, there has been increasing realization of the subtle details of pheromone composition and the complexities of insect behavior. Some of the problems and pitfalls were characterized by Silverstein, who reviewed progress towards practical goals (Silverstein, 1982). He concluded that the role of pheromone traps for monitoring and survey is now an accepted practice, but "that to firmly establish ...... large-scale use of pheromones for population reduction as part of integrated pest management programs will require cooperation of government and the evolution of special kinds of industry to overcome some inherent problems".

A recent paper by Klassen *et al.* (1982) contains an excellent summary of the status of chemical attractants and of insect control methods that should be considered in the design and use of integrated pest management systems. Potential applications of pheromones mentioned include (1) alone as a trap bait, (2) combined with a toxicant, chemosterilant, or entomopathogen in a bait or device or on a trap crop, or (3) dispersed in controlled-release formulation. As might be expected, all these methods possess a high degree of ecological selectivity. Most behavioral techniques should be effective at low or moderate population densities. Methods involving entomopathogens may be more effective at higher densities if the pathogen cycles.

Of these potential applications, the first is well established; pheromones are now widely used as lures in traps for survey and qualitative monitoring of insect populations. Additional studies on trap design and siting, lure formulation, insect behavior, etc., are necessary before quantitive relationships between trap catches and population levels can be established. Population suppression by mass trapping appears to be within reach of practical realization for several insects as more effective attractants are discovered. However, Webb (1982) comments that "mass trapping attempts with Lepidoptera have yielded erratic results".

Considerable progress has been achieved in the application of controlled-release formulations of pheromones to achieve population suppression through mating disruption, as will be seen in examples to follow. Fewer examples of the use of pheromones in combination with toxicants, pathogens or chemosterilants have been reported. The use of pheromone-pathogen combinations in the control of stored product pests may be close to practical realization (Burkholder, 1983).

The greatest value of pheromones probably lies in large-scale programmes. Recent examples (Klassen el al., 1982) of application to area-wide programs include applications such as PETE (Prediction Extension Timing Estimation), in which information on fruit tree and insect pest populations in Michigan is collected, evaluated by a computer-based system and distributed. Similar systems are functioning in other states. Also important is progress in the use of pheromone trap data to develop models of insect population growth and decline and to elucidate migratory patterns in pest species such as Heliothis (Hartstack et al., 1982).

Regulatory problems no longer appear to represent major constraints on pheromone application. Recently, new guidelines for pesticide registration were published by the EPA. Pheromones fall within the category of "Biorational Pesticides", and a tier system has been adopted as the approach to testing for safety (U.S. Environmental Protection Agency, 1982). For pheromones in general, the need for long-term toxicological tests and environmental impact studies will be obviated by the new regulations. Despite these recent reductions in regulatory constraints, however, the investment involved in demonstration of efficacy against major insect targets remains great.

In the United States, early attempts utilizing pheromones to suppress insect populations were made by scientists in universities or government departments. Results were sufficiently encouraging, in some cases, to stimulate industrial involvement. A principal contribution of industry has been the development of controlled-release formulations (laminated polymers, hollow fibers, and microcapsules) that can be applied on an area-wide basis. At least 12 pheromone formulations have been registered by the U. S. Environmental Protection Agency (EPA) for population suppression. However, this registration does not imply widespread practical application, but only the availability of these materials for use against specific insects.

The problem of satisfactory formulations has been stressed as a limiting factor in the development of pheromones. The introduction of commercial controlled-release formulations has overcome this problem to a great extent. Measurements of release rates and the effects of environmental variables have been reported by several workers (Bierl-Leonhardt, 1982, Weatherston et al., 1982), and formulations have been improved on the basis of comparative field trials (e.g., Plimmer et al., 1982). However, multicomponent pheromones present special problems, and there is a need for data on the volatilization of pheromones from formulations under field conditions. A few studies have been conducted (Caro et al., 1977, Plimmer et al., 1978), but better micrometeorological data are needed, as well as improved techniques for sampling and analysis (Taylor, 1982).

An indication of the potential of pheromones for practical use in European agriculture and forestry was provided by Minks (1979). Campion (1983) reviewed studies on the application of pheromones in programs to control various insect pests in the Mediterranean region. The following discussion is limited to selected pheromones as they are being applied in some major pheromone programs in North America.

FOREST PESTS

Control of forest pests in North America is largely the responsibility of national or local government departments. Several major forest pests, including the gypsy moth (Lymantria dispar), the eastern spruce budworm (Choristoneura fumiferana), the western pine shoot borer moth (Eucosma sonomana) and the Douglas-fir tussock moth (Orygia pseudotsugata), have been the target of large-scale pest management programs in which pheromones played an important role.

Gypsy moth

Although the area defoliated by the gypsy moth fell from 12.8 million acres in 1981 to 8.1 million acres in 1982, the area infested in the notheastern United States continues to expand, particularly towards the south. The sex attratant pheromone, disparlure (cis-7,8-epoxy-2-methyl-octadecane), has been used in large-scale monitoring programs, and the development of sprayable controlled-release formulations to disprupt mating has been a major research objective for over ten years.

A number of candidate controlled-release formulations were evaluated and season-long reduction in incidence of mating was obtained by a single application. Microcapsules (Eurand America, Dayton, OH), laminated plastic flakes (Hercon Divison, Health-Chem. Corp., New York, NY) and hollow fibers (Albany International, Needham Heights, MA) were used in large-scale field tests, in which effectiveness of treatment was determined from the number of male moths caught in disparlure-baited traps and the incidence of mating in female moths placed in the plots (Plimmer, 1982). The effectiveness of the treatment was lower in areas of medium- to high-density moth populations than at low population levels. At an application rate of 20 g ai/ha, female mating incidence was lower in plots treated with hollow-fiber or flake formulations than in areas treated with microcapsules. In later tests, mating was reduced more than 93% by a 50 g ai/ha application, indicating that effective control could be achieved. It has been suggested that at high population densities, short-range male-finding strategies may not be perturbed by changes in environmental concentrations of disparlure. Although control would be more effective at low population densities, changes in such population levels are extremely difficult to assess (Schwalbe et al., unpublished results).

Spruce budworm

There have been several large-scale attempts to suppress spruce budworm populations by mating disruption. Although male trap catch was reduced, population reduction could not be demonstrated. Inadequacy in formulation has been cited as one potential reason for failure (Sanders and Seabrook, 1982); lack of field behavioral data and unequivocal tests for assessment of effects was also a factor (Silk, 1983).

Western pine shoot borer

This insect infests a variety of pines in the western United States. the sex attractant pheromone contains a 4:1 mixture of (Z)- and (E)-9-dodecenyl acetates. The low-density population of adults suggests that mating disruption would be an effective technique for control. In a recent survey of attempts to disrupt mating, Daterman (1982) concluded that damage could be effectively suppressed in pine plantations and that the technique was suitable for operational use. Albany International hollow fiber and Hercon[R] flake formulations are now both registered by EPA.

CROP INSECTS

Pheromone formulations are now registered by the EPA for a number of agriculturally important pests. These pests include the boll weevil (Anthonomus grandis), the pink bollworm (Pectinophora gossypiella), the artichoke plume moth (Platypilia carduidactyla), the tomato pinworm (Keiferia lycopersicella), and the tobacco budworm (Heliothis virescens.

Pink bollworm

The pink bollworm is a serious cotton pest and potentially threatens 300 000 hectares of cotton in California and Arizona. Attempts to use the sex attractant pheromone (gossyplure) for mating have been intensively pursued because the growing areas are isolated and the pink bollworm emerges early in the growing season, with other insect pests occurring later. Control can be initially achieved by pheromone treatments, and conventional insecticides need only be applied later. Two formulations have been employed. The Nomate[TM] PBW formulation was applied to 50 000 hectares in 1981. Initially 6 g of formulated material (containing 7.6 percent ai) was applied per hectare. The addition of a small quantity of a pyrethroid insecticide to the adhesive material in the formulation permits a reduction in dose to 3 grams per hectare. This combination was recently registered by the EPA and presumably removes males because there is physical contact during the mate-seeking process, suggesting that a trail-following behavior is involved in mating disruption. A formulation of gossyplure based on plastic laminated flakes is also registered. Disrupt[R] PBW/Lure N Kill[TM] contains the sex pheromone in combination with a pyrethroid insecticide and is applied at 0.6 to 0.8 grams pheromone per hectare.

Artichoke plume moth

The artichoke plume moth is the primary insect pest of the globe artichoke in California. Insect damage is 2 to 10 percent each year, but may rise to 40 percent in exceptional years. For 20 years, the primary control used was methyl parathion. Initially 8-10 applications were made. However, insect resistance forced discontinuation after the number of necessary applications had risen to 20-26 by 1976. Methidathion and other organophosphate insecticides have been used since 1978. Application of the sex attractant pheromone for mating disruption rapidly followed its identification as (Z)-11-hexadecenal (Klun et al., 1981).

In one application (Haworth et al., 1982), a formulation of (Z)-11-hexadecenal in black Celcon[R] hollow fibers was aerially applied at 5g ai/ha to experimental plots in areas of low, moderate, and high population; treatments were repeated 6 times at 18-day intervals, and then individually according to the pattern of cultivation. Insecticides were applied as demanded by standard monitoring practices. Populations of the artichoke plume moth were reported to be controlled effectively by this formulation, registered as Nomate[R] Chokeguard. Mating of the artichoke plume moth was also suppressed when a Hercon Disrupt[R] flake formulation of the pheromone was applied at 7.5 g ai/ha (Kydonieus et al., 1981).

Boll weevil

The boll weevil (Anthonomus grandis) affords a potential target for mass trapping or trap cropping. The pheromone secreted by the male acts as an aggregating pheromone at certain times of the year. It has been established that low-density insect populations can be substantially reduced by trapping (Lloyd et al., 1982), and formulations of the boll weevil pheromone, grandlure, are registered for mass trapping. Trap cropping may also be an effective method of control. Weevils are attracted to pheromone traps deployed in a selected area of a field; subsequent treatment of this area with insecticide then removes a substantial fraction of the weevil population (Hardee 1982). Recently Albany International introduced NoMate[R] Blockade, a product combining grandlure with other attractants, to aggregate boll weevils in a barrier zone of a cotton field where an insecticide can subsequently be applied. Such strategies may limit the use of insecticides and reduce adverse affects on beneficial insects.

Tobacco budworm and corn earworm

Heliothis zea (corn earworm, bollworm, tomato fruitworm) and Heliothis virescens (tobacco budworm) are major pests of cotton, corn, and a variety of other crops throughout the United States. The ovipositor wash of H. zea contains four components, that of H. virescens, seven (Klun et al., 1980 a,b). In a mating disruption study, Heliothis virescens male trap catch was reduced almost completely by the 7 components of the ovipositor wash, but adult mating did not show a parallel reduction (Raulston et al., 1983). It was suggested that detailed observations of field behavior are needed to clarify the role of stimuli other than pheromone perception in mating

The importance of Heliothis species as pests has stimulated commercial interest, and intensive efforts are being made to develop formulations that will suppress populations satisfactorily.

CONCLUSIONS

Rapid progress in elucidation of pheromone composition and methods of synthesis assures that pheromones will become more readily available. Their role in the detection and monitoring of insects is established, and their utility in pest management programs has stimulated commercial development in the United States, where more than 40 formulated lures can now be purchased.

The question of the use of pheromones for population suppression by mass trapping or mating disruption cannot be simply answered. In many cases, there has been considerable technical progress and, in a few, there is a prospect of commercial success. However, commercial success of a new pest control technology depends not only on its efficacy in the field but also on its acceptance among pest management practices.

A particularly difficult problem in applying mating disruption or mass trapping is that of demonstrating efficacy (Roelofs, 1979). In the absence of well-defined prior information on the insect pest, it may prove extremely difficult to obtain satisfactory data. This situation may be remedied to some extent in future as the understanding of insect population dynamics increases. However, assessment of the effect of population suppression techniques must be based on accumulation of data over several seasons in situations where some assessment of the contribution of additional factors to population change can also be made.

Past experience has also indicated that successful application of pheromones will depend on deeper understanding of insect behavior, particularly in the field, where competing stimuli may be present. Among other factors, population structure and dynamics will influence behavior. Studies in populations of defined density and structure are important preliminaries to attempts to suppress population by air permeation with pheromones.

The importance of defining the quantitative and qualitative composition of the stimulus perceived by the male has been emphasized. This is important for behavioral study and for trapping, if traps are to compete with virgin females.

Finally, the formulation and deployment of the pheromone must be satisfactory. Performance of formulations will be affected by temperature, humidity, air movement, etc., within microenvironments where individual particles of the formulation reside. It is important that release rates of pheromones from controlled-release formulations correspond to biologically effective rates and this must be achieved by careful monitoring throughout the development of the formulation.

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NOMATE PBW, A SYNTHETIC PHEROMONE FORMULATION FOR WIDE AREA CONTROL OF THE PINK BOLLWORM

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Background and Objectives

In 1982 a Cotton Pest Abatement District (CPAD) was established to regulate all of the 36,350 acres of cotton growing in the Imperial Valley of California. The supervisory board mandated that the entire acreage be treated with gossyplure to control the pink bollworm, *Pectinophora gossypiella*, the major primary pest of cotton. The objective was early-season control of PBW without major use of hard chemical insecticides that destroy beneficials and allow outbreaks of secondary pests. In 1981 explosive outbreaks of whitefly, *Bemisia* spp. and *Heliothis* spp. resulted in increased insecticide costs and greatly reduced yields. Further losses occurred when these species dispersed and attacked fall and winter crops.

Materials and Methods

NoMate PBW,™ a hollow fiber formulation of gossyplure, produced by the Controlled Release Division of Albany International (AI) has been applied on over one million acres for control of pink bollworm. CPAD regulations called for use of NoMate PBW from early June until August 1, after which, growers were free to develop their programs as they wished. Two application programs with NoMate PBW were sanctioned by the board. The first was four consecutive applications of NoMate PBW applied at the rate of 15 grams per acre (1.14 grams a.i.) with the initial application at first bloom. The second called for six applications of NoMate PBW at the rate of 7.5 grams (0.57 grams a.i.) with the initial application at the pinhead stage of square or bud development.

The hollow fiber formulation was applied aerially with Bio-Tac,™ a polybutene sticker that holds the fibers to foliage. Special pods attached to the underside of the airplane wings were used to apply the fibers. Practically all applications were made with a pyrethroid in the Bio-Tac. This recent innovation developed commercially by AI (Attract 'n Kill) involves the use of an insecticide mixed into the polybutene sticker. Male moths attempting to mate with these fibers acquire a lethal dose of insecticide from the Bio-Tac. The pyrethroid is applied at 1/50 of its normal rate per acre and this treatment has no effect on beneficial insects.

Forty-three fields out of 482 were intensively sampled by CPAD scientists. An additional 20 fields were selected for further assessment of the two rates of NoMate PBW by Albany International personnel.

Results and Conclusions

NoMate PBW at both the 7.5- and 15-gram rates per acre were highly effective in controlling this pest through the second generation. Season-long average infestation for fields treated with NoMate PBW at both rates as measured by open boll counts was 5.8%. This was in spite of heavy spring catches of overwintering moths which indicated high likelihood of infestation. The goal of the CPAD program was achieved and few hard pesticides were used before August. Secondary pests such as *H. virescens* never became serious problems.

Most growers and pest control advisers indicated their satisfaction with the program by electing to use NoMate PBW after completion of the regulatory program on August 1. There was an average of 6.3 applications instead of 4 at the 15 gram per acre rate and 11.5 applications instead of 6 at the 7.5 gram per acre rate. The average infestation of fields treated with 7.5 grams was somewhat lower than those treated with 15 grams at bloom. This difference is attributed more to earlier treatment at pinhead square than to the effect of rate differences.

Comparison was made of the overall infestation in Imperial Valley with NoMate PBW (5.8%) with other areas in California. Cotton in nearby Winterhaven treated with conventional hard insecticides ended with 32.6% infestation. Cotton in the Palo Verde Valley where NoMate PBW significantly out-performed conventional practice, resulted in infestations in NoMate PBW fields of 3.8% vs 7.5% in the conventional practice.

2B—R2

CONTROL OF CROP PESTS WITH MICROENCAPSULATED INSECT PHEROMONES

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Background and Objectives

Pheromones show considerable promise as crop protection agents for insect pest control, and many have been identified and synthesised, including alarm, aggregation and sex pheromones. However, as major problems are encountered with field use, researchers at ICI Plant Protection Division and at the Tropical Development and Research Institute have co-operated in a programme aimed at producing a formulation which:

(a) can be applied using existing spray equipment,
(b) protects the pheromone from degradation, and
(c) acts as a controlled release system.

Formulation

The objectives outlined have been met through the development of microencapsulated pheromone formulations, which consist of small droplets (or particles) of pheromone protected by an outer shell of polymer, such as polyamide and polyurea. Such patented formulations containing stabilisers and antioxidants are easily manufactured on a large scale by interfacial polymerisation (Nesbitt et al, 1981), and possess numerous variables, including wall thickness, wall composition, internal composition and capsule size, which can be manipulated to protect the particular pheromone, and to control the release characteristics. The product can be readily applied over large areas with conventional spray equipment, and once the deposit has dried, the system provides sustained release of pheromone, which is mainly diffusion controlled.

Biological Results

A number of pheromones have now been successfully encapsulated and field trials have confirmed the effectiveness of the sex pheromone formulations in providing excellent control through disruption of pheromone-mediated communication, but emphasis in this paper is placed on the control of Pectinophora gossypiella (Saunders), the pink bollworm on cotton.

Extensive trials performed on cotton in Egypt (100ha in 1981, 100ha in 1982, 550ha in 1983) have demonstrated that control of P.gossypiella with encapsulated sex pheromone as a mating disruptant is technically achievable. Blocks of cotton were treated with five aerial spray applications of microencapsulated gossyplure - (Z,Z) and (Z,E)- 7, 11 - hexadecadienyl acetate, 1:1 (PP761)- from the end of May until mid-August.

A total of 40g of gossyplure was applied/ha/season, while control plots were treated with conventional insecticides ('Dursban'/'Dimilin', synthetic pyrethroids, etc) throughout the season. The efficacy of the treatments was determined by measuring pheromone trap catch of adult males, larval infestation of bolls, seed damage, lint quality and yield. Data from pheromone trap catches indicated that there was almost complete trap shutdown on gossyplure treated plots during June, July and most of August. Boll infestation by P. gossypiella, and yield in pheromone and insecticide treated areas were similar but sampling of open bolls in early September clearly demonstrated differences between the treatments; pheromone treated areas having appreciably fewer "partially opened" or "dried infested" bolls than the insecticide treated area. No evidence was found for unacceptable increases in the population of other cotton pests such as Heliothis armigera and Earias insulana in the pheromone treated area presumably as a result of natural biological control, as the incidence of beneficial species on pheromone treated cotton was high. Nonetheless, field data indicate that microencapsulated gossyplure may be best utilised by inclusion as 1-2 sprays in the conventional programme in Egypt, as other agents (insecticides/growth regulators/other pheromones) may still be needed for control of pests such as Earias sp.

Microencapsulated pheromone formulations have also been prepared for a number of other insect pests and field trials indicate great potential in crop protection, particularly in forestry, rice and fruit/vegetables.

Conclusions

Microcapsules provide an efficient means of controlled release for behaviour modifying chemicals which allow effective and economic use in the field. For instance, the sex pheromones used are safe to beneficials and are pest species specific, disrupting communication between the sexes and hence reducing mating and diminishing subsequent larval infestations. Microcapsules are unique in that they are the only available controlled release formulation which can be applied through conventional spray equipment. Thus, in countries like Egypt, encapsulated pheromones can be easily integrated into the existing insecticide programme, simply by replacement of one or more treatments with pheromones in the spray tanks. The system is made even more versatile as pheromone formulations for two different species can be mixed, or encapsulated pheromone can be tank mixed with selective insecticides, such as PP 145, allowing for integrated control of major crop pests (see Evans et al, 1983).

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2B—R3

CONTROL OF THE PINK BOLLWORM IN EGYPT BY MATING DISRUPTION USING PHEROMONES

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Introduction

Three different slow release pheromone formulations were tested for their ability to control Pink Bollworm, Pectinophora gossypiella (Saund.) infestations in cotton fields in Egypt.

Materials and methods

A microencapsulated pheromone formulation was supplied by Imperial Chemical Industries of the United Kingdom, a laminated plastic chip formulation by BASF of West Germany and a hollow fibre formulation by Albany International of the United States of America.

50ha blocks of cotton were treated with each pheromone formulation and no insecticides were applied in these areas for control of Pink Bollworm. Similar 50ha blocks of cotton situated 2-5Km distant from each pheromone treatment were used as control areas. These areas were subjected to the normal regime of insecticide applications as it was not possible to have completely untreated control sites.

The microcapsules were supplied in a 2% aqueous solution and applied by air using a helicopter fitted with conventional boom and nozzle spray gear. Five applications were made at a rate of about 8gms. a.i./ha/application.

The laminated plastic and hollow fibre formulations were applied by hand using local labour working in teams of 25-30 boys per team. The chips were applied individually to the cotton using a latex based adhesive, at 12.5m^2 intervals, equalling 800 chips/ha. The fibres were first mixed thoroughly with a polybutene sticker and applied in clumps of about six fibres every 6m^2 giving an average application rate of 1 fibre/m^2. Both these rates equalled about 3gms. a.i./ha/application and both formulations were applied on six occasions. One team of boys took two days to apply the chips or fibres to a 50ha block.

Assessment of the success of the pheromone treatments was made by a comparison of pheromone baited trap catch, infested flower and boll counts, open boll counts and yield of seed cotton in the pheromone treated areas and the corresponding control area. Lint quality and seed damage examinations were also made after harvest. On one occasion, after the initiation of insecticide spraying, a comparison of beneficial insects in the treatment and control areas was made using a D-Vac suction machine.

Results

There was very little difference in the results obtained using the three different pheromone formulations. Mean daily moth catch and yield losses after examination of the open bolls, were lower in the pheromone treated areas than in the control areas. Data obtained from the other methods of assessment were generally similar for all the areas. However, on average over three times as many predators were found in the treatment areas than in the control sites by D-Vac sampling.

Discussion

All three formulations tested performed as well if not better than conventional pesticides in maintaining Pink Bollworm infestations at tolerable levels in the treatment sites in Egypt. However, treatment of cotton with pheromones for control of Pink Bollworm must be undertaken prophylactically and the first applications of all three formulations were made at the flower bud stage, several weeks before the scheduled aerial application of pesticides began. Thus it was necessary to arrange for a plane to be brought into the area ahead of schedule. Labour for hand applications, on the other hand, was readily available. After the initial application, aerial applications were easily arranged and supervised.

Although hand applications required more organisation and supervision and were more time consuming, they could be used on cotton which was inaccessible to aircraft. The cost of hand applications was also considerably less.

These considerations could be of importance in other situations where labour is plentiful, resources and aircraft for aerial applications are not available or cotton cultivation is not in large contiguous blocks.

A PACKAGE OF SELECTIVE COMPOUNDS FOR CONTROL OF MAJOR CROP PESTS

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Introduction

Many economic entomologists have emphasised the vital importance of integrating biological and chemical methods of insect control. Apart from some striking successes in biological control through introduced natural enemies, and a few examples of plants bred for resistance, non-chemical strategies alone have been relatively unsuccessful as unaided methods of control. Hitherto, the most effective insecticides have been broad spectrum compounds that necessarily have adverse effects on natural enemies. Thus the discovery and development of safe, selective insecticides, which preserve natural enemies, has the highest priority for the advancement of integrated pest management (i.p.m.).

Development of a package of selective materials

In recent years a few selective materials have been commercialised, eg diflubenzuron, *Bacillus thuringiensis*, but the biological activity has been inadequate for key pests on major crops, so that non-selective broad spectrum materials have of necessity continued to be used. However, recent discoveries of new chemicals (or formulations),which have the potential to selectively control major insect pests, have now provided the possibility of developing i.p.m. strategies for a number of primary pests.

The following three materials are either available from or under evaluation by ICI, Plant Protection Division:

a) Pirimicarb - Specific aphicide, effective against all stages of the life history, discovered by ICI (Plant Protection Division).
b) PP145(IKI7899) - Insect growth regulator effective against larvae of Lepidoptera, Coleoptera and Diptera, discovered by Ishihara Sangyo Kaisha Ltd.
c) PP761 Gossyplure- Controlled release microencapsulated formulation used to disrupt pink bollworm mating, discovered by the Tropical Development and Research Institute and ICI (PPD).

Details of the chemical structure, mode of action, selectivity to pests, and safety to beneficial insects will be displayed.

Given the properties of the package of compounds described, Old World Cotton is a suitable example of a target for research and development. Many of the major pests are likely to be effectively controlled by the compounds available viz: Cotton aphid (*Aphis gossypii*), Cotton bollworm (*Heliothis armigera*), Cotton leafworm (*Spodoptera littoralis*), Pink bollworm (*Pectinophora gossypiella*)

Data are presented to demonstrate the field efficacy of the selective materials described.

Conclusions

In a recent address to the Royal Society (Geissbuhler 1981), it was asserted that in the long term,industry will successively make available a set of new and more selective biologically active products. The package of compounds described here is a significant step towards achieving this objective.

Further research must involve relevant studies on natural enemies, concurrently with the effects of selective materials on target pests, and so assess stringently the benefits of such an approach. Nonetheless, the existence of a package of selective materials challenges entomologists to devise effective strategies.

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2B—R5

TECHNIQUES FOR THE CONTROL OF COTTON PESTS IN EGYPT TO REDUCE THE RELIANCE ON CHEMICAL PESTICIDES

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Introduction

The two most serious cotton pests are the Egyptian cotton leafworm *Spodoptera littoralis* and the pink bollworm *Pectinophora gossypiella*; localised infestations of African bollworm *Heliothis armigera* and spiny bollworm *Earias insulana* also occur.

Cultural Control

Control of *S. littoralis* is currently attempted by the very labour-intensive method of collecting the egg-masses. Teams of small children go through the cotton crop once every three days and the egg masses which they collect are counted and then destroyed. The rising cost of labour and the difficulty in recruiting the necessary number of children is making this technique expensive and difficult to maintain.

Mass Trapping

Consideration was given to alternative non-insecticidal methods for controlling *S. littoralis* which would similarly conserve beneficial insects. While insect populations are low, mass-trapping by means of pheromone baited traps may reduce the rate of population increase to tolerable levels within the crop. An economic appraisal of the mass-trapping technique suggested that it would be considerably cheaper than the system of egg mass collection. However, mass-trapping at a density of 5 traps/ha in an area of 600 ha failed to significantly reduce the level of infestation.

Microbial pesticides

An alternative control method for *S. littoralis* has been the development of a highly purified virus preparation which when suitably formulated has shown adequate persistence in the field. Comparisons of effectiveness in trial areas of 10 ha have demonstrated that the sprayed virus formulation gave control of *S. littoralis* comparable with that obtained using either egg-mass collection or conventional insecticides. Consideration is therefore now being given to the development of virus production on a commercial scale in Egypt. Assessments of commercially available formulations of *Bacillus thuringensis* have also been conducted. Their field persistence is poor and control achieved inadequate.

Mating disruption

P. gossypiella is the most important pest of cotton in Egypt and in years when no outbreaks of *S. littoralis* occur then most of the insecticides used on cotton are directed against the former insect pest. Large scale mating disruption trials in areas of up to 500ha have recently been conducted using pheromone formulated in microcapsules, hollow fibres and laminated flakes. All three formulations were equally successful in that levels of control were achieved comparable to those obtained using conventional insecticides.

Work is also in progress to develop similar formulations for control of *S. littoralis* and *E. insulana* by the mating disruption technique.

Monitoring

Monitoring techniques to determine the level of insect attack within the cotton crop are adopted in Egypt using damage threshold assessments for *S. littoralis*; boll counts for *P. gossypiella*, *H. armigera* and *E. insulana* and pheromone traps for all four pest species. This has meant that insecticides are used only when necessary and emphasis has been given to insecticides of low mammalian toxicity or those possessing high insect specificity, i.e. insect growth regulators.

Conclusions

The results of the continued integration of all these insect control practices has been to achieve an acceptable level of infestation associated with relatively few applications of insecticides.

STABILIZING PHEROMONES FOR FIELD USE : PROPHEROMONES

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Background and objectives

Pheromones and other behaviour controlling chemicals have considerable potential for use in agriculture but are often too volatile and chemically unstable, particularly those containing conjugated double bonds and aldehyde groups, for direct application to crops. Special formulations, such as those involving microencapsulation or enclosure within polymeric tubes or laminates, can partially solve these problems. We have attempted to provide an alternative approach by preparing involatile and stable propheromones that would release the pheromone or other behaviour controlling chemical under field conditions. Here, attempts to prepare propheromones by 1,4-cycloaddition reactions of conjugated double bonds or by acetal or ketal formation with carbonyl groups in pheromone molecules are described. Propheromones that would release pheromonal carbonyl compounds by thermolysis, photolysis or hydrolysis were investigated.

Materials and Methods

(E)-B-Farnesene (I), prepared from (E)-nerolidol, gave 1,4-cycloaddition reaction products on heating with electron deficient dienophiles obtained commercially or prepared by established methods. (Z)-11-Hexadecenal (II), prepared from decan-1,10-diol, (E)-citral (III) and 2-heptanone (IV), obtained commercially, gave acetals or ketals either by direct treatment with alcohols or by transacetalization using the ethanol adducts. The alcohols, 1- and 4-nitrophenylene glycols were prepared from 1- and 4-nitroacetophenone, benzyl, 1-nitrobenzyl and 3,5-dimethoxybenzyl alcohols were obtained commercially. (+)-Polygodial (V), prepared from B-cyclocitral, was treated with methanol under acidic conditions.

Results and conclusions

The 1,4-cycloaddition product (VI), from (E)-B-farnesene (I) (the aphid alarm pheromone) and SO_2, regenerated I on heating (180°C) and sufficient at ambient temperatures to cause a weak alarm response in aphids. Adduct VI was also active against colonization and virus transmission by aphids whereas the pheromone I was not. Other adducts from I and dienophiles such as diundecyl butynedioate were much more active against colonization and virus transmission but did not appear to act as propheromones. Adducts between the pheromonal carbonyl compounds II (a component of several lepidopterous sex pheromones), III and IV (components of honey bee pheromones) and only 1-nitrophenylene glycol or 1-nitrobenzyl alcohol released appreciable amounts of the pheromonal carbonyl compounds under simulated sunlight. In field tests these adducts of II caught diamondback moths in sticky traps more persistently than did underivatised II. The two aldehyde groups of (+) polygodial (V) (the insect antifeedant) reacted with two molecules of methanol to form the dimethoxytetrahydrofuran (VII) which regenerated V on hydrolysis but, although active, VII was not more persistent than V against aphid colonization.

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2B—R7

USES OF THE APHID ALARM PHEROMONE AND DERIVATIVES

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Background and objectives

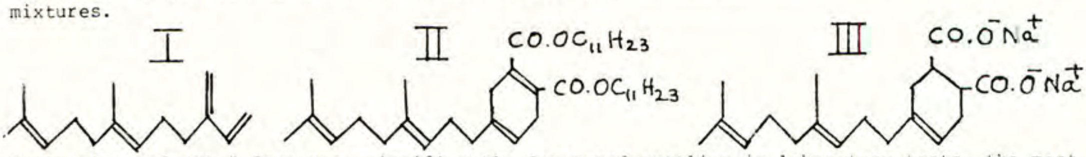
When aphids are attacked or irritated, their cornicles release a liquid secretion which quickly hardens, entangling any small parasites or predators that it contacts. Simultaneously, the cornicle secretion emits a volatile alarm pheromone, which is detected by neighbouring aphids, causing them to cease feeding and move away, or even to drop from the plants. For some years the alarm pheromone of most aphid species has been known to be (E)-β-farnesene (Bowers et al. 1972) but attempts to use the pheromone for aphid control have so far failed. This report describes recent developments in the use of the pheromone to increase contact activity of insecticides and use of derivatives of the pheromone to deter aphids from settling on plants.

Materials and methods

Samples of (E)-β-farnesene (Compound I), prepared in bulk by the method of Dawson et al. (1982) were tested against colonies of about fifty 3rd - 4th instar insecticide-susceptible *Myzus persicae* on leaves of Chinese Cabbage (*Brassica pekinensis*). The ability of the pheromone to increase contact activity of pesticides was tested by applying pheromone as a vapour from a syringe immediately before spraying with insecticide from a hydraulic nozzle, or by formulating pheromone and insecticide together and applying them by means of an electrostatically charged rotary atomiser (Arnold and Pye 1980). Derivatives of (E)-β-farnesene, prepared by the method of Dawson et al. (1982) were emulsified and painted on to leaf surfaces, and were tested for deterrent action by allowing groups of apterous *M.persicae* a choice of equal areas of treated and untreated leaf on which to settle and reproduce.

Results and discussion

Treatment of aphids with pheromone vapour immediately before spraying increased the kill of 0.05% formulation of permethrin from 38 ± 5.7% to 92 ± 2.9%. Similarly pheromone increased the kill by 0.05% permethrin applied by a charged rotary atomiser from 12 ± 5.7% to 76 ± 4.0% and by 0.04% bendiocarb from 8 ± 3.5% to 44 ± 9.1% in one test and from 22 ± 10.1% to 45 ± 14.9% in another. However, increasing the concentration of insecticide did not further improve kill and more needs to be done on formulation of concentrated pheromone-insecticide mixtures.



Derivatives of (E)-β-farnesene significantly decreased settling in laboratory tests, the most effective compound being the adduct of (E)-β-farnesene and diundecyl butynedioate (Compound II) When aphids were presented with a choice of leaf surfaces,untreated or treated with 0.5% Compound II they settled in the proportions 17:2 (variance ratio 165). This compound decreased settling significantly even when applied in 0.05% solution. Decreasing or increasing the n-alkyl chain length or introducing branching into the chain decreased deterrant activity as measured by the settling test, as did altering the chain derived from the (E)-β-farnesene. Apterous aphids turned away from filter papers treated with 0.5% Compound II, suggesting that, despite its expected involatility, it could be detected by aphids, at least over a short distance. Aphids placed directly on leaf surfaces treated with 0.5% Compound II sometimes exhibited exaggerated leg movements.

A diacid salt has also been prepared (Compound III) which is systemic. It is inactive as a surface treatment but decreases leaf colonisation by aphids when applied to the cut leaf petiole at 0.05% or via the soil at 0.1%.

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PHEROMONES IN INSECT CONTROL - ARE WE SUCCEEDING?

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Background

Interest in the use of behaviour-controlling chemicals, especially sex-pheromones, or their analogues, to improve pest control has exploded worldwide during the last decade. Yet, there are few authenticated examples of pheromones being used commercially to improve pest control programmes consistently.

Thesis

Research chemists have responded to the demands of applied entomologists for biologically-active synthetic sex-attractants for use in pest control programmes with a spate of analytical and synthetic studies. These were made possible by the development of new, sophisticated micro-analytical techniques which have been spectacularly successful in overcoming problems associated with the minute quantities of chemicals involved and the stringent requirements for purity set by the specificity of the insects' behavioural responses to them. The identities of at least the major components of the sex-pheromone, or biologically active analogues, are known for many pest species, especially among the Lepidoptera.

Despite this great research effort, there are very few examples of sex-pheromones being used commercially to improve control programmes consistently. Notable ones are:

Monitoring/Detection/Surveillance

Codling moth	-	*Cydia pomonella*
Pea Moth	-	*Cydia nigricana*
Summer fruit tortrix	-	*Adoxophyes orana*
Gypsy moth	-	*Lymantria dispar*

Control

Pink bollworm	-	*Pectinophora gossypiella*

The reason for this poor record must be the proportionally small effort put into behavioural/biological/ecological studies. The most successful programmes have included very high proportions of biological work, and future research policy should be based on their example. Unfortunately, most of the several hundred chemical identifications have not been followed up by either sufficient biological studies or relevant chemical work to solve the important problems confronting the applied entomologists. The required biological studies are time-consuming, often tedious and lack the glamour of other topics.

Although the chemists have provided an excellent basis for pest control with pheromones, their successful use involves manipulating the behaviour of insect populations, which can only be achieved with sound biological knowledge. The following are major constraints on further advance: (i) lack of detailed information on insect behaviour in response to pheromonal stimuli in the field (ii) unsuitable techniques for measuring pheromone concentrations (iii) inadequate assessment methods to measure the effects of pheromone treatment.

Conclusion

There must be a change of research emphasis if we are to improve the usefulness of this approach, which offered so much promise.

2B—R9

SEX PHEROMONES AID IN THE CONTROL OF POTATO TUBER MOTH

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Background and objectives

The potato tuber moth (PTM), *Phthorimaea operculella* (Zeller), is one of the most damaging pests of potatoes in field and storage, and is generally of greatest importance in warmer climates. The sex pheromones of *P. operculella* are released by female moths through a gland anterior to the last abdominal segment to attract males for mating purposes. These pheromones, which can be duplicated chemically, are now produced synthetically and are commercially available in several countries. The present study was undertaken to: a) determine the most effective pheromone blend that can maintain a 90-day attraction in field; b) compare unused pheromones with 90-day, field used pheromones; c) identify a suitable effective trap for use in monitoring and mass trapping the male PTM, leading to direct control in field and storage; d) relate seasonal moth population trends to tuber infestation at harvest.

Materials and Methods

Rubber caps were impregnated with the sex pheromone trans-4, cis-7-tridicadien-1-ol acetate (PTM 1) + trans-4, cis-7, cis-10-tridecatrien-1-ol acetate (PTM 2). The pheromones were used in 8 different ratios at 1 mg loading and were impregnated on the rubber caps which were suspended singly through a newly devised funnel trap. Six virgin female moths enclosed in a saran mesh cylinder were suspended in a similar trap for comparison with synthetic sex pheromones. For trapping purposes, a water trap, which was normally used and placed at ground level, was compared with the newly devised funnel trap also placed at ground level, at 40 cm and 80 cm elevation. Mass trapping studies conducted in the field involved spacing 1 funnel trap/225 mt^2 in variety Revolucion. In storage studies, 2 pheromone funnel traps were placed in one store at the same height as the stacked crates, a second store had no pheromone traps.

Results and Conclusions

All the 8 ratios of PTM 1 + 2 tested were signigicantly superior to the use of virgin female traps. Highest capture of male PTM was obtained using a 9:1 ratio. The attractiveness of 9:1 ratio to male PTM appeared to drop after 90-day field use. The 1:1.5 ratio remained attractive to PTM males during the 90-day trapping period. Storage of rubber caps with 1:1.5 pheromone ratio at -5°C for 2 months did not diminish the attractiveness of this pheromone ratio. PTM 1 alone had significantly lower captures than mixtures. In trapping studies, no significant differences ($P > 0.05$) in trap capture of PTM males were observed between the use of the water trap and funnel trap. The funnel trap is ideal for mass trapping leading to direct control. Using these traps tuber damage was significantly reduced by 23% in pheromone-treated plots. A total of 92,000 PTM males were captured in 110 days. In storage trials, the 2 funnel traps captured a total of 13,800 male moths in 120 days. Tuber and sprout damage in the store with funnel traps was 8% compared to 60% in the store without pheromone traps. Studies on seasonal, moth population trends were conducted under field conditions using the water trap. In this study, tuber damage was related to trap catches. When moth populations were high (seasonal mean 348 PTM males/week), potato clone DTO 33 sustained 42% tuber damage.

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PERFORMANCE OF PHEROMONE TRAPS AND DISPENSERS IN APPLE ORCHARDS

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Background and objectives

In recent years, five species of leafrollers have been found to injure leaves and fruits of apple and *Archips pomivora* is most predominating in Himachal Pradesh, India. Various types of traps and dispensers have been used in apple orchards to monitor the population of leafrollers in United States, Canada and Europe but no such work has been carried out in India. The objective of the present study was to investigate the relative differences in pheromone response within a tree along vertical and horizontal gradient, with regard to the aspects of the orchard and to develop an improved method of monitoring the population of leafrollers in apple pest management programme.

Materials and Methods

These studies were conducted in the experimental orchard of the Regional Fruit Research Station, Mashobra and Ekant Van Orchard, Dhalli, Shimla during 1981-82. Eight synthetic programmes viz. codling moth (CM), oriental fruit moth (OFM), fruit-tree leafroller (FTLR), fruit-tree tortrix moth (ARPO), filbert leafroller (AROS), obliquebanded leafroller (OBLR), summerfruit tortrix moth (SFTM) and gypsy moth (GM), were evaluated for their trapping response in lepidopterous pests of apple. Three types of traps: Pherocon 1C (Zoecon Corp., Palo Alto, California, U.S.A.), Traptest (Farmoplant, Montedison, Milano, Italy) and a self-made triangular trap consisting of a corrugated cardboard sheet with base surface (23cm x 26cm) coated with Bird Repellent (Rentokil Ltd., West Sussex, England) were used. The traps were examined once a week and the moths were removed and recorded. The observations were continued for eight weeks and the dispensers were not replaced during the experiment. Vertical height variables; 1.7m, 3.5m and 5.3m above the ground level were evaluated and the traps were placed on the open side of the tree. The traps, in horizontal distances, were placed 3.5m above the ground level to test three canopy positions; near the tree trunk, centre of the canopy and at the periphery of the tree. Aspects of the orchard, east, west, north and south were also evaluated separately with triangular traps, hung at 1.7m (eye level) above the ground level. In all the sets of experiments each treatment was replicated three times and the trial was laid out in split plot design.

Results and conclusions

Pheromones: OBLR pheromones attracted significantly more moths of leafroller, *A. pomivora* (73.88%) than FTLR pheromone (26.12%) in all sets of experiments. ARPO and AROS pheromones responded very poorly to *A. pomivora* and OFM, CM and SFTM pheromones did not attract any lepidopterous pests at all. *A. pomivora* was most abundant during May and least during November. GM Pheromone trapped *Lymentria concolor*. Further studies were therefore carried out with OBLR and FTLR pheromones.

Trap design: No significant differences in catches of moth in three types of traps were observed.

Trap height: Traps caught maximum moths of *A. pomivora* when placed in the upper tree canopy (5.3m). Traps located at eye level (1.7m) trapped the least number of moths. Positive response to pheromones was obtained with increase in trap elevation. Traps located at 5.3m caught on an average 15.7 moths/trap (46.0%) during eight weeks while those at 3.5m and 1.7m captured only 12.2 (25.7%) and 6.3 moths/trap (18.3%), respectively, all being statistically significant.

Horizontal gradient position: The traps located on the periphery trapped maximum number of moths (13.8/trap, 41.2%), while the traps in the centre of canopy and near the tree trunk caught on an average 11.2 (33.4%) and 8.5 moths/trap (25.4%).

Orchard aspect: The traps in the Northern aspect attracted significantly more moths (15.5/trap, 39.3%) than Eastern 10.1 (25.6%) and Western aspects (8.2, 20.7%). Traps on the Southern aspect of the orchard caught minimum moths (5.7/trap (14.3%).

These studies indicate that the trap performance is greatly affected by vertical and horizontal positions and the upper half of the tree canopy should be the optimal choice for trap location. Traps are more effective when positioned on the periphery of the tree and the Northern aspect of the orchard is the most ideal.

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2B—R11

PHEROMONE DISPERSAL - IMPLICATIONS FOR PEST CONTROL

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Background and objectives

The further development of insect sex pheromones in pest control, for monitoring population movements or as direct control agents, requires more detailed knowledge of each pest's behavioural responses to synthetic pheromonal stimuli in the field. The simplest stimulus is a single, continuously-emitting point source - e.g. a trap. Experiments with simple configurations of traps for the pea moth, *Cydia nigricana* (F.), indicated the range of attraction and flight behaviour of responding males. The effect of the absorption of sex-pheromone and its subsequent release by the vegetation around a trap on the behaviour of males was also demonstrated.

Materials and Methods

Water traps containing 100 µg (E,E)-8,10-dodecadien-1-yl acetate were placed at crop height in wheat fields (moth emergence sites). Trap interactions were shown using lines of three traps oriented either across or along the mean wind direction, with inter-trap spacings up to 100 m indicating a range of attraction well in excess of this. Absorption of pheromone by plants was shown by (i) removing traps, which had been in position for 0.5-3.0 h, and observing the numbers of males continuing to approach the vacated position, (ii) chemical analyses of leaves and (iii) electrophysiological tests on moths.

Results and conclusions

In lines of traps across the wind, competition between the traps reduced the catch in the centre trap. In lines along the wind, the upwind trap 'poached' moths from the other two traps. Moths continued to respond to absorbed pheromone up to 3 h after trap removal.

The results indicate that: (i) in wheat a trap generates a continuous field of attractant downwind, partly as a result of uptake by plants; (ii) the range of attraction can be several hundred m; (iii) moth orientation to a trap can be disrupted by the presence of other attractant sources, but (iv) some moths always reach a trap, even when surrounded by attractant from other sources.

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EVALUATION OF THE EFFICACY OF CERTAIN BACTERIAL INSECTICIDES (*BACILLUS THURINGIENSIS*) IN CONTROLLING *SPODOPTERA LITTORALIS* IN EGYPT

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Background and objectives

Experiments were carried out in the laboratory and the field to investigate the effect of five bacterial formulations on the cotton leafworm, *Spodoptera littoralis* Boisd.

Materials and Methods

In the laboratory, different concentrations of each bacterial formulation (Dipel, Bactospeine, Bactospeine/Thiodane, Thuricide and ABG-6105) were tested.

Field tests were carried out on clover (berseem) during February and March 1982 and the plants treated with different doses of each compound. The mortality percentages among larvae fed on the sprayed clover leaves were calculated.

Results and conclusions

The results in the laboratory indicated that with a practical spray concentration of 1.5-2.0% of the tested compounds, the compounds could still be classified, according to their effect, into three groups:

1) The first group (compounds with high mortality percentages): Bactospeine and the mixture of Bactospeine/Thiodane.
2) The second group (with moderate effect): Dipel and ABG-6105.
3) The third group (with the lowest effect): Thuricide.

In the field investigations the feeding stimulant "Coax" was added to an apparently promising concentration (1.0 kg/feddan) of the investigated compounds. The data showed that "Coax" activated, to a certain extent, the effect of Dipel and Thuricide on third instar larvae during the first 24 h after spraying. This effect was not reflected, however, on the weights of pupae resulting from these larvae. Bactospeine at 1.5 and 2.0 kg/feddan produced the lightest pupae when the third instar larvae were fed on the treated berseem leaves immediately after spraying.

2B—R13

TERPENOID IMIDAZOLES: NEW ANTI-JUVENILE HORMONES AND INSECTICIDAL COMPOUNDS

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Background and objectives

Most of the insect growth regulators (IGRs) with juvenile hormone (JH) activity were derived from monoterpenes and sesquiterpenes. Although some nonterpenoid IGRs such as peptides and bisthiolcarbamates have been known to show a high morphogenetic activity, their chemical skeletons seem to be similar to those of terpenoid IGRs. On the other hand, many 1- and 4-substituted imidazoles are well known potent inhibitors of mixed function oxidases and some of them have shown to inhibit the epoxidation of methyl farnesoate to JH III in the JH biosynthetic pathway *in vitro*. So we synthesized a number of nitrogen-containing heterocyclic compounds having a terpenoid moiety and examined their biological activities.

Materials and Methods

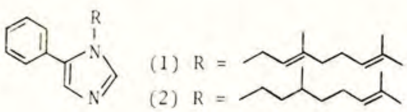
The compounds were prepared by reacting geranyl chloride or citronellyl mesylate with the appropriate nitrogen-containing heterocyclic compounds in dimethylformamide using NaH as a base. The introduction of a methoxy or ethoxy group in the 7 position of the terpene chain of the compounds was carried out by alkoxymercuration, followed by demercuration with $NaBH_4$. The compounds were applied topically in acetone solution (1-4 µl/larva) to the 0-8 hr third and fourth instar larvae of *Bombyx mori*. The larvae were reared on fresh mulberry leaves and anti-JH activity was determined by premature metamorphosis of the treated larvae. Insecticidal activity was determined by applying 1 µl of an acetone solution of each compound topically against susceptible (WHO, SSS) and resistant (Daisan Yumenoshima) strains of houseflies.

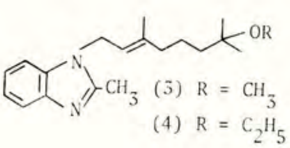
Results

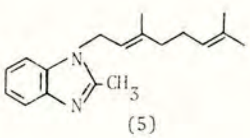
New compounds, 1-geranyl-5-phenylimidazole (1) and the 1-citronellyl analog (2) showed remarkable anti-JH activity against the silkworm, *B. mori* larvae. When treated to the third instar larvae, miniature pupae were formed after molting to the fourth instar larvae. The fifthe instars were also omitted by treatment of the fourth instar larvae. The presence of a phenyl group at the 5 position of the imidazole ring is essential for anti-JH activity. 1-Geranylimidazole, 1-geranyl-2-phenylimidazole and the 4-phenyl analog were quite inactive. 1-Dodecyl-5-phenylimidazole had much lower activity than compounds 1 and 2. The introduction of a methoxy group in the 7 position of the terpene chain of compounds 1 and 2 eliminated or decreased the activity. The anti-JH effects of compounds 1 and 2 on *B. mori* larvae were completely blocked by simultaneous application of a JH mimic such as methoprene, while the acute toxicity of those compounds which was shown at high doses for third instar larvae was not rescued by application of methoprene. The miniature pupae caused by applying compounds 1 and 2 molted to the miniature adults which underwent normal mating, ovarian development and oviposition. These results suggest that the mode of action of compounds 1 and 2 is quite different from that of precocene II.

On the other hand, benzimidazoles having a terpenoid moiety, 1-(3,7-dimethyl-7-methoxy-2-octenyl)-2-methylbenzimidazole (3) and the 7-ethoxy analog (4), showed high insecticidal activity against housefly adults. In the series of 2-alkyl-1-geranylbenzimidazole, the 2-methyl analog (5) showed the highest insecticidal activity. Substitution on the 5 or 6 position led to a drastic decrease in activity. The introduction of a methoxy or ethoxy group in the 7 position of the terpene chain of compound 5 increased the activity, while the 6,7-epoxy analog showed lower activity than 5. Compounds 3 and 4 showed higher insecticidal activity than malathion and also high activity against the organophosphorus resistant houseflies and green rice leafhoppers, *Nephotettix cinticeps*. Also compounds 3 and 4 inhibited larval molting of *B. mori* at low doses when applied topically to the larvae 1-2 days before ecdysis. The treated larvae were unable to liberate themselves from the old larval skins, so that they finally perished

On the basis of preliminary biological data it is concluded that compounds 1,2,3 and 4 represent reasonable leads for the development of new IGRs and insecticides.







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TOXICITY OF PRECOCENES AND ANALOGUES TO THE RICE BROWN PLANTHOPPER

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Background and objectives

Precocenes I and II have been shown to cause a range of biological responses, including precocious metamorphosis, in a limited number of insect species. At similar dosages required for these effects mortality also occurs. The mode of action of these morphogenic effects is thought to occur through selective necrosis of the corpora allata leading to reductions in juvenile hormone titre. However, it is thought that other sites of action may be responsible for lethal effects. Although other homopteran species have been reported to show morphogenic effects with precocenes the brown planthopper, *Nilapavarta lugens* (Homoptera; Delphacidae) has not shown these. As *N. lugens* is a widespread and important pest of rice it provides an appropriate test species and consequently the lethal action of precocenes I and II and three analogues is reported here.

Materials and Methods

A strain of *N. lugens* originating in Japan was used in this experiment. Insects were reared for many generations on growing TNI variety rice plants in the greenhouse. The compounds tested were **1** : precocene I (7-methoxy-2,2-dimethylchromene), **2** : precocene II (6,7-dimethoxy-2,2-dimethylchromene), **3** : 7-methoxy-2,2-dimethyl-6-nitrochromene, **4** : 6-amino-7-methoxy-2,2-dimethylchromene and **5** : 4-chloro-7-methoxy-2,2-dimethylchromene. The required concentration of each compound in acetone (1 ml) was applied to cover the bottom part of a 9 cm glass petri dish. After the solvent had evaporated a fresh piece of rice stem was introduced. First instar nymphs of *N. lugens* were included and the dish was sealed. The insects were kept in the dark at 25°C. Treatments were replicated at least three times. Mortality was assessed at 24 h and per cent mortality was corrected using Abbott's formula. Log dosage-probit mortality regression yielded lines of similar slopes.

Results and conclusions

The toxicities and toxicity indices relative to precocene I of the five compounds were as follows :

Compound	LC50($\mu g/cm^2$)	95% fiducial limits	slope	toxicity index
1	18.8	12.5 - 27.4	1.58	1.0
2	1.8	1.6 - 2.0	1.59	10.4
3	4.9	4.3 - 5.6	2.29	3.8
4	13.3	10.2 - 18.6	1.80	1.4
5	8.3	6.3 - 10.4	2.13	2.3

If all the compounds are considered as derivatives of precocene I, then substitution in the 6-position increased activity in the order H- $<$ H_2N- $<$ O_2N- $<$ MeO- . Substitution by chlorine at the 4-position also increased the toxicity. In general, the published structural requirements for the optimisation of anti-juvenile hormone activity do not appear to fit in with those of toxicity for this species and method of treatment.

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2B—R15

STUDIES OF LIMONOID INSECT ANTIFEEDANTS

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Background and Objectives

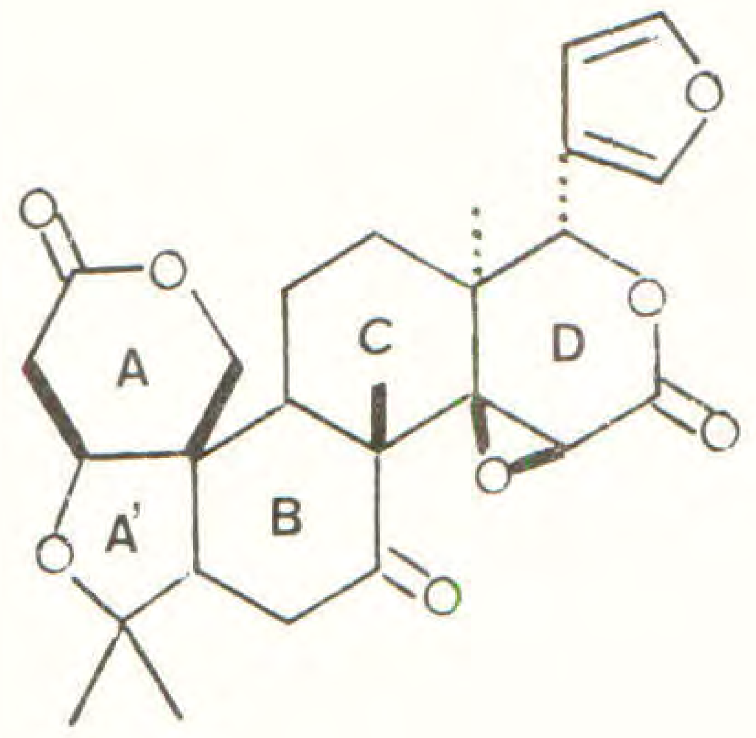
A number of naturally occurring limonoids are now known which have high activity as insect antifeedants and/or ecdysis inhibitors. Since the synthesis of these substances on a large scale is at present impractical, application in the near future will necessarily involve use of those limonoids which can easily be obtained in high yield as phytochemicals or by synthesis of compounds derived from them. Limonin can be simply obtained in pure form from various citrus seeds available in quantity as a by-product of the citrus industry. This limonoid also has potential as a useful substrate for synthetic modification for the production of analogues and as a model compound on which synthetic modification could yield structure-activity information useful in synthetic antifeedant design. The exploration of these possibilities is the objective of this research.

Materials and Methods

Antifeedant activity was determined using sixth instar larvae of the African armyworm, Spodoptera exempta in a choice assay comparing feeding rates on surface-treated disks relative to untreated controls. Synthetic modification of limonin was achieved using methods developed during early degradation studies on this molecule and the resulting compounds were evaluated in the armyworm feeding bioassay.

Results and Conclusions

Bioassay of limonin reveals antifeedant activity against African armyworm to be somewhat lower than that reported for other insects. Synthetic modification of functionality in rings, A', B, D, and the furan ring results in significant changes in antifeedant activity and when these results are compared with those reported on related limonoids allows some qualitative assessment of structure-activity relationships. The results may prove useful in design of antifeedants.



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CAN SUB-TOXIC HERBICIDE PRETREATMENTS INCREASE TOLERANCE TO HERBICIDES?

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Background and Objectives

In recent years the concept of chemical safeners to protect crops from herbicide injury has led to the development of several commercially viable compounds. In some cases the chemical safener is similar in structure to the herbicide for which it is effective. In most cases, the safener itself can be toxic to the crop when given at higher rates. What property of these chemicals makes them effective safeners at low rates but phytotoxic at higher rates? If compounds similar in structure to a herbicide can act as safeners for that herbicide (1), can herbicides themselves given at low rates protect crops from later herbicidal applications of the same chemical? Some investigators (2) have shown that prior treatment can result in increased rates of atrazine metabolism by maize. However data are lacking on actual protection at the whole plant level. The purpose of our research was to examine whether (i) crops susceptible to a particular herbicide could be made more tolerant by prior pretreatments with the same chemical (ii) whether crops already tolerant to a herbicide could be made more tolerant to higher rates of the herbicide by prior pretreatment.

Materials and Methods

The following herbicide: 2 crop combinations were studied. (i) Metribuzin on tomatoes (cv. H2653) and soybeans (cv. Evans); (ii) Alachlor on sorghum (cv. Dekalb E59+) and maize (cv. S x 111) and (iii) CDAA on maize. Seeds were planted in moist vermiculite in double styrafoam cups. Pretreatments ranged from 1/10 to 1/1000th the herbicidal rate, given as a root drench or seed pretreatment. Herbicide was applied to roots. Plants were maintained in a growth room at 25°C and 75% relative humidity (daytime) under a 16 h photoperiod and an average light intensity of 400 einsteins/m^2 from metal halide lamps. Plants were harvested 8-14 days following herbicide treatments.

Results and Conclusions

Pretreatments alone did not affect plant growth, except in some cases where slight growth reduction (15%) was observed. This surprisingly did not result in later additive injury upon treatment with herbicidal rates. Pretreatments with metribuzin gave slight protection to tomatoes and soybeans, although not significantly in all the experiments. Alachlor pretreatments did not protect sorghum from later alachlor injury. However, prior alachlor treatment did increase maize tolerance to alachlor. The most encouraging results were obtained in maize with CDAA. Prior treatment significantly reduced CDAA injury from 23% (no pretreatment) to 8% (with pretreatment). We conclude that employing early pretreatments may be a promising approach for increasing crop tolerance to a herbicide particularly when marginally adequate tolerance to that chemical at herbicidal rates already exists.

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2B—R17

THE USE OF GERMINATION STIMULANTS FOR THE CONTROL OF OROBANCHE IN TOMATO

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Background and objectives

Broomrape (*Orobanche* Spp.) is a very widespread destructive root parasite of many vegetable and agronomic crops grown in the semi-arid regions of the world. Very often crop failure occurs as a result of heavy infestations in the field. Methods attempted for *Orobanche* control are variable and include mechanical, cultural, biological and chemical methods; however, limited success has been accomplished, since most of these operations are either ineffective, inconsistent or uneconomical.

A new approach for possible control of *Orobanche* has been investigated through the use of synthetic germination stimulants of the parasite seeds in the absence of the host plants, hence causing suicidal kill of the parasite. Six stimulants were tested *in vitro* and under greenhouse conditions.

The objectives of the studies were to test the effect of new synthetic strigol analogues (GR-compounds) on *Orobanche* seed germination, investigate the effects of some soil factors on their activity, and evaluate their efficacy for *Orobanche* control.

Materials and Methods

The effects of various concentrations of GR compounds on *Orobanche ramosa* L. seed germination were studied *in vitro* in Petri dishes. *O. ramosa* seeds were spread on fiber-glass filter paper discs, 8 mm in diameter, and moistened with sterile distilled water. These seeds were then subjected to two weeks period of preconditioning at 25 °C, after which they were transferred into plastic Petri dishes, and treated with solutions of the stimulants at different concentrations.

The effects of some soil factors on the activity of GR24 and GR28 were studied *in vitro* in soil. Discs containing preconditioned *O. ramosa* seeds were placed in muslin cloth bags in a cup, and then covered with 150 g of soil. The soil was then drenched with enough solutions of GR24 and GR28, which made a concentration of 1 ppm by weight of the soil. In all the *in vitro* experiments, seed treatment was followed by a period of incubation at 25 °C for 14 days, after which the germination percentages were determined using a binocular. In greenhouse studies, tomato seedlings of a local cultivar 'Marmande' were transplanted into clay pots containing a soil-peatmoss mixture which had been inoculated with *O. ramosa* seeds. pots were drenched with solutions of the synthetic stimulants, 1 to 8 weeks before transplanting, at 1 to 3 ppm (W/W). Data on tomato fruit yield and shoot dry weight, as well as on the dry weight and number of shoots of *O. ramosa* were collected at the end of each experiment.

Results and conclusions

The results of the *in vitro* experiments indicated that the germination stimulants GR7, GR24 and GR28 at 1 ppm, were the most active compounds in inducing *O. ramosa* seed germination (81.7 %, 84.9 % and 87.6 %, respectively). GR 41 and GR53 had intermediate activity (62.0 % and 61.3 %, respectively).

Studies on the activity of various strigol analogues in different soils, revealed that GR7, GR24 and GR45, used at 1 ppm, were the most active. The activity of GR28 was not affected by soil pH ranging from 5.9 to 8.0; however, the activity of GR24 was decreased with increased soil pH. Soil texture apparently had no direct effect on the activity of GR24. The higher germination percentage obtained in coarse-textured soil, compared to the fine textured soil, was probably due to conditions which favored seed germination in such a soil.

In greenhouse studies, GR7 applied at 1 to 3 ppm (W/W), 5 to 6 weeks before tomato transplanting, reduced significantly the dry weight of *Orobanche*. GR28 applied at 1 and 3 ppm did not reduce the *Orobanche* infestation as compared to the inoculated control; whereas concentrations of 1+1 ppm and 1+1+1 ppm applied sequentially at weekly intervals, four, five and six weeks before tomato transplanting as of the initial spraying time reduced significantly the number and dry weight of *Orobanche*, as compared to the inoculated control. GR24 applied at 2 and 3 ppm, four and five weeks before tomato transplanting, reduced significantly the dry weight of *Orobanche* as compared to the inoculated control. In other experiments, GR24 applied at 1 or 2 ppm (W/W), four and six weeks before tomato transplanting, caused significant reductions in the number of haustoria and dry weight of *O. ramosa*.

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METAZACHLOR SAFENED FOR MAIZE

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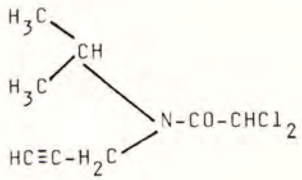
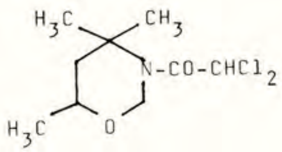
Metazachlor, N-(2,6-dimethylphenyl)-N-(1-pyrazolyl-methyl)-chloroacetamide was recently introduced commercially for the control of weeds in oil seed rape. In combination with specifically tailored antidotes (safeners) metazachlor promised to be a potent preemergence herbicide for maize. The antidotal properties of differently structured dichloroacetamides were investigated under varying soil types and moisture conditions.

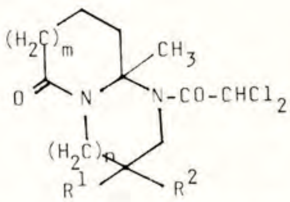
The following compounds were selected in greenhouse and field trials.

A



B





	m	n	R^1	R^2
C	0	0	H	H
D	0	1	H	H
E	1	0	H	H
F	0	1	CH_3	CH_3

Individually applied the latter did not provide any biological activity on intact plants. However, in situations where certain rates of metazachlor caused damage to emerging maize seedlings these compounds worked as superior safeners.

In the open on a light sandy soil using the model ratio for the mixture herbicide: antidote = 8:1 the safening efficacy was established corresponding to the range F = E=D > A > C = B. In trials including the same factors plus excessive precipitation the range turned out to be F > E > D = C > B > A. Additional testing under various soil conditions confirmed compound F as matching metazachlor more favourably when used at the ratio herbicide: antidote = 8:1 to 6:1 with the herbicide at 1.0 to 1.5 kg/ha a.i. or less.

2B—R19

INDIRECTLY ACTING FUNGICIDES

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Introduction

The usual way of controlling crop diseases is to use compounds that kill fungi. An alternative is to use compounds that act against disease, either through effects on pathogenic processes of fungi or on resistance mechanisms of the host. Such compounds would not be inherently lethal. Interference with any one of the processes (of host or pathogen) that are essential for disease to occur should result in disease control. This report briefly illustrates some of the ways in which this may have been achieved by indirectly acting compounds. For a comprehensive review see P. Langcake (1981) Phil. Trans. R. Soc. Lond. B. 295, 83-101.

Compounds acting against pathogenic processes of fungi

To be pathogenic, fungi must enter plants. Compounds that inhibit cutinase activity of pathogens that normally penetrate the cuticle can control disease e.g. diisopropyl fluorophosphate. This compound does not affect the growth of fungi.

The commercial rice blast fungicide, tricyclazole, is 30 times less active *in vitro* than *in vivo*. It inhibits melanin biosynthesis, essential for the rigidity of the cell wall and appressorial infection peg of *Piricularia oryzae*. When melanin production is inhibited the strength of the infection peg is insufficient and infection is prevented. Two other blast fungicides, CGA 49104 and PP 389 appear to act in the same way.

Two important avirulence factors causing disease symptoms and spread are toxins and hydrolytic enzymes. Ferulic and chlorogenic acid detoxify the toxin piricularin produced by *P. oryzae*. When applied to rice plants an increase in resistance was seen although the acids are not antifungal. The phenolic, rufianic acid, inhibits fungal pectolytic and cellulolytic enzymes *in vitro*. It also gives control of *Rhizoctonia* on bean and wilts of tomato.

Compounds causing changes in host physiology that reduce disease.

Chemicals that cause changes in host physiology can often affect subsequent disease development. A series of auxin compounds based on naphthalene acetic acid control *Fusarium* wilt of tomato. Auxins can increase calcium pectate content of plant cell walls making them resistant to fungal pectolytic enzymes. Other auxins control wilts by causing blockage of xylem vessels thus restricting disease spread.

Ethylene can induce changes in peroxidase and polyphenol oxidase activities, pectic enzymes, and induction of phytoalexins and fungal cell-wall degrading enzymes in plants, leading to increased resistance to wilts.

Compounds that act on host resistance mechanisms

Phenylthiourea (PTU) protects cucumber plants from *Cladosporium cucumerinum* by causing a strong lignification reaction around sites of fungal penetration. Genetically resistant plants respond to *C. cucumerinum* in the same way. PTU inhibits polyphenol oxidase thus making more phenolics available for peroxidase action and lignin synthesis.

Probenazole, a new rice blast fungicide, is converted to two active principles. Neither are inhibitory to *P. oryzae* in agar. Although "probenazole" inhibits *P. oryzae* appressorium formation, other effects on growth are much more pronounced in the presence of host tissue. Blast lesions on treated rice are similar to hypersensitive lesions of resistant cultivars.

The experimental dichlorocyclopropane fungicide (WL 28325) is a systemic prophylactic compound highly specific for rice blast. Treated plants respond to infection by *P. oryzae* in a manner very similar to that of genetically resistant rice.

The new systemic fungicide, aluminium tris o-ethyl phosphonate (TEPA), stimulates defence reactions and synthesis of phytoalexins in grapevine and tomato against Oomycetes. It is inactive *in vitro*. Inhibitors of phenolic synthesis (eg. glyphosate) annul TEPA's effects in plants. Phenolics are required for phytoalexin synthesis.

Some fungicides are potent inhibitors *in vitro* yet they can also induce responses in the host similar to those of natural resistance. It is difficult to exclude the possibility that these host responses contribute to the effectiveness of some fungicides, eg. acylalanines and Curzate against downy mildew on vines.

Conclusion

Commercial indirectly acting fungicides are emerging. More will do so. Few companies are prepared to ignore the possible contribution of indirect mechanisms to the effectiveness of fungicides.

RELEASE OF AN ENDOGENOUS ELICITOR OF A DISEASE RESISTANCE RESPONSE FROM SUSPENSION CULTURED FRENCH BEAN CELLS

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Background and objectives

Phytoalexins are low molecular weight, host plant-synthesized antimicrobial compounds which accumulate in plant cells in response to infection with phytopathogenic microorganisms. Their accumulation may also be induced by treatment of plant tissues with a wide range of unnatural chemical agents (abiotic elicitors) or cell surface macromolecules from the pathogen (biotic elicitors). Recent reports have suggested that induction of phytoalexins during interactions between host plants and phytopathogens may involve intercellular transmission of the response via the release, from the host, of natural, endogenous elicitor molecules. However, these studies have relied upon the direct extraction of elicitor-active molecules from host plant tissue by harsh chemical or physical treatments, and the physiological significance of the biological activity of these endogenous elicitors is therefore in doubt. We have attempted to demonstrate release of endogenous host elicitors from French bean cells which have been induced to produce isoflavonoid phytoalexins by treatment with exogenously applied biotic or abiotic elicitors. This approach does not require harsh extraction procedures, and furthermore allows analysis of the timing of events during the intercellular transmission of the elicitation response.

Materials and Methods

Cell suspension cultures of French bean variety Canadian Wonder were grown in a modified Schenk and Hildebrandt medium. Culture batches (50ml) containing a further 10ml of the same culture within a dialysis bag, were exposed to a range of macromolecular biotic and abiotic elicitors. Control experiments consisted of flasks where the external cells outside the dialysis bag were replaced by conditioned culture medium plus macromolecular elicitor. After various time intervals the external cells (those directly in contact with the elicitor) and the cells inside the dialysis bags were harvested and the activities of L-phenylalanine ammonia-lyase (PAL, EC 4.3.1.5.), chalcone synthase (CHS) and chalcone isomerase (CHI, EC 5.5.1.6.) (key inducible enzymes associated with isoflavonoid phytoalexin defence response) were measured. Enzyme induction in the cells inside the dialysis bag demonstrated transmission of the elicitation response from the outside cells.

Results and conclusions

A range of basic polypeptides (abiotic elicitors), of which denatured ribonuclease A was the most potent, induced PAL, CHS and CHI activities in the cells to which they were added; this was followed, with a lag of approximately 2 hours, by increased enzyme appearance in the cells separated from the abiotic elicitors by the dialysis membrane. Such intercellular transmission of elicitation did not occur in response to biotic elicitor preparations from Colletotrichum lindemuthianum (the causal agent of anthracnose disease of bean) or yeast. No enzyme induction was observed in control experiments where elicitor, but no bean cells, was placed outside the cells in the dialysis bag. These and other results indicated that a low molecular weight, heat stable elicitor is released from bean cells following treatment with abiotic elicitors. In ribonuclease-treated cell cultures, the endogenous elicitor appeared in the culture medium at a maximum activity level 45 min after treatment of the cells with the elicitor; it was no longer detectable 2 hours later.

The above cell culture system has potential for (a) the extraction and structural elucidation of the endogenous elicitor, (b) studies of the biochemical mode of action of the elicitor and (c) studies of the elicitor's biological specificity. The endogenous elicitor, once characterised, may provide a basis for the design of molecules aimed at activating the host plant's own resistance mechanisms.

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2B—R21

EFFECT OF CERTAIN PLANT GROWTH REGULATORS ON MANGO SHOOT GALLS CAUSED BY *APSYLLA CISTELLATA*

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Background and objectives

Shoot gall is a serious mango malady caused by *A. cistellata* in India, Bangladesh, Nepal, Pakistan and Oman. Affected shoots do not fruit and get dry in the succeeding year. Axillary and apical buds are induced to form galls when first instar nymphs feed *in situ* at the ovipositional sites (G. Singh *et al.* 1975).

Insecticidal control is costly and produces side effects, so that growth regulators were tried to disturb the physiology of gall formation and to vanish the pest automatically. Earlier workers have suggested that auxins work as active cecidogenic agents in plants (1968 a).

Materials and methods

Trials were initiated in the year 1979-80 with 1-naphthyl acetic acid (NAA), 2,4-dichlorophenoxy acetic acid (2,4-D) and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T). In 1981-82, 2,4-D 250 mg/l, 2,4,5-T 250 mg/l, gibberelic acid (GA) 50 and 100 mg/l, NAA 200 mg/l, choline chloride (CCC) 100 mg/l and maleic hydrazide (MH) 50 and 100 mg/l and abscessic acid (AA) 10 and 50 mg/l were sprayed twice at fortnightly intervals with a high volume sprayer on the affected shoots starting on 14th September. A third trial in 1982-83 included a single spray of 2,4-D on 10th October @ 20, 50, 70, 100, 120, 150 and 200 mg/l to the point of slight run off as above. Each dose was replicated on four twigs.

Observations were recorded on the number of galls formed, their shape and size, number of nymphs per five galls and flower panicles emerged.

Results and conclusions

In the present investigations auxins (NAA or IAA) did not alter the physiology of gall formation by *A. cistellata*. GA increased gall numbers slightly but did not change their shape or size. Similarly growth inhibitors CCC, maleic hydrazide and abscessic acid did not affect gall formation at above concentrations. 2,4-D and 2,4,5-T were the only plant hormones which gave rise to abnormal open galls with loose scales and elongated axis. The degree of elongation and looseness of scales was proportionate to the dose applied. Looseness of scales started after 15 days of spray with 200 mg/l of 2,4-D, 25 days with 100 mg/l and after 45 days in 70 mg/l. With 50 mg/l of 2,4-D opening of scales was partial (up to 50%). Nymphs could not survive at 200, 150, 120 and 100 mg/l doses of 2,4-D due to alteration in shape and size of galls resulting in automatic control of the pest. Such opened galls did not give rise to flower panicles.

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SEX PHEROMONE COMMUNICATION DISRUPTION IN CORN ROOTWORM BEETLES, DIABROTICA SP. *

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Background and objectives

Disruption of behavioral patterns leading to reduced mating is recognized as one of the emerging strategies toward environmentally benign insect population management. Techniques originally developed for cotton-, vegetable-, and fruit insect control are now being applied to the Chrysomelid leaf beetles on *Zea mays* where their chemical control has been plagued by acquired pesticide resistance. Based on earlier papers by Hummel and Kucera (1977), Hummel (1979), and Hummel and Andersen (1982) this report summarizes some recent progress in the control of the corn pest *Diabrotica undecimpunctata howardi* Barber by air permeation of corn fields with 1. the non-toxic, environmentally benign female sex pheromone 10-methyl-tridecane-2-one and 2. several terpene natural products derived from the camphorweed plant *Heterotheca sp*. (Compositae).

Materials and Methods

Racemic 10-methyl-tridecane-2-one has been synthesized in a 6-step procedure. Sticky traps baited with pheromone contained in cellulose hollow fibers of 0.25 mm internal diameter were displayed at ear height of the corn plants. Beetles caught were removed daily, washed off with pentane, sexed, and counted. Female sex pheromone disruptant was volatilized from an 8 x 8 array of 64 stations in a 100 m^2 area. During the months of August and September, the number of male beetles attracted to the pheromone baited trap located in the center of the treated plot was compared to the number in a similar control plot located at least 25 m upwind. Using the same arrangement, another area was permeated with an ethanolic solution of the terpenes 1-bornylacetate, 1-borneol, D,L-camphor, and 1.8-cineole evaporated from filter paper squares of 25 cm^2 area each. Control and treatment plot were interchanged at least once each week to compensate for possible positional effects.

Results and conclusions

When compared to untreated control plots, permeation with racemic 10-methyl-tridecane-2-one resulted in a reduction of 30-80% of the males caught in the sticky pheromone baited survey traps. Also, permeation of the area with any of the *Heterotheca* terpenes or their quarternary mixture resulted in a reduction of the number of males caught. A correlation exists between concentration of disruptants and disruptive effect. Concentrations of terpenes must be 100-1000 times higher (about 10g/100 m^2/day) than that of the sex pheromone for achieving equal permeation effects. The behavioral control observed is completely reversible. After exhaustion of the disruptant sources, no statistical differences remain between treatment and control plots.

The suppressive effect on sex pheromone communication obtained with natural products from *Heterotheca sp.* is reminiscent of observations by Hummel and Andersen (1982) who find significant reductions of sex pheromone communication and orientation behavior in Chrysomelid beetles in the vicinity of *Cucurbita* plants.

While the sex pheromone communication disruption seems, at first sight, to be more rational and effective in terms of savings in volatilized disruptant, natural products produced by plants may have one important advantage : their source may be interplanted with the crop to be protected. Thus, the disruptant may be continuously released during the growing season, an idea that has been practiced and found useful by native people ever since antiquity.

The results obtained so far encourage 1. similar experiments in larger areas and 2. controlled followup studies in which the level of communication disruption is compared with the level of root damage caused by larvae of the subsequent generation. Ultimately, resulting yield losses in treated and untreated plots must be determined.

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2B—R23

WITHANOLIDES AND RELATED STEROIDS FROM SOLANACEAE AS INSECT ANTIFEEDANTS

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Background and objectives

During an infestation of small field plots of various solanaceous plants by larvae of Spodoptera littoralis it was noted that cape gooseberry (Physalis peruviana) was not attacked, whereas other Physalis and Nicandra spp. suffered heavy damage. Methanolic extracts of P. peruviana leaves and aqueous methanolic solutions obtained from them were bioassayed for antifeedant activity with S. littoralis larvae and found to be quite active. This led us to investigate the feeding inhibition exerted by the main steroidal components of P. peruviana, as well as by related steroids isolated from other solanaceous plants, such as Withania somnifera and Nicandra physaloides.

Materials and Methods

The following substances were tested as to their antifeedant efficacy against last-instar larvae of S. littoralis (weighing 170-190 mg) and of Epilachna varivestis (average wt, 18 mg): withanolide E (I); 4ß-hydroxywithanolide E (II); 5ß,6ß-epoxy-1ß,14 ,17ß,20 -tetrahydroxywith-24-enolide, the sodium borohydride reduction product of withanolide E (III); withaferin A (IV); withanolide D (V); Nic-1 (nicandrenone, VI); nicalbin A (VII); and nicalbin B (VIII). The assays of S. littoralis were conducted with the 'Styropor method' (Ascher & Meisner, 1973) and of E. varivestis with the bean-leaf Petri-dish method (Rembold et al., 1980).

Results and conclusions

In the tests against S. littoralis withanolide E (I) was the most potent antifeedant in this group, followed by its reduction product (III). Addition of a ß-oriented hydroxy-group at position 4 (compound II) reduced the activity by a factor of 10. Nic-1 (VI) was only slightly active, whereas withaferin A (IV), withanolide D (V), nicalbin A (VII) and nicalbin B (VIII) proved to be inactive. Against E. varivestis, III was active at a lower concentration (0.01 %) than II (0.025 %); the latter was, in turn, slightly more active than I (0.05 %). Both IV and V were only slightly active, with activity apparent only at 0.1 %. The outstanding finding was that against E. varivestis nicalbin A was by far the most active compound. Its range of activity was comparable with that of III; the weight reductions induced by VII were, however, much stronger than those obtained with III. Nicalbin A was lethal to many of the larvae at higher concentrations, apart from being an efficient antifeedant for the survivors. We have recently found it to be highly effective also against Tribolium castaneum.

Acknowledgement

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INSECT PHEROMONES IN THE INTEGRATED PEST MANAGEMENT

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Investigations of synthetic compounds affecting insect behaviour have been carried out in the Czechoslovak Academy of Sciences by the Institute of Entomology and the Institute of Organic Chemistry and Biochemistry for about ten years in a close cooperation with several other research institutions in Czechoslovakia. The following ways of application of pheromones in the Integrated Pest Management (IPM) have been investigated:

1. The use of pheromones for pest detection and monitoring

By means of pheromone baited traps two lepidopteran pest species have been detected to occur commonly in Czechoslovakia: The Oriental fruit moth, Cydia molesta was found in high numbers for the first time in 1977. The area of distribution of this species reaches as far as Central Bohemia. Till then, the species was considered as a quarantine pest. Another economically important species, a serious pest of lucerne, Cydia medicaginis (Kuzněcov, 1962) has been detected by traps baited with acetylated codlemone. It has been found to occur commonly in lucerne fields in Southern Moravia, and new data on ethology of the moths which are necessary for standartization of the monitoring system have been obtained.

2. Supervised control, timing of sprays

The codling moth, Cydia pomonella is the key pest of apple in Czechoslovakia. Since 1974 pheromone traps have been employed with the aim (a) to delimit the areas where two generations of the pest occur regularly, and (b) to verify the Michigan model (Croft et al., 1976) of the supervised control based on combination of meteorological data with those of pheromone monitoring of the pest. The forecast of the peak of flight activity in the first generation fits the actual phenology of the moths with a sufficient accuracy.

3. Pest surveying, pheromones in IPM projects

Systems of monitoring of several agricultural pests have been developed and data on the occurrence, population dynamics, behaviour and ecology collected. Concerning the two vineyard pests, the European grape moth, Eupoecilia ambiguella and the grape berry moth, Lobesia botrana, the conventional preventive chemical treatments have been successfully substituted by the supervised control. Monitoring systems for several other lepidopteran pests are being studied and incorporated into the IPM, involving the orchard pests Hedya nubiferana, Enarmonia formosana, Adoxophyes orana, Cydia funebrana, Anarsia lineatella, Archips spp., Phyllonorycter (Lithocoletis) blancardella, the pea moth, Cydia nigricana and the wheat pest, Cnephasia pumicana.

4. Population density management

The synthetic pheromone of the stored product phycitid moths (Ephestia kuehniella, E. cautella, E. elutella, Plodia interpunctella) has been found as a useful tool for the regulation of chemical treatments and for the mass trapping of the males of E. cautella.

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2B—R25

Hand application of pheromone dispensers for control of agricultural pests.

C.v.Ramm W.W. Krone

BASF uses Hercon multilayered plastic flakes as a slow release formulation for dispensing formulations from aircraft or by hand. Hand application is described in this paper.

In Egypt young children search cotton fields for egg masses of Spodoptera littoralis at 3 day intervals from mid-May to July, at a cost of $53/ha. Later in the season Pectinophora gossipiella becomes a more important pest. "Gossiplure" applied by hand in Hercon flakes has been used successfully for 2 years to aid control and reduce pesticide use, thereby minimising damage to beneficial insects. This work has been undertaken by the Centre for Overseas Pest Research in co-operation with Ain Shams University and the Plant Protection Research Institute in Cairo.

The features of hand application are:-

1. flakes can be handled by children and are not poisonous to animals;
2. as few as 800 flakes/ha effectively prevent mating of pink bollworm;
3. children can place the flakes precisely on the upper leaf of cotton plants 3.5 m apart. This is achieved by their walking along every sixth row and sticking a flake on a plant every 5 to 6 steps taken. Fields too small, too irregularly shaped for aerial application, or containing mixed crops can be so treated;
4. hand application of the flakes is easy and rapid, and costs $.8/ha, excluding the cost of supervisors;
5. neighbouring crops are not "polluted" with the formulated pheromones;
6. local people can apply them precisely when the cotton plant is susceptible to pink bollworm;
7. where villages are scattered or trees and power lines prevent aerial application, hand application is preferable.

Where larger fields are common BASF offers aerial application. Hand application of pheromone flakes is possible because of previous experience by children with leaf worm egg mass collecting.

Hand application of pheromones to orchard crops

In Georgia, USA, mating of peach tree borers has been partially prevented by fastening 250 Hercon dispensers/ha to the trees with wire. In Switzerland 60 ha of apple orchards have been protected against Laspeyresia pomonella by distributing dispensers at 150/ha throughout the orchards. Accurate placement of pheromone dispensers for tree crops is easy by hand.

METHODS OF ANALYSIS OF SYNTHETIC INSECT SEX PHEROMONES AND JUVENILE HORMONE ANALOGUES

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Background and objectives

Sensitive and reliable analytical methods are required for purity control of synthetic pheromones and juvenile hormone analogues and for the study of their behaviour, degradation and photoisomerization.

Methods and results

A highly effective GLC method has been developed for analysing pheromone samples. Geometrical and positional isomers of aliphatic primary monoenic and dienic alcohols and acetates were separated on a capillary column (50m) coated with 1,2,3-*tris*-cyanoethoxypropane. The separation factors, selectivity and distribution co-efficients of the monoenic C_{10} and C_{12} acetates, C_{14} and C_{16} alcohols and dodecadien-1-01 and its acetates were determined.

Quantitative analytical methods with the detection limits shown were developed for the following pheromones : *Parthetria dispar* attractant in biological substrate, soil and water (0.06 - 0.10 ppm, 0.008 ppm and 0.0005 ppm respectively); *Lobesia botrana* sex attractant in soil and water (0.06 ppm and 0.0015 ppm respectively); *Laspeyresia pomonella* attractant in biological substrate (1 ppm); *Agriotes gurgistanus* Fald. and *Agriotes lineatus* L. attractants in crops, soil, water and plant material (0.02 ppm, 0.04 ppm, 0.001 ppm and 0.02 ppm respectively).

Vapour pressure and volatility curves were established by the gas saturation method. A method for the quantification of nanogram amounts of pheromones in air was also devised.

Methods were developed for the analysis of juvenile hormones (altoside, altosar, p-Chlorophenyl ester of geraniol, fluoro- and bromo-phenyl esters of dimethylmethoxyoctene and diflubenzuron in water, soil and plants with detection limits of 0.05 - 0.2 ppm depending on substrate and compound analysed.

2B—R27

ENVIRONMENTAL BEHAVIOUR OF SOME INSECT SEX ATTRACTANTS

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Background and objectives

The practical effectiveness of insect sex attractants depends greatly on the formulations used for their application and on their physico-chemical properties and stability under field conditions. The objective of this work was to determine the influence of these factors on performance of several different attractants.

Materials and methods

The persistance of attractants of codling moth (Cydia pomonella) European vine moth (Lobesia botrana), cabbage moths (Mamestra brassicae, L.), cotton bollworm (Heliothis armigera Hb.) and wireworms (Agriotes gurgistanus Fald.) was studied in laboratory model studies and field experiments. Chemical transformation of attractants for european vine moth and codling moth in rubber formulations during storage was investigated. Auto-oxidation and photolysis of some aldehydes, alcohols and corresponding acetates considered to be sex attractants have been studied. The influence of attractant dose, temperature, storage time and time of exposure was studied under field conditions.

Results and conclusions

The persistance of the attractants was shown to be determined by their physical properties and the performance of different formulations was related to the volatility of the active ingredient. Temperature and wind speed significantly influenced release rates to the atmosphere.

When exposed to air and sunlight, the attractants underwent chemical transformations. Decomposition under uv-irradiation and/or sunlight was mainly due to free-radical oxidation. Cis-trans isomerization was the main photolysis process in solution. It was established that the stability of the alcohols and acetates was mainly determined by the number and position of double bonds. Degradation of aldehydes was due to the instability of the carboryl group and occurred by free radical oxidation initiated by light and temperature.

The results obtained make possible forecasting of the persistence of the active ingredient in relation to its biological efficiency.