Session 9A

Developments in Pheromones and other Semiochemicals

Chairman	Dr M G Solomon
Session Organiser	Mr R A Umpelby
Papers	9A-1 to 9A-4

THE CURRENT AND FUTURE PROSPECTS FOR SEMIOCHEMICALS IN THE INTEGRATED MANAGEMENT OF INSECT PESTS

O.T. JONES

AgriSense BCS Ltd, Treforest Industrial Estate, Pontypridd, Mid Glamorgan, CF37 5SU

ABSTRACT

While the use of semiochemicals for monitoring insect pest populations has now become well established, their use in direct population reduction is still restricted to some well documented but successful niche opportunities. Much progress has been made to establish semiochemical products as part of robust IPM systems and this paper reviews the areas where critical technical and commercial advances have been made over the last 5 years. The future directions and challenges that face the industry over the rest of the decade are also discussed.

INTRODUCTION

Conventional insecticides have been successfully used for nearly half a century to control insect pests. However, concerns about pesticide residues in food and environmental contamination by pesticide residues, combined with increasing numbers of pests showing signs of resistance to insecticides, are all factors which are driving the search for alternative methods of pest management. It is probably, however, legislative pressures on insecticide users to reduce the amounts they use, that is the greatest force driving this issue world-wide. With some European countries now setting themselves targets for percentage reductions in pesticide use, the pace of change is increasing rapidly.

The search for environmentally friendly alternatives to conventional insecticides has taken many paths but has generally looked at biological control agents (predators and parasites) and biorational pesticides (biochemical and microbial agents). The Environmental Protection Agency (EPA) in the USA describes biorational pesticides as naturally occurring substances which are target specific and are usually active in small amounts. They 'mitigate' pest populations when they are used at levels which are higher than those which exist already in nature. In contrast, conventional insecticides are innately toxic to insects, less selective, and human exposure to them is usually greater.

Of the biochemical pesticides, the hormonal pesticides (Insect Growth Regulators) and semiochemicals constitute the largest groups. Law and Regnier (1971) coined the term semiochemicals for substances which transmit messages between living organisms, both plants and animals. Semiochemicals which are emitted by an individual and produce a response in another individual of the same species are referred to as pheromones, and the bulk of our knowledge of semiochemicals comes from this group.

Semiochemicals, and pheromones in particular, have several desirable characteristics including a high level of species specificity, activity at low concentrations, and an innocuous nature to living organisms (Hodosh et al., 1985).

The practical application of pheromones and other semiochemicals was thoroughly reviewed at the end of the 1980's in two landmark books on the subject (Jutsum & Gordon, 1989; Ridgway et al., 1990b). This review will attempt to highlight what has been achieved with this technology since that time and point to some critical issues for its future.

THE CURRENT TECHNOLOGICAL STATUS OF SEMIOCHEMICALS IN PEST MANAGEMENT PRACTICES.

A.   Monitoring pest populations.

Monitoring pest populations by means of traps baited with semiochemical attractants has become very well established as part of integrated pest management practices. Monitoring for quarantine pests such as the Asian Gypsy Moth, a potentially very damaging race of *Lymantria dispar*, in North America, Europe and Australasia as well as several tephritid fruit fly pests such as the Mediterranean Fruit fly , *Ceratitis capitata*, is widely practiced with a high level of satisfaction generally amongst end users (Beroza & Knipling, 1972; Hagen et al., 1981). Similarly where trap thresholds for spray timing have been established they have made for a much more rational use of insecticides which in turn should avoid or delay the onset of insecticide resistance. Such thresholds have been refined over the last five years and more sophistication will undoubtedly follow in terms of determining optimum spray timing.

Obtaining quantitative information about pest populations from trap catch data has proved difficult in many cases, especially in strong flying, highly mobile species such as *Heliothis spp* (Srivastava et al., 1992). It should be remembered, however, that semiochemical-based trapping systems measure insect behaviour and are not direct measures of populations such as sweep netting or 'sondage' sampling. In many cases, species have been trapped successfully in the field but their presence in traps has not correlated well with subsequent sampling of eggs, larvae or damage. This has been put down in many cases to the fact that sex pheromones, in Lepidoptera at least, usually attract the males and a correlation between male and female numbers in the field does not always exist. Indeed the females may not be present, or are in a migratory phase and not laying eggs or have already oviposited elsewhere before the males moved to the site being monitored. Conversely, there have been examples of pest damage in the field but where insects have not been caught in traps. In such cases, only mated females have flown into the crop.

Such problems have not occurred with such frequency, however, as to dissuade researchers from continuing to seek quantitative data from trapping systems and many useful correlations have been established especially with the smaller less dispersive moth species. The olive moth, *Prays oleae*, serves as a good example of what is sometimes required to establish such correlations (Ramos, et al., 1989). It took ten years of studies before researchers were able to establish a correlation between male trap catch in pheromone traps and subsequent infestation of fruit which worked every year. The degree of synchrony between pest emergence and the olive tree phenology was crucial in determining the subsequent infestation level; a high degree of a-synchrony meant that only the late emerging

moths could attack fruits. Once this factor was included in the equations then correlations between trap catch and subsequent infestations were established which were valid over many years.

Future developments in the use of insect monitoring systems based on semiochemicals are likely to fall into two categories:

1. *Improvements to traps and lures.*

Lures will be optimised for specificity and attractiveness and the dispensers used will release the same amount of attractant at the start of its field life as it does at the end. Most current dispensers release more when fresh and less as they get older with the corresponding effect of over-sampling the population at the start and under sampling it at the end. Traps have received little attention in terms of design, both for user friendliness and optimisation of insect capture. This area will receive increasing attention as attempts are made to correlate trap catch with populations; only if the efficiency of the trap is constant throughout the season will it be possible to establish good correlations.

2. *Improvements to data collection and interpretation.*

Data collection and interpretation has been left very much to the individual trap operator and without much sophistication in data recording and collating. An increasing use is foreseen of data loggers, centralised data collation, expert systems and population modelling as an integral part of pheromone monitoring systems, For instance, a trap operator could log the position, time and date of catch and number of insects caught in a trap using a bar code reader, data logger and modem link to a centralised data assessment facility. At that centre, pest management decisions would be taken with reference to data inputs from the current or previous years.

The decision to spray, however, will be based on the cost-benefit ratio of any intervention together with other factors such as the phenology of the crop, the behaviour of the insect, climatic factors, natural enemy activities, all incorporated into a model which would predict the benefits in detail of chemical, or indeed biological, interventions.

B. Controlling Pest Populations.

Three main strategies (mass trapping, 'lure and kill' and mating disruption) have been pursued in an attempt to control insects using semiochemicals alone or integrated with insecticides.

1. *Mass trapping.*

By employing suitable densities of pheromone-baited traps, pest suppression can be achieved by male and/or female annihilation. With Lepidoptera, successes have only been

achieved where populations have been low, where moths have a low dispersal tendency or where populations have been physically isolated, e.g. warehouse moth. Bearing in mind that it is the male of the species that is normally attracted by the sex pheromone of moths, then a very high rate of removal of males from the population has to be achieved in order to reduce the fecundity of the females. The cocoa pod borer (*Conopomorpha cramerella*) is a good example of a moth pest exhibiting the characteristics noted above where control through mass trapping has been possible (Beevor *et al.*, 1993).

Success has been greater with this technique in instances where the pheromone used attracts both sexes. The mass trapping of bark beetles in pine forests (Lie & Bakke, 1981; Bakke & Lie, 1989) and the boll weevil in cotton (Ridgway *et al.*, 1990a) provide good examples in this context. In both these examples the attractant used has an aggregation effect on both sexes. Beetle pheromones are also synergised in many cases by host plant volatiles so that powerful attractants are available for mass trapping of the two sexes, explaining once again perhaps why this technique has found favour in controlling Coleopteran pests.

Apart from the limitation of pheromone attractants being attractive to only one sex in other insect orders, there is also a need to improve the efficiency of the traps used with these attractants. The catching efficiencies of some traps can be as low as 5 or even 1% (Lingren *et al.*, 1978). Although the attraction of the insect to the trap vicinity is relatively efficient, of those that come to within 0.5 m of the trap only 1 to 5 % may be caught. Recent work by Quarty and Coaker (1992) has shown that visual cues on the outside of funnel traps can significantly enhance entrapment of the almond moth, *Ephestia cautella*, emphasizing the need for further development in trap design for capture efficiency which will be required before mass trapping as a form of insect control can be applied to a range of insect pests from different orders.

2. *Lure and Kill.*

This technique is similar to mass trapping in that insects are lured to a source of attractant chemicals but instead of being trapped, the responding insects come in contact with a killing agent, usually a conventional insecticide. This technique overcomes the problem of low trapping efficiencies, since the responding insects need only alight in the vicinity of the attractant source to pick up a lethal dose of the killing agent which is applied to the area where the insect lands.

Lure and kill techniques have been used for several decades in the control of tephritid fruit flies. Protein hydrolysate is tank mixed with an organophosphorous insecticide and applied to selected areas of fruit crops where the fruit fly pest is then lured by the protein bait to the insecticide. This technique saves effort and cost in that only a percentage of the crop is treated. Using a general food attractant such as a protein hydrolysate is however often detrimental to beneficial insects in that they are also attracted by the toxic mixture. Work over the last 8 years has shown that the protein can be substituted in the case of the olive fly, *Bactrocera oleae*, by a microencapsulated formulation of the fly's sex pheromone, which is then tank mixed with dimethoate or malathion for ground or aerial spraying (Montiel Bueno,

1992). In aerial sprays, 20m-bands of olive grove are sprayed and 80m left unsprayed between the bands. Terrestrially, 1 m square areas of each tree are treated with the pheromone-insecticide mixture from a knap sack sprayer. In either case the fly is maintained at below the economic threshold but with no adverse effects on the natural enemy populations. Evidence has emerged recently that the success of the technique is due to the fact that the pheromone is attractive not only to the males where it produces a strong oriented response, but also through a milder attraction which it produces in the females which also brings them to the treated areas where they are killed by the insecticide.

With most examples of the successful application of this technique coming from Dipteran pests; most notably commercial lure and kill formulations for the common housefly, *Musca domestica*, involving its sex pheromone Z-9-tricosene, one is left with the question why is the technique not being applied to other insect orders?. The answers here are probably similar to those already discussed under Mass Trapping namely, one sex only being attracted, population density, etc. However, the recent exploration of stimulo-deterrent diversionary strategies (Miller and Cowles, 1990) or push-pull systems may provide the answer as to how lure and kill systems may be integrated in the future into robust insect pest management systems. In essence, semiochemicals are used to divert insect pests from the crop being protected and are then aggregated on other parts of the crop, or even non-host crop, where they are destroyed using conventional insecticides, or better still, biological ones. Early indications are that the sum of the two effects is not just additive but is likely to be synergistic.

3.   *Mating disruption.*

As far as the control of insects through the use of pheromones is concerned, this is probably the area of activity which has received the greatest amount of attention and effort over the last five years. The development of hand applied dispensers for season long control of moth pests has undoubtedly been a significant advance in applied pheromone technology. Sprayable formulations, which were suitable for advanced agricultural practices and much in favour during the 1980's, suffered from short persistence in the field - two to four weeks duration at best. However, the season-long formulations which have been introduced more recently, have opened up the technology for many cropping systems and they have found application in both developing and developed countries.

Although there are many examples of the successful commercial application of this technique in rice (Casagrande, 1993; Hall *et al.*, this volume), grapes (Neumann, 1992,1993; Dennehey *et al.*, 1990), top fruit (Charmillot, 1990; Vickers,1990) and forestry (Daterman, 1990) perhaps the best known example is that of the pink boll worm, *Pectinophora gossypiella*, in cotton where formulations based on its sex pheromone have now been successfully introduced to many cotton growing regions of the world (Campion *et al.*, 1989).

What has accounted for the success that has been achieved with this pest? The first set of factors relate to the technology. Good reliable controlled-release formulations have been developed which give the required duration in the field. The pheromone active ingredient is now available at prices which make the technology competitive with other forms of control.

The second factor relates to the pest itself. It seems to respond well to the technique and is a key early pest in the complex of insects which attack cotton. If sprays of conventional insecticides are applied against it early in the season then the detrimental effects of those sprays on the natural enemy populations are such that secondary pest problems often arise. The avoidance of such early sprays is key in IPM strategies for cotton pests and pheromone-based technology is highly suitable in this context. The third factor relates to the attitude of the authorities and public bodies who have advocated the use of the technology. The Plant Protection Authorities in many countries have played a crucial role in the promotion of this new technology and have shown great faith in it. In most countries too, the regulatory authorities have taken an enlightened attitude towards the registration requirements for the technology and have granted a number of data waivers where the normal requirements were clearly not applicable and the benign nature of the technology had been clearly demonstrated. A fourth factor which has become clear over the last 5 years is that Mating Disruption works best when carried out on an area-wide basis in a co-ordinated fashion. The control of Oriental Fruit Moth (*Grapholitha molesta*) in a 2,000 ha mixed stonefruit area of the Tulbagh valley in South Africa (Kirsch & Lingren, 1993), the control of *Chilo suppressalis* in a 2,500ha rice growing area of Spain (Jones, *et al.*,1990) and the control of Pink Bollworm in 150,000 ha of cotton in Egypt in 1994 shows what can be achieved when area wide programmes are undertaken. The major future advances in this field must come from such projects and our understanding of the parameters which limit the application of this technology will be much improved provided the industry has the continued support of government and academic researchers in such endeavours.

COMMERCIAL EXPLOITATION OF PHEROMONES AND OTHER SEMIOCHEMICALS.

In 1990 a survey was carried out of 41 companies world-wide which were offering semiochemical-based insect monitoring and control systems. Products for 257 pest species were recorded (Inscoe *et al.*, 1990). Most of the species reported were moths (189) and beetles (27), and the crops in which these systems were being used were field crops (89), vegetables (79), orchards (63) and forests (55). Almost half the companies surveyed were based in the US. Since the survey, many of the companies which took part have changed hands, but generally very few have dropped out of the field altogether, although some have changed emphasis and redirected effort to some other areas of biorational pest control.

Over the last 5 years a number of new companies have entered the pheromone market, but no new major participant from the conventional insecticide sector has entered the market and gained a significant share to date. Most of the companies that have entered the market have been small, often start up, operations offering traps and lures for monitoring pests since neither technological nor commercial barriers of any significance exist to dissuade them from entering the market. In some cases, this has caused problems of product quality and consistency. Consumers of pheromone products increasingly demand of their suppliers products which conform to certain specifications and quality standards; it is incumbent on the semiochemical industry to establish high levels of product specification, quality and performance so that barriers to entry are sufficiently high to exclude the amateur, or worse still, the unscrupulous operator. The pheromone industry, as far as the supply of traps and lures is concerned, has come of age and standards need to be established to protect both the

consumers and the credibility of the industry. The activities of the USDA-ARS and APHIS in establishing such standards for monitoring lures and traps have to be applauded (Leonhardt, *et al.*, 1990).

The companies which are actively involved in the commercialisation of semiochemicals for pest suppression have been, if nothing else, persistent. Many companies have continued their development programmes despite set-backs of control failures, shortages of resources and changes of ownership. The number of cases where semiochemicals have been successfully applied, however, continues to grow and the industry must build on these successes. The future must be a time of consolidation for the companies involved where the technology becomes an established part of IPM in those species where it has proved successful. This will also be an opportunity for gaining a greater understanding of the way in which the technology works. This is an area where the continued support of the public sector will be vital. The commercial companies involved, collectively do not have the resources to research the fundamental principles involved in this technology. At a time when politicians are legislating for reductions in conventional pesticide use, it is difficult to reconcile this with their fervour in reducing also the world wide capacity for scientific research into alternative pest control technologies at government and academic institutions. By the same token, there is considerable political pressure world-wide for privatisation of all aspects of pest control activities. However laudable this may be for consumer choice and competition, it will make the introduction of these new technologies that much more difficult. Government authorities have played a crucial role in promoting novel technologies such as those involving semiochemicals both from the point of view of facilitating the regulatory process and in pump-priming and co-ordinating their introduction, that their forced withdrawal from the technology transfer process can only mean an increased barrier to entry for these new technologies. The pioneering role of the Egyptian Ministry of Agriculture's Plant Protection Division in introducing pheromone technology for the control of the pink bollworm in cotton, exemplifies the crucial co-ordination and planning activity which is required to mount an area-wide programme over 150,000ha (Campion *et al.*, 1989). The challenge for the future of the programme in Egypt, as in many other countries, will be to ensure their continued involvement as programme co-ordinators while at the same time satisfying the desire for privatisation of the pest control industry in that country.

The importance of flexibility in the regulatory control of products based on semiochemicals has been raised earlier in this paper. This was the subject of a symposium and workshop held in Brighton in 1990 under the auspices of the BCPC and the issues were explored in depth in a most constructive way by all parties involved. The proceedings were published (Ridgway *et al.*, 1992) and the Monograph serves as a very useful reference on the subject in all matters related to the registration of semiochemicals. Several suggestions were made for improvements in the registration procedures which would facilitate pheromone registrations provided the industry made available information and rationale for the authorities to effect these desirable changes. Following the meeting, two associations were formed - the American Semiochemical Association (ASA) and the European Semiochemical Association (ESA) with the specific task of maintaining a dialogue with the EPA in the case of the former and with the competent authorities in the Directorate General for Agriculture (DGVI) of the European Commission in the case of the latter, to complete these objectives.

While the ASA has made significant progress in its dialogue with the EPA, the ESA has achieved nothing in its attempts to open up discussions with the European Commission, despite making representations, suggestions for data requirements and endless requests for comment. The European companies involved with pheromones have made a lot of progress in establishing norms for registration in individual countries of the European Union (EU) but to date, the procedures for handling pheromones and other semiochemicals as far as the Plant Protection Directive is concerned is as undetermined as it was four years ago and not for the lack of trying on the part of the industrial community involved with these compounds.

CONCLUSIONS AND FUTURE PROSPECTS.

The successful commercial exploitation of semiochemicals will depend essentially on the integration of two disciplines, chemistry and insect behavioural ecology. We have made great strides forward in the former as it relates to semiochemicals but there is still a lot to learn about the latter. The perseverance, tenacity and ingenuity of researchers, commercial companies and farmers are beginning to pay-off; semiochemical based products are becoming more reliable and robust as they become integrated into everyday pest management practices, legislators and government extension services are lending more support to the field implementation of this technology, the demand from the farmers and the consumers is increasing for biorational pest control products. With so many positive trends, the future role for semiochemicals in integrated pest management practices is assured.

ACKNOWLEDGEMENTS

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THE INTERACTION OF SEX PHEROMONE AND PLANT VOLATILES FOR FIELD ATTRACTION OF MALE BIRD-CHERRY APHID, *RHOPALOSIPHUM PADI*

JIM HARDIE, J. ROBERT STORER, STEPHEN F. NOTTINGHAM, LYNN PEACE

Deparment of Biology, Imperial College at Silwood Park, Ascot, Berks SL5 7PY

RICHARD HARRINGTON, LYNDA A. MERRITT, LESTER J. WADHAMS, DEBBIE K. WOOD

Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ

ABSTRACT

Two monoterpenoids, (-)-(1R,4aS,7S,7aR)-nepetalactol and (+)-(4aS,7S,7aR)-nepetalactone, have been identified in sex pheromones released by a number of aphid species and there is some evidence that different blends confer a degree of species-specificity. In field experiments using transparent plastic water traps releasing different ratios of these components, male *Rhopalosiphum padi* (L.) were caught in significantly larger numbers by the nepetalactol alone than by mixtures of the two components. Catches were increased if volatiles from a steam-distilled extract of leaves from the winter host, *Prunus padus*, were released simultaneously with the nepetalactol. Synergism was also observed by combining the nepetalactol and benzaldehyde, a major component of the volatiles from the *P. padus* extract.

INTRODUCTION

The major agricultural and horticultural pest aphids are prevalent during summer and comprise the asexual/parthenogenetic generations. In temperate regions, short day lengths induce many species to produce sexual generations in autumn. These latter generations comprise both males and sexual females (oviparae) which lay overwintering eggs after mating. Males of many species are winged while the oviparae are usually wingless (Hille Ris Lambers, 1966) and it is the male which locates the ovipara. Studies in the early 1970s showed that oviparae released volatile sex pheromones from the tibiae of the hind legs (Pettersson, 1970a, 1971; Marsh, 1972, 1975). However, the results indicated that aphid sex pheromones were not species specific and acted only over very short distances (a few cm). These conclusions led to the idea that male aphids, in search of a mate, would firstly locate the host plant. Since aphids are host-plant specific, the primary hosts of host-alternating species are one or a few closely related species, this strategy of increases the possibility of the male finding conspecific females (Steffan, 1987; Guldemond, 1990). Recent work has, however, indicated that the pheromones may work over greater distances and are more species-specific than previously thought (Pickett *et al.*, 1992).

Aphid sex pheromones were first identified in 1987 and for a number of species the major components are (-)-(1R,4aS,7S,7aR)-nepetalactol (I) and (+)-(4aS,7S,7aR)-nepetalactone (II) (Fig. 1) (Dawson *et al.*, 1987a; Pickett *et al.*, 1992). Air entrainment of volatiles released by sexual females followed by gas chromatography-mass spectrometry analysis has shown that a number of species produce both components but in different proportions (Pickett *et al.*, 1992). Laboratory olfactometer studies and mating bioassays have shown that males respond most strongly to synthetic blends which mimic those released by conspecific females (Dawson *et al.*, 1990; Hardie *et al.*, 1990), although this specificity is not absolute. In closely related species there may be no species-specific cue associated with the ratio of these two components and other, as yet

unidentified components, have been proposed (Guldemond *et al.*, 1993). Field experiments, however, have revealed a high degree of specificity in response to some synthetic pheromones.
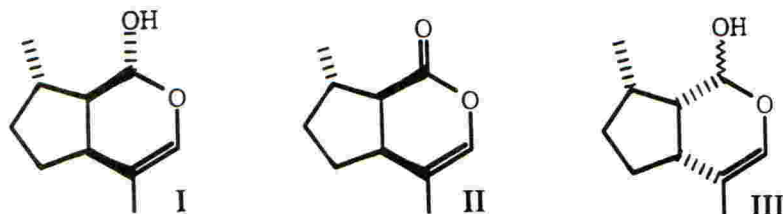

Figure 1. Structures of the identified components of aphid sex pheromones; (-)-(1R,4aS,7S,7aR)-nepetalactol (I), (+)-(4aS,7S,7aR)-nepetalactone (II) and (4aR,7S,7aS)-nepetalactol (III).

Oviparae of the damson-hop aphid, *Phorodon humuli*, produce a single component pheromone, the (4aR,7S,7aS)-nepetalactol (III, Fig. 1), which is highly attractive to males of that species in the field (Campbell *et al.*, 1990). This isomer of nepetalactol has, so far, only been found in the damson-hop aphid sex pheromone. Further field experiments have shown that water traps with lures releasing the nepetalactone (II) caught only male blackberry-cereal aphids, *Sitobion fragariae*, in large numbers, even though it was one of twenty-one species recorded in the water traps and the eighth most common species flying (Hardie *et al.*, 1992). It thus appeared that species specificity to sex pheromones occurs in the field and further evidence was sought by releasing different blends of nepetalactol (I) and nepetalactone (II) from water traps. This paper details the findings with respect to the bird-cherry aphid, *Rhopalosiphum padi*, a major pest of cereal crops.

The idea that male aphids firstly locate the host plant (see above) and then search locally for receptive oviparae has lost some of its credibility as it is now known that males can locate a specific odour source in the field without host-plant cues being present. However, it is also evident that insects can integrate volatile cues from plant and insect sources. Thus green leaf volatiles synergised attraction to the aggregation pheromone of pea and bean weevil, *Sitona lineatus* (Blight *et al.*, 1984). A similar situation has been observed with the boll weevil, *Anthonomus grandis* (Dickens, 1989). In aphids, the alarm pheromone response of the turnip aphid, *Lipaphis erysimi*, was significantly increased by the presence of a plant-derived isothiocyanate (Dawson *et al.*, 1987b). For *P. humuli*, the activity of the sex pheromone was synergised by odour from a primary host, *Prunus cerasifera* (myrobalan), as catches of males were increased by the presence of a fresh bark extract (Campbell *et al.*, 1990). Laboratory experiments have also shown that male peach-potato aphid, *Myzus persicae*, are behaviourally responsive to odour of their primary host (Tamaki *et al.*, 1970) as are male *R. padi* (Pettersson, 1970b). However, host-plant volatiles are weakly or not attractive to male cabbage aphids, *Brevicoryne brassicae* (Pettersson, 1973) and have no role in the attraction of male *Cryptomyzus galeopsidis* to conspecific females (Guldemond *et al.*, 1993).

In the present experiments, the effects of nepetalactol (I), nepetalactone (II) and volatiles from the bird-cherry host plant, *Prunus padus* on the attraction of male *R. padi* in the field are reported. Results are also presented for the response of male *R. padi* to benzaldehyde, alone and in combination with sex pheromone. This compound is a major component of *P. padus* volatiles and is known to attract *R. padi* males in olfactometer experiments (Pettersson, 1970b).

MATERIALS AND METHODS

The field experiments took place in woodland sites at Imperial College, Silwood Park. Four sites were used in each study with two replicates at each site. Transparent water traps were manufactured from plastic Petri dishes, 14 cm diam by 2 cm deep, with the pheromone released from a glass vial (08 CPV Chromocol) with a plastic cap. A 1 mm hole was drilled through the cap to allow volatile release while a plastic cover ensured that rain did not penetrate the vial and interfere with release. The traps were filled with a dilute detergent solution and set 1.1 m above the ground, 2 m apart. Treatments were initially randomised and then re-randomised three times per week when aphids were removed from the traps and male *R. padi* identified. Vials and volatiles were renewed twice during the experiment.

Three of the trials were set up as 2 X 2 factorials, in which the pheromone components and/or the plant volatiles were factors, each at two levels, present or absent. Data were analysed using the mean site counts but are presented as totals in the figures. As the data were in the form of counts, differences were assessed by measuring the Poisson deviance by using Poisson errors declared in the GLIM statistical package. The other trial, with seven pheromone blends, was analysed as a straight comparison between treatments again, using Poisson deviance in the GLIM package.

Sex pheromone blend experiments
Vials were prepared containing 10 mg of both nepetalactol (I) and nepetalactone (II) in 50 μl diethyl ether or just 50 μl diethyl ether as a control. In the first experiment, two glass vials were suspended above each of the water traps and contained solvent only (*i.e.* controls), nepetalactol (I) plus solvent blank, nepetalactol (I) plus nepetalactone (II), nepetalactone (II) and solvent blank. These experiments were conducted over a twelve-week period from September 16 1991.

A second experiment, using more blends of nepetalactol (I) and nepetalactone (II), was carried out over a four-week period from October 5 1992. Each transparent water trap had six pheromone release vials placed at the center. The following nepetalactol (I):nepetalactone (II) ratios, 1:0, 3:1, 3:2, 1:1, 2:3, 1:3, 0:1 and blank controls were tested. In order to eliminate differences in the visual cues, each treatment comprised the same number of vials. Thus, for the 1:0 blend, three vials of nepetalactol (I) were placed alongside three solvent vials, while a 3:1 blend comprised three nepetalactol (I) vials, one nepetalactone (II) vial and two solvent vials etc.

Sex pheromone, plant volatile interactions.
The above experiments demonstrated that nepetalactol (I) was the most effective lure for male *R. padi* and subsequently the interaction of this pheromone and host-plant volatiles was investigated. A steam distilled extract was prepared from freshly-collected leaves of bird cherry, *Prunus padus*, in November 1992. Glass vials were made up containing 14 gm (wet weight) leaf equivalents in 50 μl diethyl ether. Water traps were arranged as above, in association with two glass vials, and treatments comprised - leaf extract plus a control vial, a nepetalactol (I) plus a control vial, extract plus a nepetalactol (I) vial and two solvent control vials. As in previous trials, two replicates were used at each of four sites. The trial ran from September 29 to October 27 1993, coinciding with the main period of autumn migration to the primary host.

Concurrently with the *P. padus* extract trial, a further study was undertaken to examine the interaction of nepetalactol (I) and benzaldehyde. To prevent oxidation, 10% of the antioxidant, butylatedhydroxytoluene (BHT), was added to vials containing 50 mg of benzaldehyde, a major component of the *P. padus* extract. Four treatments comprised - a benzaldehyde plus a control vial, a nepetalactol (I) plus a control vial, a benzaldehyde plus a nepetalactol (I) vial and two solvent control vials.
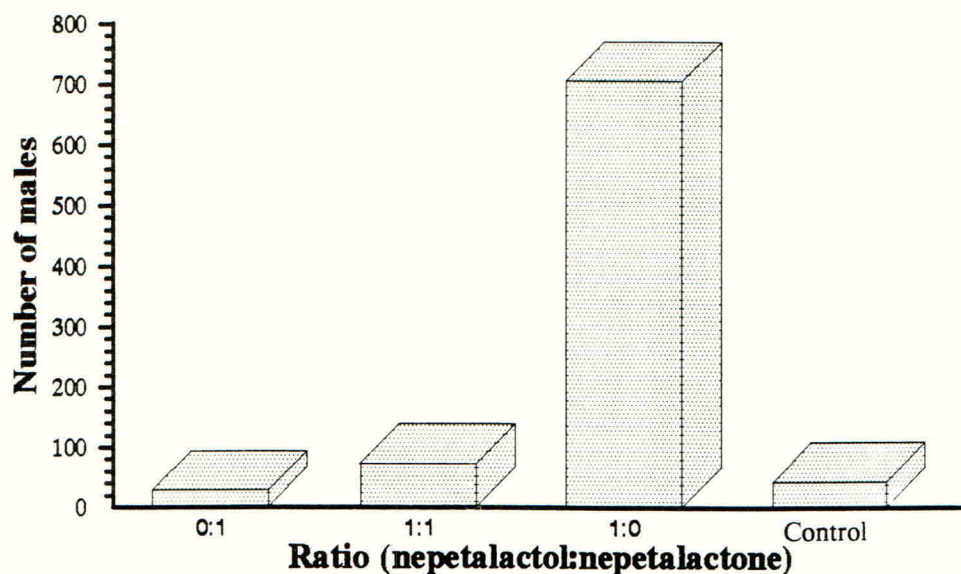
RESULTS

Figure 2. Numbers of male *R. padi* caught over twelve weeks in water traps with different ratios of nepetalactol (I) and nepetalactone (II) and a solvent control.
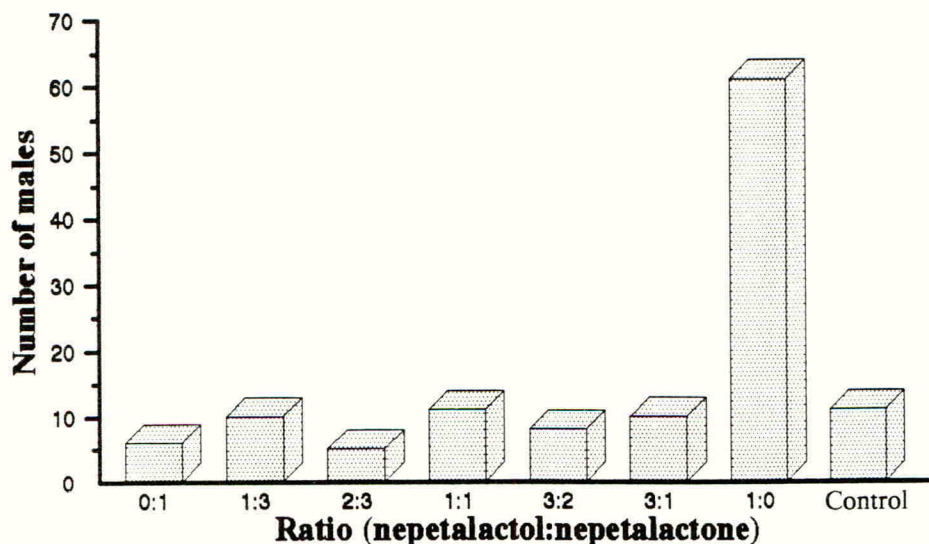

Figure 3. Numbers of male *R. padi* caught over four weeks in water traps with different ratios of nepetalactol (I) and nepetalactone (II) and a solvent control.
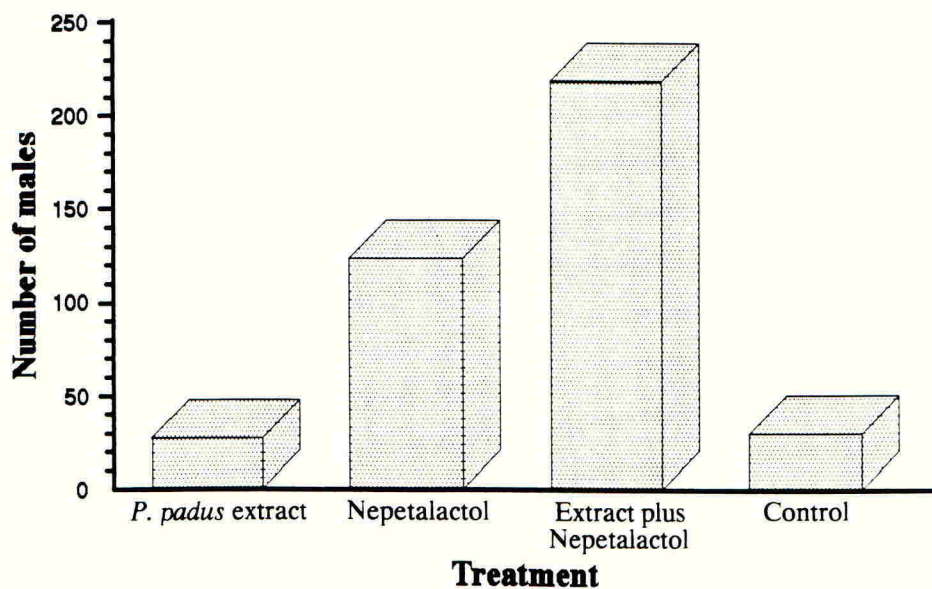
Figure 4. Numbers of male *R. padi* caught over four weeks in water traps with nepetalactol (I), volatiles from a steam-distilled extract of leaves from bird cherry, *P. padus*, and a combination of the aphid and plant volatiles.
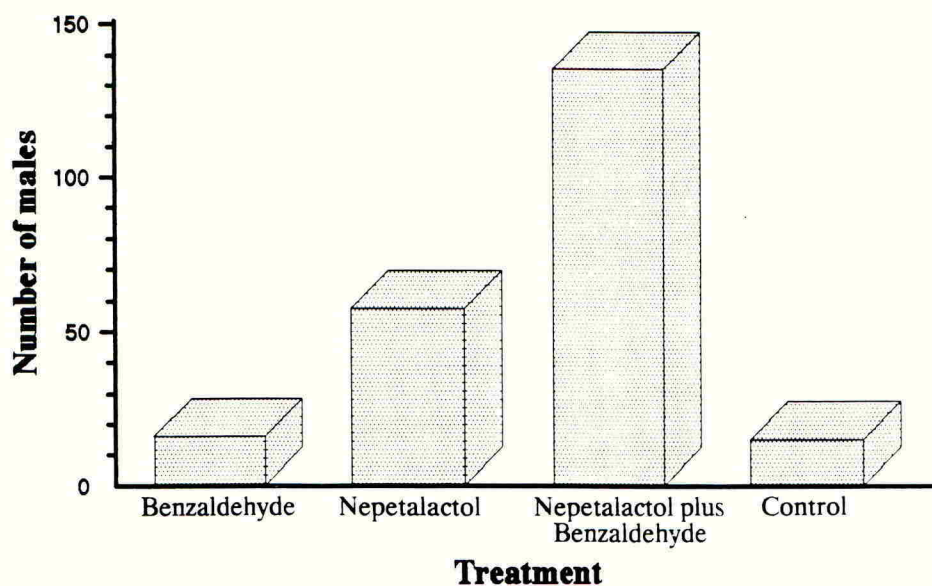

Figure 5. Numbers of male *R. padi* caught over four weeks in water traps with nepetalactol (I), benzaldehyde and a combination of the two.

Male *R. padi* were caught over the whole period of the first trial, from mid September to early December, although peak catches occurred in October. Statistical analysis showed an interaction between the two pheromone components (P < 0.001). The water traps releasing only nepetalactol (I) were highly attractive. However, when combined with nepetalactone (II), the catch was significantly reduced. Nepetalactone (II) alone and the solvent control traps caught similar numbers of males (P ≥ 0.05), although when combined with nepetalactol (I) there was an increase in catch compared with nepetalactone (II) alone (P < 0.001) or with controls (P ≤ 0.01; Fig. 2). The expanded, but shorter, blend trial in the following year caught fewer male *R. padi*, but again they were specifically attracted to the traps releasing nepetalactol (I) alone (Fig. 3) and there was a significant effect of treatment (P < 0.001). The co-release of even a small amount of nepetalactone (II) reduced catches to the level of controls.

The catches of male *R. padi* in the study comparing host-plant odour and nepetalactol (I) are shown in Fig. 4. The nepetalactol (I) again proved attractive on its own. Although the plant-odour releasing traps caught similar numbers of males to the controls, there was an interaction between plant odour and nepetalactol (I) (P < 0.05) indicating a synergistic effect. The comparison of the effects of nepetalactol (I) and benzaldehyde on trap catches shows similar results (Fig. 5) and an interaction (P < 0.05) again indicates synergism between the sex pheromone and a plant volatile.

DISCUSSION

Pettersson's original experiments demonstrating the occurrence of sex pheromones in olfactometer assays used four aphid species of the genus *Schizaphis* (Pettersson, 1970a, 1971). However, with the exception of the wingless males of *S. rufula*, there appeared to be no species-specific recognition of sexual females. Marsh (1975) used two species, the vetch aphid, *Megoura viciae*, and the pea aphid, *Acyrthosiphon pisum*, and again males detected and responded behaviourally to odours from sexual females of either species. More recently, Eisenbach and Mittler (1987) have indicated that *Schizaphis graminum* males may be able to discriminate between females from different biotypes and male *Cryptomyzus galeopsidis* spend more time in the odour of conspecific females than odour of closely related species (Guldemond *et al.*, 1993).

The chemical identification of sex pheromone components and the observation that different species, although releasing the same two components, released different blends (Pickett *et al.*, 1992) indicated that some species-specificity might be derived from the sex pheromone composition. Laboratory experiments with synthetic chemicals using olfactometers and mating bioassays confirmed this, but the specificity was not absolute (Hardie *et al.*, 1990). Thus, the pheromone blends released by *M. viciae* and *A. pisum* sexual females were sufficiently similar for male-female interactions to occur between these species, as reported by Marsh (1975). Certain other anomolies remain, the observation that the pheromone blend changes with the age of the female in *M. viciae* (Hardie *et al.*, 1990) and significant discrimination was recorded in *Cryptomyzus* species which could not be explained by odour composition relating to the major nepetalactol (I) and nepetalactone (II) components (Guldemond *et al.*, 1993).

In the field, males show a high degree of specificity to traps releasing synthetic pheromone. Thus, the damson-hop aphid nepetalactol (III) was specific to *P. humuli* males (C.A.M. Campbell pers. comm.) while nepetalactone (II), released by *S. fragariae* oviparae, was specific to males of that species (Hardie *et al.*, 1992). The present results demonstrate that nepetalactol (I) is highly attractive to *R. padi* males and preliminary results indicate that sexual females release this compound (L.J. Wadhams, unpublished). The initial experiments demonstrated that while nepetalactone (II) on its own was not active, nepetalactol (I) significantly increased catches. However, a 1:1 mixture of the two components significantly reduced catches compared to nepetalactol (I) alone. This finding is demonstrated even more clearly in the later experiments with a considerably expanded range of ratios of the two components. Even the minimum co-release of nepetalactone

(II) with nepetalactol (I) decreased the catches to control levels. Thus, there is an integration of the volatile signal and the presence of nepetalactone (II) inhibits the response to nepetalactol (I).

The observations that male aphids can locate synthetic sex pheromone sources in the field in such a specific manner in the absence of host-plant cues questions the previously proposed role of the host plant as the primary target in mate location. However, male *A. fabae* have olfactory receptors for plant volatiles and peripheral perception of plant volatiles is as sensitive in males as it is in asexual females (Hardie *et al.*, 1994). There is also laboratory evidence for males responding to host-plant odour (see above). Although attempts to show host-plant preference in male aphids failed with *A. fabae* as the males did not settle (Hardie and Glascodine, 1990). Whilst male host preference was observed in three *Cryptomyzus* species, it was less marked than that for the pre-sexual females, that would undertake host selection prior to larvipositing the sexual female forms (Guldemond, 1990). In the experiments of Campbell *at al.* (1990), there was evidence that male *P. humuli* responded to host-plant volatiles both in the laboratory and field. Trap catches were increased in the field by co-release of fresh host-plant volatiles along with the sex pheromone. The present experiments demonstrate conclusively that a steam distillation extract of host-plant leaves synergistically increases the catch of male *R. padi* in the presence of nepetalactol (I), while being unattractive on its own. Co-release of benzaldehyde, a major component of the volatiles from the primary host, with the pheromone also synergistically increased catches. It would appear that in some aphid species there is an interaction between host-plant cues and sex pheromone that enhances the mate location process and possibly contributes to species-specific mate location and thus species isolation. Other species may possess mechanisms that do not utilise this combined insect/plant odour strategy to mate location (Pettersson, 1973; Guldemond *et al.*, 1993). It should, however, be noted that benzaldehyde is a relatively common volatile and further work is in progress to further characterise plant volatiles involved in mate location by male *R. padi*.

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THE USE OF PHEROMONES FOR MATING DISRUPTION OF COTTON BOLLWORMS AND RICE STEMBORER IN DEVELOPING COUNTRIES

D.R. HALL, D.J. CHAMBERLAIN, A. CORK, K. DESOUZA, L.J. McVEIGH

Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB

Z. AHMAD

Central Cotton Research Institute, PO Box 572, Old Shujabad Road, Multan, Pakistan

K. KRISHNAIAH

Directorate of Rice Research, Rajendranagar, Hyderabad 500 030, India A.P.

N.J. BROWN, E. CASAGRANDE, O.T. JONES

Agrisense-BCS, Treforest Industrial Estate, Pontypridd, Mid Glamorgan CF37 5SU

ABSTRACT

Slow-release pheromone formulations have been used for control by mating disruption of cotton bollworms (*Pectinophora gossypiella, Earias vittella and E. insulana*) in Pakistan and yellow rice stemborer (*Scirpophaga incertulas*) in India. Single applications of pheromone formulations gave season-long control and final yields at least as good as those achieved with conventional insecticides, and savings of up to five applications of insecticides against cotton bollworms and two against rice stemborer.

INTRODUCTION

Mating disruption with pheromones is a technique for control of insect pests, particularly Lepidoptera, that avoids the use of toxic chemicals and is specific for the target pest. The technique is thus compatible with all other chemical, microbial, biological and cultural control methods and has no direct effects on non-target organisms that may themselves play an important part in pest control.

Several slow-release formulations for pheromones are now commercially available, and mating disruption is becoming increasingly accepted as a pest control option particularly on cotton, fruit, grapes and some horticultural crops. One of these formulations was developed at NRI (Cork *et al.*, 1989) and commercialised as "Selibate" by Agrisense-BCS, and this paper describes recent work on the use of these polymer formulations for control of cotton bollworms (*Pectinophora gossypiella, Earias vittella and E. insulana*) in Pakistan and yellow rice stemborer (*Scirpophaga incertulas*) in India.

MATING DISRUPTION OF COTTON BOLLWORMS IN PAKISTAN

Background

The pink bollworm (*Pectinophora gossypiella*) is a major pest of cotton world-wide. Once the eggs have hatched and the larvae have entered into the bolls they are difficult to control with conventional insecticides. Several controlled-release formulations containing the synthetic female sex

pheromone, a 1:1 mixture of (Z,E) and (Z,Z)-7,11-hexadecadienyl acetate known as "gossyplure" (Bierl *et al.*, 1974), are now commercially available, and have been used to control this pest in the developed world (e.g. Staten *et al.*, 1987), and in developing countries such as Egypt (e.g. Moawad *et al.*, 1991) and Pakistan (Critchley *et al.*, 1991). In Egypt, 350,000 acres of cotton were treated with pheromones against *P. gossypiella* in 1994.

In Pakistan, the spiny bollworm (*Earias insulana*) and spotted bollworm (*E. vittella*) may also inflict serious losses. The female sex pheromones of *E. insulana* (Hall *et al.*, 1980) and *E. vittella* (Cork *et al.*, 1988) have been identified and during 1986-1988 trials were carried out in Pakistan with a single twist-tie formulation containing gossyplure and a 10:1 blend of (*E,E*)-10,12-hexadecadienal (EE10,12-16:Ald) and (*Z*)-11-hexadecenal (Z11-16:Ald), the two common components of the pheromones of the two *Earias* species (Chamberlain *et al.*, 1992). Season-long control of the bollworm complex was achieved using the pheromone formulation applied alone or together with one conventional insecticide treatment, while conventional spray programmes required up to five applications of insecticide per season to achieve a similar level of control.

The polymer formulation for pheromones is particularly effective at stabilising labile aldehydes such as EE10,12-16:Ald against oxidation and isomerisation (Cork *et al.*, 1989), and small-scale trials carried out in Pakistan during 1988 showed that a polymer formulation containing the pheromones of *P. gossypiella* and the two *Earias* species was as effective as the twist-tie formulation at controlling the bollworm complex. However, as the *Earias* species attack the cotton some five to seven weeks later than *P. gossypiella*, application of formulations containing both pheromones at the beginning of the season resulted in wastage of over half of the *Earias* pheromone before the appearance of the target species. Thus trials were carried out on farmers' fields in the Multan region of Pakistan during 1991 and 1992 with separate formulations applied sequentially.

1991 trials

Two adjacent plots (total 5.6 ha) were treated with pheromones and insecticides, two plots (total 6.0 ha) with insecticides only and one small plot (0.4 ha) was not treated with any pest control agents. The *P. gossypiella* formulation consisted of polymer strips containing 4% gossyplure. These were applied by local labour at 1000 point sources ha^{-1} giving an application rate of 40 gm AI ha^{-1} approximately 50 days after sowing, before formation of fruiting bodies and before capture of significant numbers of adult male moths in pheromone traps. The *Earias* formulation consisted of similar polymer strips containing 1.6% of a 65:35 mixture of (*E,E*)- and (*Z,E*)-10,12-hexadecadienal. This was applied at 750 point sources ha^{-1} giving an application rate of 16.5 g AI ha^{-1} six weeks after application of the *P. gossypiella* formulation when catches in pheromone traps indicated the presence of both *Earias* species. Both formulations were attached to cotton plants by stapling the strips round the terminal shoot.

The trial was monitored by weekly sampling of larval infestations by the three bollworm species in pin squares, flowers and bolls as appropriate. Infestations by American bollworm (*Helicoverpa armigera*) were also determined as were populations of sucking pests (Thysanoptera and Homoptera) and mites (Acarina). Adult populations of the four bollworm species were monitored with pheromone traps. At the end of the season, plant density, boll numbers and boll weight were determined and estimated yields calculated. It was agreed with the farmers that insecticide would be applied against bollworms if infestation levels in pin squares or flowers exceeded 10% early in the season (July/August) or 5% later (September/October). As a result, the pheromone treated plots received only one spray each against bollworms, while the two insecticide-treated plots received four and five sprays respectively. An early season spray of methamidophos specific for sucking pests was applied to all four plots.

Following application of the pheromone formulations, suppression of catches of adult moths in the pheromone-treated plots relative to catches in the insecticide-treated plots averaged 95.7% for *P. gossypiella* over 84 nights, and 99.0% for *E. insulana* and 100% for *E. vittella* over 42 nights, indicating very effective communication disruption by the pheromone formulations. Infestation levels by all four bollworm species were lower in the pheromone treated plots at all growth stages, and mean boll infestations during the sampling period are shown in Table 1. As a result, estimated yields of seed cotton were significantly higher in the pheromone-treated plots than in either the insecticide-treated or untreated plots (Table 1).

TABLE 1. Bollworm damage and yield data from mating disruption trials, Pakistan, 1991

Treatment	No. sprays insecticide[2]	Mean % of bolls containing larvae $\pm$ SEM[1]			Estimated yield (kg ha^{-1})[1]
		P. gossypiella	*Earias* spp.	*H. armigera*	
pheromone + insecticide	1	0.00a	0.13$\pm$0.12a	0.00a	2855 a
insecticide only	4.5	3.44$\pm$0.66b	1.25$\pm$0.27b	0.69$\pm$0.29b	2495 b
untreated	-	3.75$\pm$0.42b	2.75$\pm$0.68c	0.75$\pm$0.49b	2595 b

[1] means followed by different letters in each column are significantly different at 5% level
[2] insecticide applications specifically against bollworms

1992 trials

During 1992, similar trials were carried out using improved formulations and application parameters. The *P. gossypiella* formulation consisted of extruded circular bands containing 2.6% gossyplure, applied at 500 point sources ha^{-1} giving an application rate of 45.5 g AI ha^{-1}. The bands were looped over the terminal shoots and 1 ha could be treated by one person in 3 h. The *Earias* formulation consisted of extruded "strings" containing 2.4% 10,12-16:Ald (95% *E,E*) with 0.24% Z11-16:Ald. These were tied round the terminal shoots of the plants at 1000 point sources ha^{-1} giving an application rate of 34 g 10,12-16:Ald ha^{-1}. The pheromone formulations were applied to five plots (total 10.4 ha). Results were compared with those in five plots (total 12 ha) treated with conventional insecticides only and in a single untreated plot (0.6 ha).

For *P. gossypiella*, mean trap catch suppression in the pheromone treated plots was 98.7% during the first 70 nights of the trial and 88.8% over 98 nights. Mean trap catch suppressions for *E. vittella* and *E. insulana* were 99.8% and 99.8% respectively over the first six weeks and 99.2% and 82.5% over the whole ten weeks of the trial. All plots except the untreated plot were treated once with methamidophos at the beginning of the season against sucking pests and at the end with furathiocarb against aphids. The plots under conventional insecticide treatment were sprayed additionally five times with pyrethroids against bollworms. Severe flooding in the Multan region meant that it was impossible to apply insecticide in the middle of the season and infestations of *P. gossypiella* in the plots treated only with insecticides and the untreated plots were very high. In contrast, infestations in the plots treated with pheromone were much lower, and this resulted in significantly higher estimated yields in the plots treated with pheromone (Table 2).

TABLE 2. Bollworm damage and yield data from mating disruption trials, Pakistan, 1992

Treatment	No. sprays insecticide[2]	Mean % of bolls containing larvae $\pm$ SEM[1]			Yield[1] (kg ha^{-1})
		P. gossypiella	Earias spp.	H. armigera	
pheromone + insecticide	0	6.35$\pm$0.77c	1.10$\pm$0.34b	0.00	1615 a
insecticide only	5	23.35$\pm$2.38b	1.75$\pm$0.56b	0.00	1198 b
untreated	-	36.50$\pm$3.14d	1.75$\pm$0.68b	0.01$\pm$0.01	1083 b

[1] means followed by different letters in each column are significantly different at 5% level
[2] insecticide applications specifically against bollworms

MATING DISRUPTION OF YELLOW STEMBORER IN INDIA

Background

The yellow stem borer (*Scirpophaga incertulas*) is now regarded as the major insect pest of rice in the Indian subcontinent, and is also a key pest of rice in much of South East Asia and China. The recent elevation of its pest status can largely be explained by the lack of any adequate varietietal resistance, while varieties have been developed with resistance to most other major pests. Traditionally, rice farmers in southern India have very low pesticide inputs, but in recent years pesticide use has steadily increased even in marginal rice growing areas. Systemic insecticides such as phorate are now commonly used on irrigated rice during the early part of the summer (rabi) season with organophosphates such as chlorpyrifos and monocrotophos and the organochlorine endosulfan normally applied as foliar sprays after the vegetative growth stage of the summer crop and during the monsoon (kharif) season crop. The use of endosulfan, a pesticide highly toxic to fish and not normally recommended for use in paddy, is particularly worrying.

The female sex pheromone of *S. incertulas* was identified as a 3:1 blend of (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-9-hexadecenal (Z9-16:Ald) by Cork *et al.* (1985) and the presence of a third component, (Z)-9-octadecenal (Z9-18:Ald) was reported by Tatsuki *et al.* (1985). The polymer formulation is well suited for use with aldehydes such as these, and a polymer formulation containing the pheromone of the related striped rice stemborer (*Chilo suppressalis*) has been used commercially in Spain for several years (Casagrande, 1993). After initial trials in West Bengal during 1992 (Cork and Basu, in preparation), trials with polymer formulations have been carried out in Andhra Pradesh aimed at developing mating disruption as a commercially-viable control technique for *S. incertulas* in India.

Rabi 1993

This first trial aimed to compare two polymer formulations containing components of the *S. incertulas* pheromone (Table 3, treatments 2 and 3) with the commercially available formulation containing the *C. suppressalis* pheromone (Table 3, treatment 1). The latter is a 1:10:1 blend of Z9-16:Ald, Z11-16:Ald and (Z)-13-octadecenal (Z13-18:Ald) (Nesbitt *et al.*, 1975; Tatsuki *et al.*, 1983), and thus has two components in common with the *S. incertulas* pheromone.

approximately 20% AI remained after 70 days field exposure. During the kharif monsoon season, longer duration varieties are grown with an average of 135 days between transplant and harvest, and there was concern that with an early application of the pheromone formulation there might be insufficient pheromone towards the end of the season to protect the crop adequately. Thus the effectiveness of early application of the formulation at 9-12 DAT was compared with that of later application at 39-44 DAT.

The polymer formulation (Table 3 treatment 1) was used in both cases and applied as previously. Each treatment was applied to three replicate plots of 10 ha each, and results were compared with those in three nearby 10 ha areas treated according to farmers' practice with conventional insecticides.

TABLE 5. Damage levels and yields in *S. incertulas* mating disruption trials, kharif 1993

Growth Stage	Maximum % damage $\pm$ SEM[1]		
	Early application	Late application	Farmers' practice
tillers	1.22 $\pm$ 0.33 a	1.73 $\pm$ 0.45 ab	2.87 $\pm$ 1.78 b
panicles	1.68 $\pm$ 0.26 a	2.00 $\pm$ 0.17 a	6.12 $\pm$ 1.06 b
Yield (kg 25m^{-2})	12.64 $\pm$ 0.36	11.79 $\pm$ 0.37	11.27 $\pm$ 0.47

[1] means followed by different letters are significantly different at the 5% level

Catches of male *S. incertulas* moths in pheromone traps in the plots treated with pheromone were suppressed by over 87% up to 50 DAT relative to catches in traps in the farmers' practice plots. Infestation data in Table 5 shows that there were no significant differences in stem borer damage between plots treated with pheromone early or late, and damage levels remained at less than 2% throughout the season. However, damage levels in the farmers' practice plots increased during the season and were significantly different from those in the pheromone treated plots from 64 DAT to harvest. Yield estimates indicated that the pheromone treated plots produced higher yields than the farmers' practice plots, although the differences were not significant. The highest yield was obtained with the early pheromone application.

Rabi, 1994

Large scale field trials were repeated at two locations in Andhra Pradesh, Medchal and Warangal, using a polymer formulation containing a 1:3 blend of Z9-16:Ald and Z11-16:Ald which is the most attractive blend for *S. incertulas*. In both areas three replicate plots of 10 ha were treated with the pheromone formulation and results were compared with those in three plots of 1 ha located around each treatment plot at a distance of approximately 500 m and treated according to farmers' practice with conventional insecticides. The formulations were applied according to parameters developed previously between 9-15 DAT at 625 point sources ha^{-1} and 40 g AI ha^{-1}.

Catches of male moths in pheromone traps in the plots treated with pheromone were suppressed by over 90% up to 72 DAT relative to catches in traps in farmers' practice plots. Populations of *S. incertulas* were exceptionally high during this season and damage levels were correspondingly high. Results of damage assessments summarised in Table 6 indicate that reasonable control was achieved in

TABLE 3. Composition of formulations tested in rabi season 1993

| | Relative proportions | | | |
Treatment	Z9-16:Ald	Z11-16:Ald	Z9-18:Ald	Z13-18:Ald
Treatment 1	0.84	10	-	0.91
Treatment 2	0.77	10	-	-
Treatment 3	0.96	10	0.76	-

The formulations were in the form of extruded lengths containing 4% of the pheromone blends. Applications were made at between 25 and 30 days after transplant (DAT) by attaching individual dispensers to bamboo stakes (0.75 m) at 4 m intervals giving 625 point sources ha^{-1} and application rates of 40 g AI ha^{-1}. Each treatment was made to a single 10 ha plot, although these were made up of many individual farmers' fields which were typically less than one acre in size. Within each 10 ha plot, three subplots of 1 ha were sampled weekly for stemborer damage to tillers and panicles in 25 hills at 5 m intervals along a randomly selected transect. Results were compared with those in nearby plots of similar size and planting date which were treated with conventional insecticides according to farmers' practice. Three additional farmers' practice plots were selected at random within the locality but more than 5 km from any treatment area.

Catches of male *S. incertulas* moths in pheromone traps in the treatment plots were suppressed by over 96% for 51 days relative to catches in the farmers' practice plots. Table 4 shows the maximum damage levels recorded for tillers and panicles in each pheromone treated plot compared to those in the respective nearby farmers' practice plots and the distant farmers' practice plots.

TABLE 4. Damage levels in *S. incertulas* mating disruption field trials, rabi 1993.

| | Maximum % damage | | | |
| Treatment | Treatment | | Farmers' practice | |
	Tillers	Panicles	Tillers	Panicles
Treatment 1	3.94	2.35	7.80	5.68
Treatment 2	4.44	2.28	11.90	8.74
Treatment 3	2.70	2.12	4.06	8.10
Distant Farmers practice			7.11	10.52

Results show no obvious differences between the effects of the three formulations. All showed lower levels of infestation than the farmers' practice plots which received at least two applications of insecticide.

Kharif, 1993

In this trial, the effect of the timing of pheromone application was examined. Persistence studies on the pheromone formulations carried out during the previous trial indicated that

the Medchal area with infestation levels significantly lower in the plots treated with pheromone than in the farmers' practice plots. At Warangal catches of adult moths in pheromone and light traps indicated that populations of *S. incertulas* were even higher than at Medchal, and high infestation levels were recorded in both pheromone-treated and farmers' practice plots.

TABLE 6. Damage levels in *S. incertulas* mating disruption field trials, rabi 1994.

Growth Stage	Maximum % damage $\pm$ SEM[1]			
	Medchal area		Warangal area	
	Pheromone	Farmers' practice	Pheromone	Farmers' practice
tillers	$2.06 \pm 1.93a$	$5.55 \pm 0.92b$	$2.76 \pm 1.32a$	$6.96 \pm 4.54a$
panicles	$5.28 \pm 1.71a$	$13.04 \pm 3.20b$	$15.67 \pm 8.63a$	$20.89 \pm 10.12a$

[1] figures in each pair followed by the same letter are not significantly different at the 5% level.

CONCLUSIONS

Control of the cotton bollworm complex in Pakistan can be achieved by application of pheromone formulations against *P. gossypiella* and the two *Earias* species. Combined with use of selective pesticides against sucking pests, this approach provided an IPM package for cotton pest control. Other pests such as *H. armigera* and whitefly did not cause problems in the trial areas, at least in part because of the high levels of beneficial insects in the plots treated with the pheromone formulations. At current prices, the cost of a single, season-long application of the polymer formulation against *P. gossypiella* could be less than the cost of two applications of conventional insecticides, and use of this pheromone formulation alone is thus already cost-effective. However, the cost of the main component of the *Earias* pheromone is still too high for use of the two formulations to be justified on purely economic grounds at present.

A single application of a polymer formulation can give effective, season-long control of *S. incertulas* in India. Under normal pest pressure this can be at least as good as control achieved with several applications of conventional insecticides, but under high pest pressure both approaches failed to give adequate control. These trials required coordinated application by very many small-scale farmers, and although they are used to cooperative activities because of water supply constraints on rice cultivation, achieving this will be important for successful use of the technique and its economic viability in India.

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ATTEMPTS TO CONTROL APHID PESTS BY INTEGRATED USE OF SEMIOCHEMICALS

J.A. PICKETT, L.J. WADHAMS, C.M. WOODCOCK

Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ

ABSTRACT

The paper describes practical studies ensuing from the identification of aphid sex pheromones and the discovery that these can effect long range attraction of aphids and also their parasitoids. An important aspect of recent research has been the contribution made by electrophysiological and behavioural studies and these have given rise to a series of aphid repellents active in field trials with cereals. Attempts at integrated use of sex pheromones and other semiochemicals together with aphid pathogens are described.

INTRODUCTION

Recent studies have shown that semiochemicals play a more important role in aphid chemical ecology than originally thought, particularly in relation to longer range interactions. This increased understanding has enhanced prospects for aphid control by means of semiochemicals. Already, there has been some success and commercial developments with the aphid alarm pheromone. After the first identification of an aphid sex pheromone (Dawson et al., 1987), this testing has now proceeded to the extent that commercially funded programmes are developing these pheromones for agricultural and horticultural use. This paper charts earlier investigations into aphid semiochemicals, the identification of the sex pheromones and recent developments in aphid chemical ecology (Pickett et al., 1992) in the context of aphid control.

APHID PEST CONTROL

Aphids represent the main insect pests on many agricultural and horticultural crops in temperate and northern climates. They cause direct feeding damage and also transmit virus diseases to crop plants; for example, the peach-potato aphid, *Myzus persicae*, transmits Beet Yellows Virus in the semi-persistent mode and Potato Virus Y in the non-persistent mode. Control is usually by systemic aphicides, although these have been partly superseded by more selective direct-acting agents such as the carbamate pirimicarb. Resistance has developed markedly to the organophosphorus compounds and is now beginning to cause problems with pirimicarb. New agents such as imidacloprid have been developed with greatly enhanced selectivity, but resistance is likely to develop if such compounds are over-used or used inappropriately. Other approaches to aphid control need to be explored to deal with increasing demands for reduced pesticide inputs and also to provide alternatives where resistance to conventional pesticides develops. Novel control methods based on use of semiochemicals should also include exploitation

of pathogens, predators and parasitoids that naturally regulate aphid populations on crop plants.

SEMIOCHEMICALS IDENTIFIED FOR APHIDS

Alarm pheromones

Hille Ris Lambers initially attempted to use the aphid alarm pheromone, (E)-β-farnesene, which causes dispersal of aphids when attacked by predators, to reduce landing of alate aphids on seed potatoes (Hille Ris Lambers and Schepers, 1978). Attempts were made to combine the alarm pheromone activity with other control agents against the cotton aphid, *Aphis gossypii*, on glasshouse ornamental crops, particularly chrysanthemums. Although the alarm pheromone gave only a small increase in mobility of *A. gossypii*, this was sufficient to increase pick-up of the pathogen *Verticillium lecanii* and thus to cause acceptable mortality (Pickett et al., 1986). Similar results were obtained in the field using contact pesticides such as permethrin. In both cases, electrostatic spraying systems were employed to present the alarm pheromone to the aphids in the most efficaceous way. (E)-β-Farnesene is now available commercially and there have been further attempts to use this material in the field, including higher levels of treatment where some direct toxicity can be observed (Ester et al., 1993), presumably via a hormonal effect noted previously (Mauchamp and Pickett, 1987).

Antifeedants

Antifeedants have been successfully employed in the field against transmission of virus by aphids. The drimane antifeedant (-)-polygodial, extracted from the water-pepper *Polygonum hydropiper* (Polygonaceae), was used against transmission of Barley Yellow Dwarf Virus and gave results statistically similar to those obtained with the broad-spectrum pyrethroid cypermethrin (Dawson et al., 1986). However, three applications of the antifeedant were required, compared to one application of the pyrethroid. It is hoped that recent studies on the mechanisms by which polygodial inhibits aphid feeding, and thereby virus transmission, will lead to further practical developments (Powell et al., 1993).

Sex pheromones

Depending on species and climate, many aphids feed and reproduce partheno-genetically on the secondary or herbaceous summer host, where the main pest impact is observed. In the autumn, presexual female morphs, the gynoparae, migrate to primary or winter hosts. Here, true sexual females, the oviparae, are produced and on maturity, these release a sex pheromone which is used by the males to locate mates. The sex pheromones are detected principally by olfactory cells in the secondary rhinaria on the male antenna and electrophysiological studies played an important role in the identification of the active components. Using tungsten microelectrodes, single cell recordings (SCR) can be obtained from individual olfactory sensilla and these, combined with high resolution capillary column gas chromatography (GC) (Wadhams, 1990), were used to locate physiologically active peaks in volatiles released by the females. For many species of aphid, the sex pheromones were found to comprise one or both of the

cyclopentanoids nepetalactone and nepetalactol (A and B, Figure 1) in different ratios (Pickett et al., 1992). It was interesting to observe that there are two different cell types in the secondary rhinaria responding separately to the two compounds, and dose-response data showed high specificity between the two cell types for the respective compounds (Dawson et al., 1990).
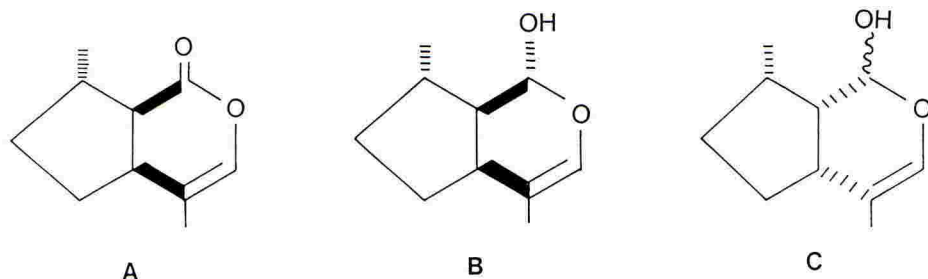

FIGURE 1. Structure of aphid sex pheromone components.

Other aphid semiochemicals

In the spring, alates which form on the primary host migrate back to the herbaceous summer host. Coupled GC-SCR on the primary rhinaria on the fifth and sixth antennal segments has been used in the identification of attractant compounds from the host plant, e.g. for *P. humuli* (Campbell et al., 1993). However, other cells located in the primary rhinaria respond to volatiles from non-host plants. These compounds may be employed by aphids to avoid inappropriate hosts and thus could provide candidate repellents. Indeed, one compound identified in this way for *A. fabae* is (-)-(1R,5S)-myrtenal, which in the linear track olfactometer acts as a repellent and also inhibits attraction to host volatiles (Hardie et al., in press). Pettersson (pers. comm.) had suggested that, whilst autumn migrants may find volatiles from the primary host attractive, the spring migrants may find such compounds repellent. By GC-SCR on spring migrants of *R. padi*, using volatiles from the winter host, *Prunus padus*, methyl salicylate was identified as having strong electrophysiological activity and was found to reduce attraction to cereal leaves (Pettersson et al., 1994).

INTEGRATION OF SEMIOCHEMICALS IN PEST CONTROL STRATEGIES

Despite initial success with the alarm pheromone and antifeedants, it was considered that a more sophisticated strategy for using aphid semiochemicals should be developed in line with other work (Miller and Cowles, 1990). A push-pull or stimulo-deterrent diversionary strategy against aphids has been proposed: treatments on the harvestable crop would include inhibitors of kairomones acting as attractants for aphids,

antifeedants where appropriate, and attractants for aphid predators and parasitoids. In addition, a sacrificial or trap crop would be selected with kairomonal attraction enhanced by choice of cultivar or application of attractant formulations. On the sacrificial crop, selective control agents such as fungal pathogens would be employed.

Sex pheromones

The damson-hop aphid, *Phorodon humuli*, was selected for initial field work, in collaboration with Horticulture Research International, East Malling, and provided the first demonstration of long-range attraction of flying aphids (Campbell et al., 1990; Pickett et al., 1992), which had previously been disputed. Hops, *Humulus lupulus* (Cannabaceae), are the summer host for this species and *Prunus* spp. provide the winter host. Combined GC-SCR on the males, using an extract of volatiles from calling oviparae, showed that the sex pheromone for *P. humuli* comprised two diastereoisomers of a nepetalactol with a different stereochemistry (C, Figure 1) from that observed for most other aphid species (Campbell et al., 1990). In an autumn field trial, six Petri dish water traps containing this compound caught over 3,000 male *P. humuli*, whereas a suction trap, sampling over 500 cubic metres of air per hour, caught less than 400. In these trials, male aphids were observed to orientate towards the pheromone traps in surprisingly strong wind conditions and preliminary work indicates that male behaviour can be influenced by a sex pheromone lure several metres away from the source. Although mating disruption and trap-out are being considered commercially as control methods, collaborative efforts between Rothamsted and East Malling involve use of the pheromone to attract male *P. humuli* into traps containing a strain of *Verticillium lecanii* that works well at the relatively low field temperatures occurring during the autumn migration. The study has now been extended to other species, e.g. *Sitobion fragariae* (Hardie et al., 1992), and in Korea, catches of over 1,000 aphids per day per water trap have been obtained for a number of species, including *Tuberocephalus momonis*, *Lachnus tropicales* and *Aphis citricola* (K.S. Boo, Seoul National University, Suwon, pers. comm.).

It was considered likely that volatiles from the primary or winter host might also have an influence on the attraction of males. In initial trials, an extract of *Prunus* bark synergised the activity of the sex pheromone for *P. humuli* (Campbell et al., 1990) and a similar situation was found for the bird-cherry-oat aphid, *Rhopalosiphum padi*, with volatiles from the bird-cherry, *Prunus padus* (J. Pettersson, pers. comm.; Hardie et al., submitted). However, it appeared that host volatiles did not have a role in the attraction of male *Cryptomyzus galeopsidis* to conspecific females (Guldemond et al., 1992).

Beneficial insects: aphid parasitoids

During field trials on the sex pheromone components, it was observed that certain aphid parasitoids were also caught in the traps containing the nepetalactone A (Hardie et al., 1994). EAG studies, initially on *Praon volucre* but now including many other parasitoid species such as *Aphidius* spp. and *Diaeretiella rapae*, showed that there was a high inherent sensitivity to this component, with a lower response to related compounds, including nepetalactone isomers and nepetalactols (Hardie et al., 1993). It therefore appeared that an innate response was possible with these parasitoids. Indeed, in initial field trials, increased parasitism on the grain aphid, *Sitobion avenae*, by *P. volucre* was observed in nepetalactone treated plots (C.P.M. Tripathi, pers. comm.). In these trials,

P. volucre was the main parasitoid attracted. However, for certain crops, it would be preferable to attract other aphid parasitoids, such as *Aphidius ervi* against the pea aphid, *Acyrthosiphon pisum*. *A. pisum* employs both the nepetalactone A and the nepetalactol B as its sex pheromone, in a 1:1 ratio (Pickett et al., 1992). By using lures with this formulation in a pot trial, parasitism of *A. pisum* by *A. ervi* was increased by more than 300% (C.P.M. Tripathi, pers. comm.). This work has now formed the basis of a commercially funded programme in which set-aside strips are used to build up aphid populations so that parasitoid searching behaviour can be stimulated in cereal crops by means of aphid sex pheromones. A semiochemical released by hyperparasitoids causing dispersal of aphid parasitoids has recently been identified (Höller et al., 1994) and this development could lead to ways of improving parasitoid aggregation within the crop canopy for aphid control.

Non-host repellents

In Sweden, in a field trial on barley, two formulations of methyl salicylate, identified as a repellent for spring migrant *R. padi*, reduced colonisation by this aphid by approximately 50%, a level which, by model predictions (Wiktelius and Pettersson, 1985), would be sufficient to allow parasitism to have a further useful impact. *R. padi* is not found at high population densities in the United Kingdom in the summer, but further trials using methyl salicylate on wheat demonstrated similar reductions in populations of *S. avenae* and the rose-grain aphid, *Metopolophium dirhodum* (Pettersson et al., 1994). The combined use of repellent compounds, initially methyl salicylate, and aphid sex pheromones to attract parasitoids is now being explored, with the benefit of some commercial funding.

Plant molecular genetics

Aphids, as phloem feeders, represent a difficult target for development of resistant crop cultivars by molecular genetics. However, promising results are now being obtained, for example at the University of Durham. In order to preserve such genetic lines against resistance by aphids, integration with other control measures would be beneficial (Boulter, 1993), including the semiochemical strategies described here. Furthermore, there are now real prospects for genetically modifying crop plant secondary metabolism to generate aphid semiochemicals directly, and the biosynthesis by which the sex pheromones are produced is currently under investigation.

Knowledge of the pathways by which aphid semiochemicals are biosynthesised could allow further exploitation of biotechnological methodology. Use of sex pheromones for attracting aphids and disrupting mating, and also as attractants for aphid parasitoids, is creating a need for increased amounts of these compounds for field development. They are relatively difficult to synthesise on a large scale and a new programme has been established under a "clean technology" initiative to explore the production of these pheromones by fermentor technology. During the search for the identity of aphid sex pheromones, very small amounts of the open-chain compound citronellol were found in volatiles from scenting females. This compound could be the biosynthetic precursor of nepetalactones and the possibility is being investigated for aphids by feeding studies. However, a more convenient route to the biochemistry is to use plants in the family Labiatae (= Lamiaceae), such as *Nepeta* spp. which biosynthesise

nepetalactone with the appropriate stereochemistry (Eisenbraun et al., 1980). By synthesising labelled precursors, the early steps in the biosynthesis of the *P. humuli* sex pheromone have been studied from geraniol through to compounds oxidised in the so-called 10-methyl position. A partial clone has been obtained for the gene that encodes the cytochrome P450 in the initial oxidative step (Hallahan et al., 1992) and the enzyme effecting the next oxidation to the aldehyde groups is now characterised (Hallahan et al., in press). In collaboration with the Institute of Food Research, attempts are being made to establish tissue cultures from *Nepeta* spp. as another route to providing fermentor production of the sex pheromone. Currently, the most convenient method for producing these compounds is to cultivate *Nepeta* spp. plants in small plots, extract the material by steam distillation and effect the chemical conversion in the laboratory (Dawson et al., 1989).

CONCLUSIONS

The search is on for non-host plant chemicals that will act as repellents for pest aphids. The identification of sex pheromones has been extended to a wide range of aphid pests and could include more, where there is a need. The use of these semiochemicals is being attempted in a wide European collaboration from southern Italy to Sweden, and attempts are being made to exploit some aspects by biotechnology.

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Session 9B

Biological Control of Plant Pathogens: Pre- and Post-Harvest

Chairman   Dr K Powell

Session Organiser   Dr N Magan

Papers   9B-1 to 9B-4

PROSPECTS FOR BIOLOGICAL CONTROL OF FOLIAR PATHOGENS

N.J FOKKEMA, M. GERLAGH, J. KÖHL, P.H.J. JONGEBLOED, G.J.T. KESSEL

DLO Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, NL-6700 GW Wageningen, The Netherlands

ABSTRACT

Prospects for biological control of foliar pathogens are reviewed with respect to different strategies corresponding to different stages in the disease cycle of necrotrophic and biotrophic pathogens. Advantages and disadvantages of different strategies of pathogen control based on microbial suppression of infection, sporulation and survival are discussed. Strategies aimed at the reduction of dissemination and survival of the pathogen resulting in a delay of epidemics have in general a greater prospect than strategies imitating fungicides in protecting individual plants. Protection of wounds, however, is an important exception. Integrated with chemical control, biological control may play an important role in reducing the development of fungicide-resistance in pathogen (e.g. *Botrytis cinerea*) populations.

INTRODUCTION

Prospects of biological control of foliar pathogens are determined by the effectiveness and reliability of the biocontrol agents (BCA's) in controlling disease, the need for biocontrol products and ensuring the market for such products. These factors have to be considered at an early stage in the research and development process.

A lot of research on microbial interactions in the phyllosphere is labelled as biocontrol research, without any experiments under agricultural conditions. Antagonists selected in bio-assays, if not dual culture interactions, are upgraded to potential biocontrol products in discussions of scientific papers. Studies on the mechanisms of interaction, including genetic modification, are scientifically rewarding and largely outnumber tedious ecological studies on performance of BCA's under commercial agricultural conditions. In contrast to the development of agrochemicals, initial research on potential BCA's is carried out at universities and public institutes. This has the advantage in that we can have a sensible discussion of the prospects for biocontrol of foliar pathogens, while actually having only one product on the market ('Trichodex', Makhteshim Chemical Works). Further research on population dynamics of introduced BCA's in relation to disease control in repeated field experiments, however, is not attractive for scientists whose career depends on the number of publications.

Until recently research on biological control of plant diseases has been focused mainly on soil-borne diseases which are often difficult to control chemically. Because of the change in public attitude towards pesticide use, biocontrol is also considered as a desirable environmentally friendly alternative for the control of foliar diseases, which up

till now have been controlled satisfactorily by chemicals. In addition, the risk of occurrence of field resistance to fungicides (De Waard *et al.*, 1993; Elad *et al.*, 1993) will be reduced if non-chemical and chemical control could be integrated. Although breeding for host-resistance, however, is the first choice for non-chemical control, for several pathosystems it is unlikely to be achieved in the near future. Considering the needs, biocontrol research should target those pathogens currently requiring large amounts of chemicals for control and without much prospects on breeding for resistance.

The prospects for biocontrol can best be estimated by comparing the following three main strategies viz. microbial suppression of infection, microbial suppression of sporulation and microbial suppression of survival. The first strategy is characterized by a short period of interaction between antagonist and pathogen, because the pathogen may escape from antagonism by penetration. The other strategies allow a much longer period of interaction affecting dissemination and survival, resulting in an indirect protection of the crop. State of the art and prospects will be discussed for each strategy.

SUPPRESSION OF INFECTION

Saprophytic bacteria, yeasts and filamentous fungi are natural colonizers of the phyllosphere. If not affected by broad spectrum fungicides (Fokkema, 1988), yeasts may deprive the phyllosphere from nutrients like pollen grains and aphid honeydew which stimulate infection of a variety of necrotrophic pathogens (Blakeman & Fokkema, 1982; Dik *et al.*, 1991). The presence of this natural microbial buffer on wheat leaves could be demonstrated under field conditions (Dik *et al.*, 1991). With the exception of excessive occurrence of sooty moulds, there are no known detrimental effects of phyllosphere saprophytes on the host. Awareness of the natural presence of a saprophytic microflora in the phyllosphere together with the relatively easy demonstration under controlled conditions of potential antagonism of individual isolates against a great variety of mainly necrotrophic pathogens on various hosts has boosted research in this area (Andrews, 1992; Sutton & Peng, 1993a). Under practical conditions, however, an effect on disease is mostly difficult to demonstrate.

Yeasts and hyphal fungi

On field-grown wheat leaves the accumulation of naturally occurring yeast-like antagonists is such that applications of yeasts to sparsely colonized young leaves has a temporary effect only (Fokkema *et al.*, 1979). Prophylactic application of yeasts and other fungal antagonists is, therefore, likely to be effective only when applied to naturally very poorly colonized plant parts with a sudden presence of infection stimulating nutrients such as young flowers (Dubos, 1987; Johnson *et al.*, 1993; Peng *et al.*, 1992; Redmond *et al.*, 1987) and fresh wounds of leaves, stems and fruits (Köhl *et al.*, 1991; Wilson & Wisniewski, 1989). Pollinating domesticated insects may transfer BCA's precisely to the right site at the right time in order to prevent blossom infections (Peng *et al.*,1992). Man-made wounds create excellent opportunities for biological control because of the possibility of timely application of the BCA's. This explains the success in biocontrol of post-harvest fruit diseases (Droby *et al.*, 1993; Droby & Chalutz, 1994). Infections of healthy tissue via invasion from adjacent necrotic plant parts by pathogens, like *Botrytis*

cinerea and *Sclerotinia sclerotiorum*, may be reduced by antagonists suppressing the pathogen in the necrotic tissue (Peng & Sutton, 1993a).

The use of *Trichoderma* spp. in biocontrol of *Botrytis cinerea* in grapes was thoroughly explored by Dubos (1987). During eight consecutive seasons, Dubos achieved an average control of rot of 65% with four carefully timed sprays between flowering and three weeks before harvest. It is likely that *Trichoderma* competes with *B. cinerea* for nutrients from pollen and necrotic flower remains. Trichodex (Makhteshim Chemical Works), based on *Trichoderma harzianum* (T-39) is registered in Israel for control of *B. cinerea* in cucumber and grapevine. Use in alternation or combination with fungicides gives reliable control (Elad, *et al.*, 1993), reduces the input of chemicals into the environment and reduces the risk of fungicide-resistance. Combination of antagonists and fungicides may even result in synergism, since Di Pietro *et al.* (1993) showed that endo-chitinase from *Gliocladium virens* increases the sensitivity of *Botrytis* spores to toxins.

Bacteria

Use of bacterial antagonists against fungal pathogens has the disadvantage that bacterial populations are less adapted to the fluctuating humidity conditions in the phyllosphere than yeasts and filamentous fungi. Despite interesting reports that sprayings with bacterial preparations, and with watery compost extract can reduce the severity of biotrophic as well as necrotrophic pathogens (Weltzien, 1991) applications in the field seem restricted to situations where humidity is not limiting e.g. cabbage in polyethylene tunnels (Edwards *et al.*, 1994). Protective amendments may reduce the effect of drought on bacterial antagonists. Although induction of host resistance and production of antibiotics are among the mechanisms attributed to bacterial antagonism, it appears that these are not effective after decline of the antagonist population.
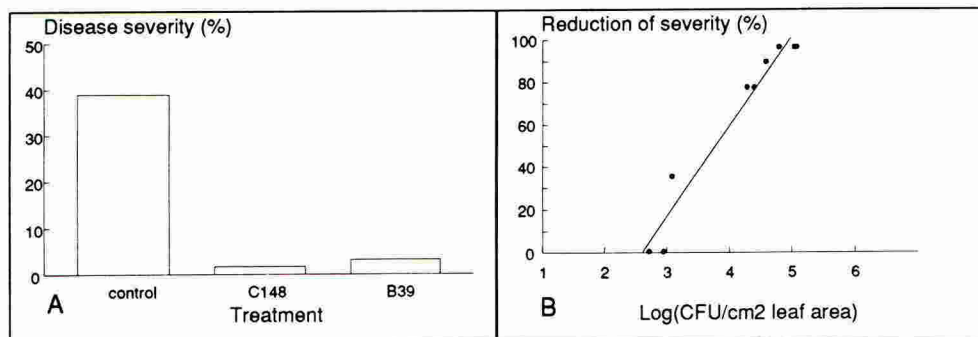

Fig. 1.A. Control of *P. infestans* with *Pseudomonas* sp. (isol. C148) and *Bacillus* sp. (isol. B39) on whole potato plants (n=6)
B. The effect of population density of C148 on potato leaves on antagonism against *P. infestans*

The promising control of potato late blight by *Pseudomonas* (C148) and *Bacillus* (B39) (Fig. 1 A, B) isolates under controlled conditions is correlated with high densities in the phyllosphere which now difficult to maintain in the field (Jongebloed *et al.*, 1993).

The main merits of research on bacterial antagonism for crop protection may be the direct applications of newly discovered mechanisms such as induced resistance, pyrrolnitrin-based fungicides and perhaps the role of bio-surfactants.

The use of bacterial antagonists has much better prospects against bacterial diseases than against fungal diseases because the target pathogen is equally affected by drought as the antagonist and, in contrast to fungal pathogens also colonize the phyllosphere saprophytically resulting in a long interaction time between antagonistic and pathogenic bacterial populations. A nice example is the control of fire blight in pear orchards, caused by *Erwinia amylovora*, by 50 per cent or more following applications of a mixture of *Pseudomonas fluorescens* and *Erwinia herbicola* during bloom (Johnson *et al.*, 1993).

Conclusion

Microbial prevention of infection is not recommended as a general strategy for biological control of foliar fungal pathogens because the period of interaction is generally short, and uneconomic repeated prophylactic applications of BCA's are required in order to create an adequate density of the antagonist before the pathogen arrives. Interesting exceptions may be situations where antagonists can be applied at the right time and place (e.g. man-made wounds) or where the infection originates from mycelium in necrotic tissue (longer interaction times) instead of conidia.

SUPPRESSION OF SPORULATION

Biological control is determined both by the intensity of the interaction as well as the duration of the interaction. The latter is much more favourable when biological control addresses the colonization and sporulation of the pathogen. Microbial suppression of sporulation is a well known strategy for the control of biotrophic rusts and mildews, but relatively unexploited in the control of necrotrophic pathogens.

Biotrophic pathogens

Mycoparasitic antagonists interfering with sporulation of the pathogen have been tested for control of biotrophic rusts (Kranz, 1981) and mildews. Reduction of sporulation in these polycyclic diseases has a direct effect on the development of the epidemics. Many mycoparasites are potential biocontrol agents (Hijwegen, 1988) and from this natural source the opportunity exists for the selection of those with the best performance under adverse (micro)climatic conditions, the broadest host range and best prospect for mass production. Most research is concentrated on powdery mildews of which the biomass is almost entirely on the leaf surface and consequently well exposed to mycoparasitism.

The main bottle-neck to overcome is the requirement for high humidity for successful mycoparasitism. Selection of appropriate species and strains in this respect for the control of rose powdery mildew was rewarding (Hajlaoui & Bélanger, 1991). *Sporothrix flocculosa (Stephanoascus flocculosus)* was as effective as fungicides in commercial-scale experiments in glasshouses in which the humidity could be kept at 80% (Bélanger *et al.*, 1994). *Ampelomyces quisqualis* is the most intensively studied mycoparasite of powdery

mildew. Amendments based on paraffin as well as selection of drought-resistant mutants are strategies to reduce the dependence on high humidity. Ecogen Israël Partnership currently develops a product based on *Ampelomyces quisqualis* isolate AQ10 (Sztejnberg *et al.*, 1989) against powdery mildew in grapes, apples and cucurbits (Hofstein & Fridlender, 1994).

<u>Necrotrophic pathogens</u>

In contrast to mycoparasitic suppression of sporulation of biotrophs, suppression of sporulation of necrotrophs mostly results from saprophytic competition in necrotic leaf tissue or crop debris. The natural occurrence of competition between pathogenic and saprophytic fungi in dead leaf tissue indicates the feasibility of this approach. In this niche, the pathogen can no longer take advantage of its pathogenic character by colonizing living tissue, although the pathogen might be the first colonizer of dead plant tissue. Pathogen and antagonist are equally subjected to environmental conditions.

Most research has dealt with reduction of primary inoculum of the apple scab fungus (*Venturia inaequalis*). Applications of decomposers like *Chaetomium globosum* and *Athelia bombacina* reduce ascospore production by more than 90% (Heye & Andrews, 1983). This as a stand-alone treatment, however, is not sufficient for disease control because of the production of secondary conidial inoculum but might be useful in IPM. Similarly Pfender and coworkers demonstrated that *Limonomyces roseipellis*, a secondary invader of wheat straw, suppressed the production of ascospores of the tan spot pathogen *Pyrenophora tritici-repentis* on wheat straw (Pfender, 1988). Particularly in no-tillage farming practices, surface-borne plant residues are an important inoculum source which can considerably be reduced by selected microbial competitors (Pfender *et al.* 1993). In these examples only initial infection by ascospores is hampered while subsequent dissemination by conidia remains unaffected.

Microbial suppression of sporulation of *Botrytis* spp. does not have this disadvantage because dissemination is only by conidia produced on necrotic leaf parts, mature fruits and plant debris. Although *B. cinerea* is ubiquitous the main inoculum during epidemics comes from within the crop as has been demonstrated for strawberries (Braun & Sutton, 1987) and onions (Köhl *et al.*, 1994). *Gliocladium roseum* applied to green and overwintered strawberry leaves previously inoculated with *B. cinerea* suppressed sporulation of the pathogen after artificial killing of the leaf tissue (Sutton & Peng, 1993b). *G. roseum* is as effective as chlorothalonil when applied to green leaves which may be colonized endophytically by both the antagonist and *B. cinerea*, whereas the antagonist failed to suppress sporulation in dead leaves.

A *G. roseum* isolate did not reduce sporulation of *Botrytis* spp. in field-grown onions but removal of dead onion leaf tips reduced the spore load above the onion plots and the number of leaf spots (Köhl *et al.*, 1994). This observation led to selection of antagonists among the saprophytic fungi naturally colonizing dead onion leaves. Many fungi are able to suppress sporulation of *Botrytis* spp. on dead onion leaf pieces under conditions of high humidity, but only a few potential BCA's remain effective when the humid conditions are

Table 1. Effect of *Ulocladium atrum* and Daconil M (chlorothalonil/maneb) on sporulation of *Botrytis cinerea* on dead lily leaves. Leaves had been exposed to field conditions for 5-6 days and had been subsequently incubated in moist chambers for seven days.

Treatment	Leaf area (%) covered with conidiophores of *Botrytis cinerea*				
	Experiment number				
	1	2	3	4	5
water	15 a[*]	24 a	19 a	30 a	23 a
Daconil M	16 a	31 a	13 a	26 a	16 a
U. atrum	2 b	1 b	1 b	3 b	2 b

[*] Numbers within a column with a common letter do not differ significantly (LSD-test, $P < 0.05$).

interrupted with dry periods (Köhl & Fokkema, 1994). In a field assay with dead lily leaves naturally colonized by *B. cinerea* a *Ulocladium atrum* suppressed sporulation consistently better than several known antagonists and chlorothalonil/maneb (Table 1).

Conclusion

Important advantages of interference with sporulation on dead plant tissue are that:
1. The fungal antagonist may colonize the entire dead substrate as well as the pathogen does. Since the pathogen cannot escape by penetrating the living leaf there is a long interaction time.
2. The interaction based on competition for substrate is not pathogen-specific like many mycoparasitic interactions with biotrophs. Consequently many necrotrophic pathogens may form the market for one single biocontrol product.
3. Post-harvest treatments of crop debris, which would make sense for the following crops, may also require a less strict registration procedure.

SUPPRESSION OF SURVIVAL

Several foliar pathogens produce survival structures which are susceptible to mycoparasitism. Microbial interference with surviving fruiting bodies has already been discussed above. Less differentiated melanized structures like sclerotia are formed by, e.g. *Botrytis* spp., and *Sclerotinia* spp. and are particularly interesting targets for biological control when cycles of vegetative spores are absent in the life cycle of the pathogen. This is the case with *Sclerotinia sclerotiorum* causing disease in many vegetables and arable crops, which is initiated by ascospores released by apothecia produced on sclerotia in the top layer of the soil. The possible use of the mycoparasite *Coniothyrium minitans* in biocontrol is well documented (Whipps & Gerlagh, 1992). In protected crops application of the mycoparasite to the soil in order to destroy the sclerotial inoculum might be rewarding (Whipps, 1994). The use in arable crops by foliar application at the end of the season is aimed at interference with the formation as well as the survival of sclerotia in plant debris and will be discussed here.

Management of *Sclerotina sclerotiorum* in field crops

Coniothyrium minitans easily invades diseased tissue and kills under field conditions more than 70 % of the sclerotia produced in a bean crop within one month (Gerlagh *et al.*, 1993). Elimination of sclerotia in the crop debris will certainly continue since the number of apothecia produced in the following season is generally reduced by 90%. The remaining 10% is likely to originate mainly from sclerotia already present in the soil for a number of years. Once introduced in a diseased crop, *C. minitans* maintains itself easily and spreads rapidly via conidia to neighbouring plants.

Table 2. Effect of *C. minitans* applied as annual late season sprays on apothecia and infection by *S. sclerotiorum* in 1992.

Crop	Number of apothecia/100 m		Number diseased plant/100 m	
	contr.	CONIO	contr.	CONIO
potato	7	5	0	0
bean	5	2	163	177
carrot	-	-	0	0
chicory	68	6	3	0
rotation: potato, bean, carrot, chicory				

The hypothesis that annual end-of-the-season applications may eventually clear the naturally heavily infested soil from inoculum and eliminate disease has been tested in a five year experiment with four crops in rotation (Gerlagh *et al.*, 1993). Among the crops, potato, bean, carrot and chicory, bean is extremely susceptible with high disease incidence and consequently contributing to an enormous accumulation of inoculum for the following seasons. Spraying with *C. minitans* neutralizes this accumulation which, because of ploughing, becomes evident two years later in the chicory plots (Table 2). The impact on disease is difficult to demonstrate, in this experiment because of the extreme differences in susceptibility of the crops chosen. However there was an effect evident in a biennal carrot crop for seed production (Table 3).

Table 3. Effect of *C. minitans* applied in the first year on apothecia and infection by *S. sclerotiorum* in the second year.

Treatment	Row width cm	Apothecia no/m^2	diseased flowers % incidence
WATER	20	3.07	3.3
	40	1.35	4.8
	60	0.6	3.7
CONIO	20	0.11	0.73
	40	0.04	0.26
	60	0.13	0.39

Conclusion

Convincing evidence under agricultural conditions shows that application of *C. minitans* is a reliable measure to reduce inoculum of *S. sclerotiorum*. Such a treatment may reduce disease in important arable crops like bean, lettuce, rapeseed and sunflower (McLaren *et al.*, 1994) and protected crops (Whipps, 1994). The advantage of spraying above-ground plant parts above soil treatment is the immediate contact with newly formed sclerotia and the simplicity of the treatment. A post-harvest treatment may simplify the registration procedure. A slight disadvantage might be that this application method is not directly aimed at sclerotia already present in the soil.

GENERAL CONCLUSION

Several strategies for biological control of foliar pathogens are presented. Research over the last five years has indicated that microbial interference with the dissemination of the pathogen may have greater prospects for success in agricultural practice than attempts to prevent infection which are imitating chemical control strategies. Microbial protection of man-made wounds, however, seems an important exception.

In studying biological control we should consider the ecology of the pathogen and the antagonist and attack the pathogen stage where the combination of duration and intensity of the interaction is optimal. It is not difficult to find antagonists performing well in bio-assays or in controlled environment. Research should concentrate on prolonged establishment of the antagonist under conditions prevailing in agricultural practice.

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ADVANCES IN BIOLOGICAL CONTROL IN PROTECTED CROPS

J.M. WHIPPS

Department of Microbial Biotechnology, Horticulture Research International, Wellesbourne, Warwickshire, CV35 9EF

ABSTRACT

Several new disease biocontrol agents for use in protected crops have become commercially available in the last few years. Each has a different pathogen-host target but all are registered for use in protected crops rather than in the field, emphasising the significance of stable environmental conditions for achieving reproducible biological disease control. Technological advances in the areas of inoculum production and formulation leading to ease of application are identified as key features in the successful commercial acceptance of disease biocontrol agents. Improvements in selection and screening procedures, further mode of action studies and development of genetically modified microorganisms are suggested as areas where further advances could be made.

INTRODUCTION

Interest in biological disease control continues to grow on two fronts. Consumers are beginning to embrace the concept of 'green', pesticide-free, products and companies are having to find alternatives for chemicals as they are withdrawn from the market or their use is restricted. In addition, for many soil-borne pathogens there are still no satisfactory chemical controls available.

In the USA, costs for registration of an indigenous, non-manipulated microorganism can be $c.$ $0.5 million in contrast to $c.$ $20 million for a pesticide (Becker & Schwinn, 1993) and this can act as a further incentive to the development of biological disease control agents. The lower potential cost should make it possible to exploit small, but profitable, niche markets such as vegetables and ornamentals in the protected crops sector, particularly as there is little true competition between such products at the moment. This contrasts with traditional rationales for developing pesticides which are aimed at large-acreage field crops such as wheat.

NEW PRODUCTS

It is interesting to note that since Lynch (1988) reviewed the availability of disease biocontrol agents to farmers and growers at the B.C.P.C. only one, a *Pseudomonas fluorescens* product (Dagger G, Ecogen Inc., USA) for control of *Pythium* and *Rhizoctonia* on cotton has been withdrawn whereas at least four have either become

available or are nearing the market for use in protected crops rather than in the field. These include a *Gliocladium virens* product (GlioGard or GL-21, W.R. Grace & Co, USA) for control of damping-off in bedding plants caused by *Pythium* and *Rhizoctonia* (Lumsden *et al.*, 1992); a *Streptomyces griseoviridis* product (Mycostop, Kemira Oy, Finland) for control of fusarium wilt (*Fusarium oxysporum* f. sp. *dianthi*) of carnation and damping-off in brassicas caused by *Alternaria brassicicola* (Lahdenperä *et al.*, 1991); a *Trichoderma harzianum* product (Trichodex, Makhteshim Chemical Works Ltd, Israel) for control of grey mould (*Botrytis cinerea*) of tomato, cucumber and grape (Elad *et al.*, 1993; Elad 1994); and a vesicular-arbuscular mycorrhizal preparation (Vaminoc, AGC MicroBio, UK) for promoting yields of cucumber, possibly by protecting roots from diseases, such as those caused by *Pythium*. Significantly, all these products have been developed for use, at least initially, under glasshouse conditions where environmental variability is controlled or reduced. In addition, the three products controlling root pathogens work in artificial growing media based either on peat or partially sterilized soil (Gliogard and Mycostop) or in rockwool (Vaminoc), again situations with simplified or more standardized microbial and physicochemical environments.

One of the other features contributing to their successful introduction into the market concerns their mode of application or use. All are simple to use, requiring little change to normal horticultural practice. For example, GlioGard and Vaminoc are produced as granular materials resembling fertilizers already in use and are simply added to the growing medium and Trichodex and Mycostop are available as powders which can be easily sprayed onto foliage or the soil as recommended. Trichodex also has an advantage that it can be integrated with some fungicide treatments. Thus, little new methodology has to be invoked although some care has to be taken to ensure that storage temperatures are appropriate and environmental extremes are avoided as this may affect viability of the living preparation. This is little different to following label instructions on any pesticide and perhaps belies the amount of background preparatory work that has been done with some of these new commercial disease biocontrol products.

TECHNICAL ADVANCES

Numerous technical advances in the area of formulation and application have been made that have helped, or may help in the future, with the commercial acceptance of biological disease control agents. This area is covered in detail by Hofstein (1994) but some comment is required here. For instance, the easy to use GlioGard prills are produced by a patented process (US patents nos. 4668512 and 4724147) (Lumsden & Lewis, 1989). Here, fermenter biomass containing largely fungal spores, is mixed with wheat bran in a sodium alginate solution. This suspension is dripped into calcium chloride solution to form insoluble gelatinous beads which dry into stable, hard pellets of even size. When incorporated into growing media, moisture stimulates the spores to germinate; they then utilize the wheat bran as a food base to grow throughout the growing media.

Application of biocontrol agents to seeds has also been widely investigated and has advanced considerably from simply soaking seeds in suspensions of antagonists. Seeds can now be primed in the presence of antagonists such as *Trichoderma* or *Enterobacter cloacae* to provide improved disease control (Harman & Taylor, 1988); antagonists can be applied to seeds as thin-film coatings or in pellets, the latter providing the option of adding nutrients to enhance growth of the biocontrol agent or even physical separation from a pesticide treatment (McQuilken *et al.*, 1990a; Taylor & Harman, 1990). Such procedures result in easily-handleable biocontrol-treated seeds which once again can be used successfully by the grower with few changes in working practices.

Optimization of inoculum production both in quality and quantity has also been examined. For instance, *Pythium oligandrum* has been shown by many workers to control damping-off in a variety of crops and, indeed, a commercial preparation, Polygandron, has been produced on solid substrates in Czechoslovakia (Vyzkummy ustav rostlinne vyroby). However, to permit successful seed coating, large quantities of relatively pure oospores are required and a molasses-based liquid medium rather than a solid substrate based one was developed to achieve this (McQuilken *et al.*, 1990b, 1992). A range of fermentation procedures has also been developed to improve the quality and types of spores produced by *Trichoderma* and *Gliocladium* (Taylor & Harman, 1990; Lewis & Papavizas, 1991). Of particular interest is the finding that spores produced under water stress were associated with superior survival and desiccation tolerance (Harman *et al.*, 1991) and this has significant implications in prolonging shelf life.

It is important to note that many of these advances have involved production of cells or biomass in liquid fermentation systems. This has been driven by companies wishing to utilise existing fermentation facilities. At the same time, these systems provide good control of the environmental and nutritional conditions during production and result in batches of inoculum with reproducible biocontrol activity. Nevertheless, it should be realised that some of the most potentially interesting biocontrol agents have yet to be grown successfully in large quantities in liquid fermentation systems. For example, the sclerotial mycoparasites *Sporidesmium sclerotivorum* and *Coniothyrium minitans*, have both given reproducible levels of control of Sclerotinia disease on lettuce when applied to the soil as solid substrate inocula, the former controlling *Sclerotinia minor* in the field and the latter *S. sclerotiorum* in the glasshouse (Adams & Fravel, 1990; Budge & Whipps, 1991). This immediately raises the question as to whether such solid substrate inocula can be commercially viable (Adams, 1990). This may hinge on the costs of inoculum production overall, the amounts needed to be applied to the soil to achieve acceptable control and whether use of fungicides or soil sterilization procedures remain suitable alternative options for control of sclerotial pathogens. The fact that both these mycoparasites survive well in soil after application and continue to attack sclerotia over one or two years at least (Adams & Ayers, 1982; Budge & Whipps, 1991) and that *C. minitans* may be related to suppression of Sclerotinia disease in sunflower and oilseed rape (Huang & Kozub, 1991; Whipps *et al.*, 1993) and *S. sclerotivorum* to suppression of sclerotinia disease in lettuce (Lumsden, 1992), must indicate that further work on inoculum production of these antagonists is required.

TARGET AREAS FOR THE FUTURE

A large number of diseases are possible targets for future biological control in protected crops but those causing damping-off (*Pythium* spp. and *Rhizoctonia solani*), fusarium wilts and rots (*Fusarium* spp.), grey mould (*Botrytis cinerea*) and sclerotium-forming pathogens (*Sclerotinia* spp. and *Sclerotium* spp.) are likely to remain targets of choice. *Botrytis cinerea* is still the overall largest problem affecting the greatest number of crops whilst the other pathogens are perennial problems despite the routine use of careful hygiene procedures. Add to this problems with fungicide resistance or poor chemical control in general and the targets are clear.

Selection and screening systems should be optimised to identify novel antagonists and perhaps modified or expanded to obtain more isolates of known antagonists. *In vivo* bioassays would play an important part here (Campbell, 1994). In some cases, screens could be designed to identify antagonists which operate through the same mode of action as known, successful antagonists, for example, through antibiotic production. However, care should be taken to avoid screens based solely on *in vitro* assessments as these ignore ecological parameters which may be important for successful biocontrol.

Perhaps the final area where advances could be made involves genetic manipulation. Procedures for transferring or deleting genes and decreasing or increasing gene expression are now well-established. Indeed, the first commercial genetically modified biocontrol product, Nogall (Bio-Care Technology Pty Ltd, Australia) containing *Agrobacterium radiobacter* K1026 for control of crown-gall (caused by *A. tumefaciens*) went on sale in Australia in 1988 (Ryder & Jones, 1990). Numerous other genetically modified biocontrol isolates are potentially available (e.g. see Ossanna & Mischke, 1990; Vincent *et al.*, 1991) but commercial success will depend upon whether registration is simple and of low cost. Consumer resistance to such products would seem to be low if the Nogall experience in Australia is a good guide and bodes well for the future.

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SUCCESSFUL BIOCONTROL OF POSTHARVEST PATHOGENS OF FRUITS AND VEGETABLES

S. DROBY, E. CHALUTZ

Department of Postharvest Science of Fresh Produce, Institute for Technology and Storage of Agricultural Products, ARO, The Volcani Center, Bet Dagan 50250, Israel

ABSTRACT

The increasing demand of the public and health autherities for produce free of chemical residues has been the driving force in the search for methods to the control of postharvest diseases of fruits and vegetables. One of the emerging technologies is the use of microbial antagonists. The postharvest environment may be one in which the best chance to develop successful biological control exists because many aspects of it can be controlled. Of particular interest among antagonists reported to control postharvest diseases are yeasts because of their ability to multiply rapidly and colonize a surface for long periods of time under a variety of environmental conditions. Yeast antagonists may inhibit postharvest pathogens by successfully competing with the pathogens for nutrients and space, by inducing host resistance, or by interacting directly with the pathogen.

INTRODUCTION

The treatment of fruits and vegetables with fungicides is the primary method for controlling postharvest diseases (Eckert, 1990). Due to postharvest use of fungicides, in order to maintain high quality, there is a potential for human exposure to these chemicals. The restriction of several fungicides and banning of the use of others have already raised serious disease control problems for certain crops in major production areas. In addition, decreasing efficacy of key postharvest fungicides due to development of resistant strains of fungal pathogens has contributed to a weakening our ability to control postharvest losses.

One of the emerging technologies is the use of microbial antagonists which have been reported to control several rot pathogens of diverse commodities (Table 1). The field of biological control of postharvest diseases is relatively new research area. In recent years we have witnessed a surge in activity on biological control of postharvest disease. This has been reflected in the number of publications in professional literature and proceedings of international symposia and workshops. The topic has also been the subject of a book and several reviews (Droby *et al.*, 1991; 1994; Janisiewicz, 1988a, Jeffries and Jeger, 1990, Wilson *et al.*, 1991; Wilson and Pusey, 1985, Wilson and Wisniewski, 1989; 1994;).

THE POSTHARVEST ENVIRONMENT - THE KEY FOR SUCCESS OF BIOCONTROL

One of the keys to the successful implementation of biological control of postharvest diseases lies in understanding and controlling the postharvest environment. The postharvest environment may be one in which the best chance to develop successful biological control exists because many aspects of it can be controlled. This control could lessen the problem of

introducing the biocontrol agent into an unpredictable and highly variable environment, which previously has been the limiting factor in field-released biocontrol agents. In addition,

TABLE 1. Reports of postharvest biological control

Crop	Disease	Biocontrol agent	Reference
Potato	Soft rot	*Pseudomonas putida*	Colyer and Mount, 1984
	Fusarium rot	*Pseudomonas spp., Entreobacter spp., Pantoea agglomerans*	Schisler and Slininger, 1994
Cabbage	Gray mold	*P. fluorescens, Serratia plymuthica, S. liquefaciens*	Leifert et al., 1993
	Alternaria rot	*P. Fluorescens, Serratia plymuthica, S. liquefaciens*	Leifert et al., 1993
Carrots	Gray mold	*Trichoderma harzianum*	Tronsmo, 1993
	Mycocentrospora rot	*Trichoderma harzianum*	Tronsmo, 1993
	Rhizoctonia rot	*Trichoderma harzianum*	Tronsmo, 1993
	Sclerotium rot	*Trichoderma harzianum*	Tronsmo, 1993
Tomato	Gray mold	*Pichia guilliermondii*	Chalutz et al., 1991
	Rhizopus rot	*Pichia guilliermondii*	Chalutz et al., 1991
	Alternaria rot	*Pichia guilliermondii*	Chalutz et al., 1991
Apple	Blue mold	*Pseudomonas syringae*	Janisiewicz, 1987
	Blue mold	*Pseudomonas cepacia*	Janisiewicz and Roitman, 1988
	Blue mold	*Cryptococcus spp.*	Roberts, 1991
	Blue mold	*Pichia guilliermondii*	Mclaughlin et al., 1990
	Blue mold	*Sporobolomyces roseus*	Janisiewcz et al., 1994
	Blue mold	*Aurebasidium pullunas, Epicoccum purpurascens, Sordaia fimicola*	Falconi and Mendgen, 1994
	Blue mold	*Pichia anomala, Candida sake*	Jijakli and Lepoivre, 1993
	Blue mold	*Candida sp., Trichosporon sp.*	Gullino et al., 1994
	Gray mold	*Pichia guilliermondii*	Wisniewski et al., 1988, McLaughlin et al., 1990b
	Gray mold	*Aureobasidium pullunas, Epicoccum purpurascens, Sordaia fimicola*	Falconi and Mendgen, 1994
	Gray mold	*Sporobolomyces roseus*	Janisiewicz et al., 1994
	Gray mold	*Pseudomonas cepacia*	Janisiewicz and Roitman, 1988
	Gray mold	*Endophytic bacteria*	Pratella et al., 1994
	Gray mold	*Cryptococcus laurentii*	Roberts, 1990a
	Gray mold	*C. laurentii, C. albidus*	Roberts, 1991
	Gray mold	*Pichia anomala, Candida sake*	Jijakli and Lepoivre, 1993
	Gray mold	*Acremonium breve*	Janisiewicz, 1988b
	Gray mold	*Candida sp., Trichosporon sp.*	Gullino et al., 1994
	Mucor rot	*Pseudomonas cepacia*	Janisiewicz and Roitman, 1987
	Monilinia rot	*Endophytic bacteria*	Pratella et al., 1994
	Monilinia rot	*Aureobasidium pullunas, Epicoccum purpurascens, Sordaia fimicola*	Falconi and Mendgen, 1994
	Rhizopus rot	*Endophytic bacteria*	Pratella et al., 1994
Pineapple	Penicillium rot	Attenuated strains of *Penicillium spp*	Tong-Kwee and Rohrbock, 1980
Strewberry	Gray mold	*Trichoderma sp.*	Tronsmo and Dennis, 19800
Grape	Gray mold	*Trichoderma harzianum*	Dubos, 1984
	Gray mold	*Pichia guilliermondii*	Ben-Arie et al., 1991
	Rhizopus rot	*Pichia guilliermondii*	Ben-Arie et al., 1991
	Gray mold	*Kloeckera apiculata*	McLaughlin et al., 1992
	Rhizopus rot	*Kloeckera apiculata*	McLaughlin et al., 1992

TABLE 1. Continued.

Crop	Disease	Biocontrol agent	Reference
Peach	Rhizopus rot	*Enterobacter cloacae*	Wilson *et al.*, 1987
	Brown rot	*Bacillus subtilis*	Pusey and Wilson, 1984
Cherry	Alternaria rot	*E. aerogenes*	Utkhede and Sholberg, 1986
	Brown rot	*Bacillus subtilis*	Utkhede and Sholberg, 1986
Plum	Brown rot	*Bacillus subtilis*	Pusey and Wilson, 1984
Nectarine	Brown rot	*Bacillus subtilis*	Pusey and Wilson, 1984
Pear	Blue mold	*Pseudomonas cepacia*	Janisiewicz and Roitman, 1988
	Gray mold	*Pseudomonas cepacia*	Janisiewicz and Roitman, 1988
	Gray mold	*Pseudomonas gladioli*	Mao and Cappellini, 1989
	Mucor rot	*Cryptococcus laurentii, C. flavus, C. albidus*	Roberts, 1990b
Apricot	Brown rot	*Bacillus subtilis*	Pusey and Wilson, 1984
Cirtus	Green mold	*Pichia guilliermondii*	Wilson and Chalutz, 1989, Droby *et al.*, 1991
	Green mold	*Bacillus subtilis*	Singh and Deverall, 1984
	Green mold	*Trichoderma viride*	Borras and Aguilar, 1990
	Green mold	*Myrothecium roridum, M. verrucaria*	Appel *et al.*, 1988
	Green mold	*Bacillus pumilus*	Huang *et al.*, 1992
	Green mold	*Candida sp., Trichosporon sp.*	Gullino *et al.*, 1994
	Sour rot	*Pichia guilliermondii*	Chalutz and Wilson, 1990
	Sour rot	*Bacillus subtilis*	Singh and Deverall, 1984
	Sour rot	*Trichoderma sp.*	De Matos, 1983
	Stem end rot	*Bacillus subtilis*	Singh and Deverall, 1984
Avocado	Anthracnose	*Bacillus subtilis*	Korsten *et al.*, 1991
	Anthracnose	*Colletotrichum magna*	Prusky *et al.*, 1994
Mango	Anthracnose	*Bacillus cereus*	Koomen and Jeffries, 1993
	Anthracnose	*P. fluorescens*	Koomen and Jeffries, 1993
Banana	Anthracnose	*Talaromyces flavus*	Magan and Baxter, 1993
Kiwi	Gray mold	*Candida SP., Trichosporon sp.*	Gullino *et al.*, 1994
Roses	Gray mold	*Exophiala jeanselmei, Coryneform-type bacterium*	Redmond *et al.*, 1987
Gerbera	Gray mold	*Bacillus brevis, P. aureofacien*	Kerssies, 1993
Tulip bulbs	Penicillium rot	*P. fluorescens, Bacillus sp., B. polymyxa*	Smid *et al.*, 1993

the ability to target the biocontrol agent to the site needed for activity is enhanced in postharvest application. Furthermore, the high value of harvested crops may make the application of elaborate biological control procedures more cost-effective than similar procedures in the field. Although it appears that the postharvest environment may be especially favorable for the development of biological control agents, a considerable investment of time and money is required to establish whether any particular organism has commercial potential. Therefore, the isolation, screening and selection of potential antagonist should receive careful deliberation. Of particular interest are yeast antagonists such as *Pichia guilliermondii* isolated and developed by Wilson and Chalutz (1989) and subsequent workers (Droby *et al.*, 1989, 1991, 1993; McLaghlin *et al.*, 1990; Wisniewski *et al.*, 1991) for the control of postharvest rots of citrus and other fruits. More recently, the yeast *Candida oleophila* (182) was evaluated for its ability to control postharvest diseases of apples under semi-commercial conditions (Wisniewski *et al.*, 1993).

FROM LABORATORY TO LARGE SCALE TESTING

Various microbial antagonists has been reported to effectively control postharvest diseases of diverse commodities when tested under laboratory or small scale conditions. However, when efficacy of these antagonists was evaluated under large scale or commercial conditions it was difficult to achieve a level of control similar to that with laboratory tests. Our ability to explain and control failures and inconsistencies in biological control treatments often stems from a lack of fundamental understanding of the biology that underlies biological control.

In order to minimize the prospects for failure at the stage of transition from the laboratory to "real world conditions" we must gain knowledge on the following: (1) Identity of the antagonist; (2) Etiology of the disease/s to be controlled and postharvest physiology of the commodity; (3) Efficacy of the antagonist under different nutritional, temperature, humidity and other environmental conditions; (4) Mode/s of action of the antagonist; (5) Efficacy and host range under laboratory conditions; (6) Compatibility of the antagonist with postharvest treatments (fungicides, waxes etc.); (7) Changes in antagonist population on the commodity.

ENHANCEMENT OF BIOCONTROL AGENTS

It is doubtful that biocontrol agents by themselves alone will provide the efficacy and consistency associated with conventional fungicides. However, the probability is increasing that they will eventually constitute a significant part of integrated systems that can provide adequate control of postharvest diseases. Because conditions and factors affecting microbial colonization on commodity surfaces can be controlled to a high degree after harvest, the opportunities to enhance the activity of antagonists are exceptionally good.

In our work, we were able to demonstrate the compatibility of yeast antagonists with the current commercial practices and conditions. In pilot tests involving the application of the yeast antagonists *Pichia guilliermondii* (US-7) and *Candida oleophila* (182) to citrus fruit, yeast combined with the fungicide thiabendazole (TBZ) at one tenth of the recommended rate reduced decay to a level equal to that of the current commercial treatment of TBZ at the full rate (Droby *et al.*, 1993). A number of strategies for reducing postharvest diseases have been also evaluated for enhancement of biocontrol activity of yeast antagonists. Methods used in combinations with the yeast treatment include: storage at low temperatures, $CaCl_2$ treatment and ultraviolet light.

MODE OF ACTION OF POSTHARVEST BIOCONTROL AGENTS

Our knowledge on the mode of action of most postharvest biocontrol agents is meager. The lack of a thorough knowledge, other than antibiosis, may be attributed to our limited understanding of the interactions taking place between the host, the pathogen, and the antagonist at the site of infection. Yet, information on the mechanisms of antagonism is critical for developing successful biocontrol strategies. Specifically, such information is essential for: (1) optimization of the method and timing of application of the antagonist; (2) developing appropriate formulations to enhance antagonist efficacy; (3) registration of biocontrol agents for commercial use; and (4) developing of a rationall for selecting new or more effective isolates of the antagonists.

Since the yeast antagonists described above do not exhibit antibiosis in culture against a number of rot pathogens of fruit and vegetables, it was assumed that antibiosis is not an important aspect of their mode of action. Other possible modes of action are:

Competition for nutrients and space

In our studies we observed (Droby *et al.*, 1989) that the US-7 isolate of the yeast antagonist *P. guilliermondii* multiplied very rapidly at the wound site under a wide range of temperature, humidity and nutritional conditions and that it may increase in numbers by one to two orders of magnitude within 24 h while, at the same time, the pathogen spores had just started to germinate and grow.

Several lines of evidence have supported the assumption that the inhibition of the pathogen by the antagonist results from competition for nutrients (Droby *et al.*, 1989). Such competition was demonstrated by *P. guilliermondii* in culture when both the antagonist and the pathogen were cultured in a minimal synthetic medium or in wound-leachate solutions. The efficacy of the yeast could also be markedly reduced by the addition of nutrients to the spore suspension used for inoculation.

In most reports on biological control of postharvest diseases a quantitative relationship has been observed between the antagonist cell concentration and its biocontrol efficacy. Thus, a delicate balance apparently exists at the wound site between the number of antagonist cells and pathogen propagules, which affect the outcome of the interaction and determines whether the wound becomes the site of infection. Manipulation of the initial concentration of the antagonist cell and or fungal spores would, therefore, affect the infection.

Induction of resistance in the host

We have observed, during the interaction of the US-7 isolate of the yeast antagonist *P. guilliermondii* with the host tissue, processes similar to the wound healing response. Development of resistance of host tissues to infection has also occurred in direct response to the application of yeast to the wound site. When citrus peel discs were treated with cell suspensions of the antagonist, ethylene production increased, a phenomenon not observed when a non-effective yeast isolate was applied (Droby and Chalutz, 1994). In citrus and other commodities, ethylene induces activity of phenylalanine ammonia-lyase (PAL), an enzyme which catalyzes the branch point step reaction of the shikimic acid pathway leading to the biosynthesis of phenols, phytoalexins and lignin.

Direct interactions with the pathogen

Wisniewski *et al.*, (1991) have shown that the yeast antagonist *P. guilliermondii,* when co-cultured with *Botrytis cinerea,* appears to strongly attach to the fungal hyphae. This attachment was blocked when the yeast cells or the pathogen hyphae were exposed to compounds that affect protein integrity, or when respiration was inhibited. A lectin-type binding was suggested in this attachment. In addition, *P. guilliermondii* was found to posses a high level of β-1,3-glucanase activity when cultured on various carbon sources or on cell walls of several fungal pathogens (Wisniewski *et al.*, 1991). The attachment of this yeast antagonist to fungal cell walls would enhance the effectiveness of any cell wall hydrolases secreted by the

yeast to the extracellular matrix. When yeast cells were dislodged from the fungal hyphae, a concave appearance of the hyphal surface and partial degradation of *B. cinerea* cell wall was also observed at the attachment sites. Thus, the firm attachment of the yeast cells along with the production of hydrolases, may be responsible for the observed degradation of fungal cell wall following interaction with the antagonist.

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DEVELOPMENT OF PRODUCTION, FORMULATION AND DELIVERY SYSTEMS

R. HOFSTEIN, B. FRIDLENDER

Ecogen Israel Partnership, Jerusalem 91042, ISRAEL

ABSTRACT

Production of biological fungicides consists of two major steps, namely fermentation of the microorganism which represents the active ingredient, and formulation of the end-use product by mixing the microorganism with carriers/stabilizers. The most economical means for fermentation of the organism is attained when its cells/spores undergo propagation while submerged in liquid growth medium which comprises cheap waste material originated from the food industry. Following fermentation, a slurry is subjected to a series of Down Stream Processes which involve de-watering of the biomass and mixing it with a selection of carriers and additives to establish the end-use product.

Fermentation and formulation processes were employed during the development of biological pesticides of the hyperparasite *Ampelomyces quisqualis* (AQ) and the naturally-occurring yeast *Candida oleophila* (CO) for the respective control of powdery mildew disease and fruit rot of a variety of crop commodities. These products represent the basic features of product development which is essentially identical, whether the pathogen is controlled pre-harvest as a foliar treatment or post-harvest as a drench/spray/dip along the processing line.

INTRODUCTION

A bridge between theory and practice can be constructed when, and only when, a microbial pesticide performs (its function) reproducibly in a commercial environment. The vehicle that is always used for the transition from an event in the petri dish to a profound/solid production process is the tedious process of scaling-up (Trilli, 1986). The concept of scaling-up biopesticide production is the exploitation of fundamental phenomena in biological processes, once they have been elucidated during the basic research period. Such phenomena have to be brought out during the execution of a biotechnological process in an industrial environment (Cate, 1990).

Biological control of fungal pests is still in its infancy, and there is very little information to be referenced during establishment of guidelines for process scaling-up. Therefore, industrialization of an event that has been worked out in the laboratory heavily relies on implementation of existing technology for mass production of bacteria. Development of a biological pesticide such as yeast and fungal spores for the control of pre- or post-harvest fungal pathogens provides the best scenario for discussion of industry's perspective on the ideal approach of scale-up (Hofstein *et al.*, 1990, 1993). Certain criteria have to be met, primarily the fact that an organism has to be propagated in a cost-effective fashion, and thereafter remain viable for an extended period of storage at room temperature. These goals can be reached by the adjustment of standard equipment and methodology to the needs of

the product.

Industry's main concerns are related to the successful extrapolation of techniques that have been established in the laboratory to natural field or packing house conditions. It is now generally accepted that a biocontrol agent can provide acceptable control across many habitats only if an imbalance of the natural population dynamics is created which favors the biocontrol agent (Jutsum, 1988; Whitesides *et al.*, 1994). The most common means to achieve the imbalance is by the introduction of abnormally high numbers of the biocontrol agent in a timely manner such that it can have a competitive advantage as an antagonist or parasite against the host. To reach this goal, fermentation has to be a cost-effective process which allows the farmer to utilize the product without many economical constraints. The formulation has to be designed in such a way that the biological agent can restore its biological machinery so that the mode of action is fully expressed under authentic field or packinghouse conditions (Pusey *et al.*, 1988).

Furthermore, once details of fermentation and formulation have been established, it becomes the industrialist's responsibility to select the right strategy for product application. The most common strategy to date is that of Integrated Pest Management (IPM) whereby both chemical and biological agents are used for satisfactory pest control (Papavizas & Lewis, 1988). We will discuss mainly two management strategies. One being the mixture of the biological agent and a low concentration of compatible chemical compound such as in the case of post-harvest rot control with CO (Wilson *et al.*, 1991; Wisniewski & Wilson, 1992; Hofstein *et al.*, 1994). The other option is to alternate applications of bio-and chemical control, as in the strategy of powdery mildew disease control with AQ (Feldman *et al.*, 1993; Sztejnberg *et al.*, 1990). Reduced exposure of target pests to chemicals will contribute to the alleviation of ecological hazards which are the main cause for public concern, as well as reduced risks of pesticide resistance whose development has become a major concern among pesticide manufacturers as well as end-users (Fokkema, 1992).

We will attempt to highlight key elements regarding mass-production (i.e., fermentation), product quality (i.e. formulation and quality assurance bioassays) and finally, product application as a stand-alone treatment or within a more comprehensive approach of IPM systems.

PRODUCTION

The first phase in the development of a production process is selection of a naturally occurring microorganism that can be adapted relatively easily to propagation while being submerged in a deep-tank liquid fermentor. In the case of yeast - the agent for the control of post-harvest rot - the task is probably the simplest - because yeast has been mass-produced ever since baker's yeast was employed by mankind. However, mass production of fungal spores such as that of AQ is a much more cumbersome event, particularly since in this instance, the organism is a hyperparasite of a plant parasite (i.e., the pathogen causing powdery mildew), and hence propagates most efficiently only in the presence of its host.

The biological agent has to be amenable to growth on very cheap waste material originating from the food industry. Once physical parameters are established, primarily after a series of optimization cycles in shake flasks and benchtop chemostats, the conditions can

be adjusted and monitored in the industrial vessel. The main advantage of a deep-tank fermentation is that important information on the physiological state of the organism can be monitored and controlled to a greater precision in the industrial fermentation vessel. Essential parameters such as temperature, aeration and pH that have been determined in a standard laboratory screening process, can be duplicated without too many hurdles. This is in contrast to difficulties of scaling-up a solid/semisolid process.

The complexity in the production processes is apparently proportional to the complexity of the organism. Thus, while propagation of a yeast is a relatively simple undertaking, that of a complex organism such as AQ spores requires more sophisticated manipulations of the growth environment. One way to overcome the complexity is by selection of strains that may readily grow in a deep-tank vessel. Such is the case of AQ_{10} which was selected so that one of its properties is the capability for active growth and sporulation in a submerged fermentation process (Sztejnberg *et al.*, 1990). That, however, is still insufficient and the conditions inside the fermentor have to be fine-tuned to meet the physiological requirement of AQ spores. Evidently, follow-up work on the growth conditions in a submerged fermentation process turned the mass production into a plausible event which is economically justifiable.

Common to both CO yeast and AQ spores is the fact that the intact cell has to be preserved since the mode of action involves competition for space/nutrients at the wound site in the case of yeast for post-harvest rot control (Wilson *et al.*, 1991) and germination of AQ spores into hyphae of the fungal pathogen in the case of powdery mildew control (Sztejnberg *et al.*, 1990). Therefore, the conditions inside the fermentor have to be selected so that the cells grow without distortion for the entire period of the fermentation cycle. The situation could be different in respect to biological agents which are expected to produce active secondary metabolites such as antibiotics or enzymes; these conditions have to be selected in favor of enzyme induction and preservation of the active component whether it is a metabolite or a lytic enzyme (Chet *et al.*, 1994).

FORMULATION

Intact cell concentrates which are the output of fermentation, and centrifugation are the subject of product formulation. In the development of biological pesticides, this stage is probably the most critical and demands an innovative attitude. The formulation process has to account for certain predominant standards of a product which are the following:
- Stability during storage at ambient climatic conditions.
- Restoration of functional properties upon contact with the target pathogen.
- Simple handling of the formulation prior to application on the infected commodity.

These standards have been set by the chemical-pesticide industry but are clearly valid for biological pesticides as well, since they ought to compete in the same areas (Rhodes, 1991). By definition, it is however, much more cumbersome to harness biological agents into the framework of such criteria, primarily when the intact cell is the active ingredient responsible for the fungicidal activity. A prerequisite is that the units of the active ingredient (i.e. cells or spores) remain intact following a minimum of 6 months of storage, and preferably for 1-2 years (Carlton, 1990). An example of stability curves is that of AQ spores which retain viability (expressed as rate of spore germination on agar) for at least 6 months

of cold or ambient temperatures.

There are several end-use formulation alternatives, some of which are derived from recent advances in respect to cell adherence to carriers as well as encapsulation technology using cross-linked matrices of organic polymers. These are expected to establish a protective micro-environment which surrounds the cells or the spores from over-desiccation during dehydration into a wettable powder (or granules) and "insulate" them from typical environmental instability during storage (i.e., drastic changes in temperature and relative humidity, which can be destructive to the formulated cell).

Spores of AQ as well as cells of yeast are a concrete example of a successful formulation process whereby following research aimed at exploring the metabolic and structural features of the organism, a specific formulation was tailored for each of them. The constituents of the formulation have been selected so that they can indeed assist in preservation of the biological agent. The resulting product should also be simple to handle, similar to a standard pesticide whether in the form of wettable powder (WP) or Water Dispersable Granule (WDG) The requirement to design a universal formulation procedure for several organisms acting as cores of different biofungicides is of the utmost importance from the economic point of view. Otherwise, the industrial plant will have to consist of variable formulation setups for different products which could jeopardize economic feasibility. The CO and AQ product lines were indeed harnessed into a similar formulation process, in a strategy that turned out to be a major advantage in the development of the product(s).

Selection of tank additives is an integral factor in the design of product formulation, but can equally be part of application technology. Two examples are the pretreatment of fruit with salts (e.g. $CaCl_2$ to enhance yeast activity against Penicillium rot - McLaughlin et al., 1990) or the more classical packing-house sanitation treatment with sodium orthophenylphenate (SOPP). However, tank additives are much more important in the design of pre-harvest disease control since deterrent climatic conditions such as desiccation and ultraviolet radiation are more profound. Not every protectant or enhancer can be included in the main formulation and therefore has to be applied as part of the tank formulation. Many optional tank-additives have been screened in the laboratory as enhancers of AQ, but their actual capability to be implemented in a comprehensive pest control program could only be evaluated in the field. Another alternative is based on the assumption that certain nutrients can selectively enhance growth and/or performance of the microbial antagonist over the pathogen. Successful examples of such an approach, despite its attraction, are only a handful, but the limited achievement indicates that the approach merits further search for selective nutritional components (Gullino et al., 1989; Janiscewicz et al., 1992).

Once formulation is established, the next predominant assignment is the development of quality assurance (QA) tests. A useful QA test is a bioassay system that can monitor, relatively rapidly, the quality of each production batch. Every QA test has to take into account the main properties of the biological agent. Thus, germination rates of AQ spores into powdery mildew and growth of yeast at the wound site reflect the quality of the end products. Therefore, hyperparasitism tests (an in-situ quantitative evaluation of AQ spores) have been employed as a standard test of the end-use product for powdery mildew control. Similarly, an in situ test using artificially wounded and inoculated apples or citrus varieties was developed for the postharvest program. The common feature of the two is that they have proven useful forecasters of product quality to be determined in authentic field trials.

PRODUCT APPLICATION AND PRODUCT EFFICACY

The location selected for field evaluations should be representative of the geography where targeted commodities are of economic importance as determined by market assessment. In contrast to the open field, that is a lesser constraint in the development of a product to be used in the packing house, since the latter is a relatively confined environment with much less environmental fluctuations. However, access to commercial lines is not readily available or practical during fruit processing periods and therefore a substitute has to be allocated at least for the early stage of product development. Thus, for the development of the post-harvest yeast product, a scaled-down version of a process line, typically referred to as a pilot-line was designed and erected for processing of pome fruit and citrus varieties; product efficacy was evaluated on that line.

The pilot-line is a flexible setup which provides opportunities to manipulate rot-inducing pathogens from different perspectives. For instance, disinfection prior to fungicide application is plausible, likewise fungicide application prior to or concomitantly with waxing. Of greater importance is the capability to compare various application methods from drenching through dumping to spraying. Typical performance magnitudes can be exemplified by the illustration in Figure 1 which summarizes the capability of the CO yeast to control Penicillium rot on apples (Fig. 1a) and citrus (Fig. 1b) as a sole treatment or in conjunction with thiabendazole (TBZ) used at one tenth of the label-recommended rates.
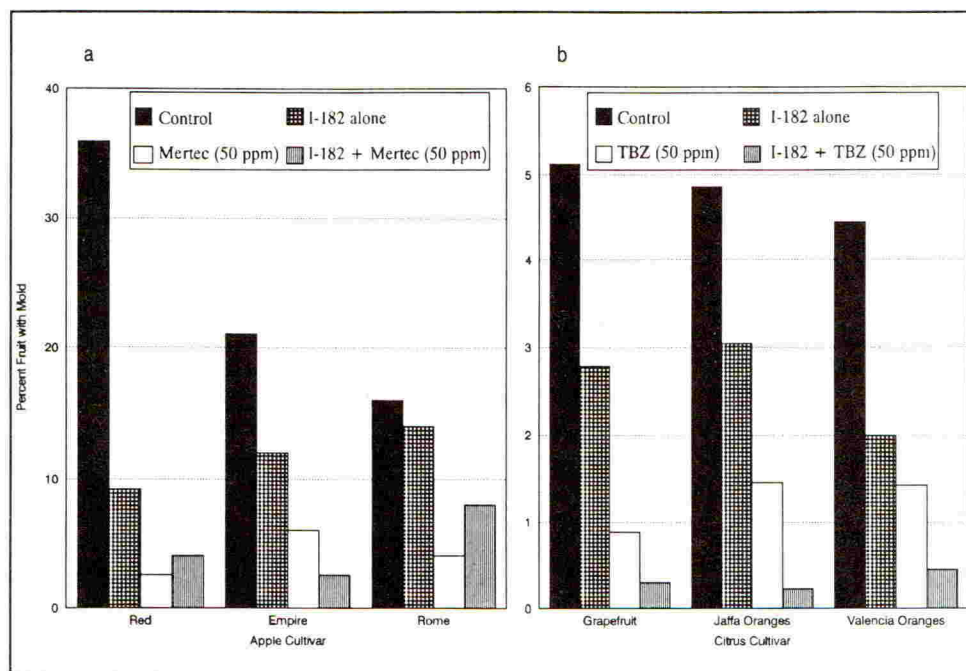

Figure 1: Pilot-line testing of Penicillium spps. control on apples (a) and citrus (b) cultivars. Commercial rate of control was attained by combination of CO and TBZ.

Pilot experiments led to the conclusion that an IPM system whereby yeast is employed at low rates of chemical fungicides reduce fruit decay to below economically damaging levels. However, even though the pilot line is a crucial step in product evaluation, an authentic trial program in a commercial packing house is the ultimate judgement of product acceptability. Results from such a scenario are presented in Figure 2 in which the rates of fruit decay in oranges are depicted after long periods at variable temperature.
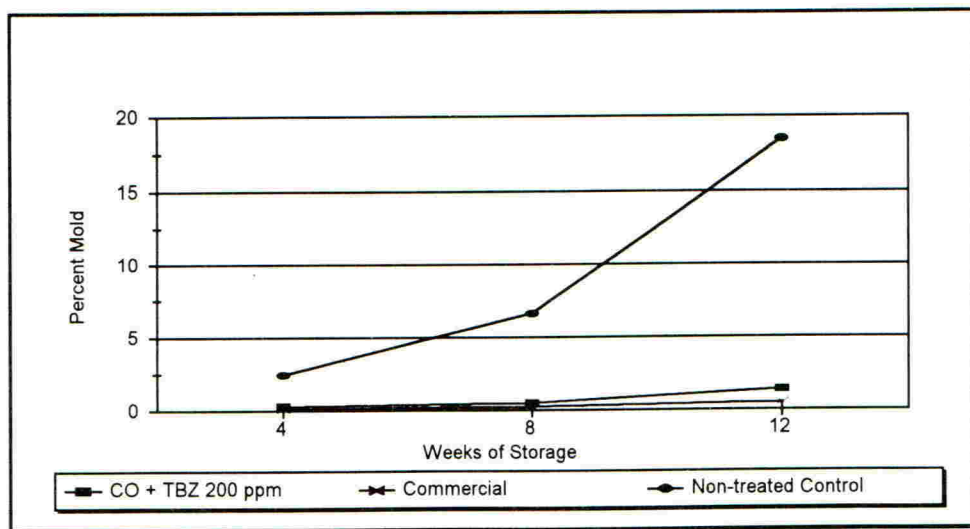

Figure 2: Penicillium rot development during cold storage (5-7°C) following Packing-house treatment of oranges with CO+TBZ or a standard mix of chemical fungicides (2,000ppm TBZ, 1,000 Imazalyl)

The open field offers a much harsher environment for testing the performance of the product than the controlled environment of the packing house. The main problems, as were viewed during the development of the AQ, are the result of (1) rapid dehydration due to insufficient relative humidity, (2) damaging UV-radiation, (3) inaccessibility of spores to location of the developing pathogen due to excess vegetation (foliage), and (4) incompatibility of the biological treatment with other routines for the control of unrelated disease or insect pests. These hurdles can be resolved by proper instructions, such as were implemented in a protocol for the application of AQ in vineyards. Periods of the day with high humidity and less light exposure (i.e., early morning or late evening) are highly recommended for spray application, as well as pruning conducted to expose bunches to attain effective coverage of berries with spore suspensions. Furthermore, incorporation of adjuvants and stickeners that are compatible with the germinating spores of AQ are an essential aspect of the tank additives and so is the presence of humectants which can, by virtue of their physical nature, create a protective film around the germinating spore.

The whole array of requirements has been implemented in a vineyard program whereby AQ_{10} has been employed within an IPM program. The concept was to alternate AQ applications with those of various chemical treatments at different phases of grape developments.
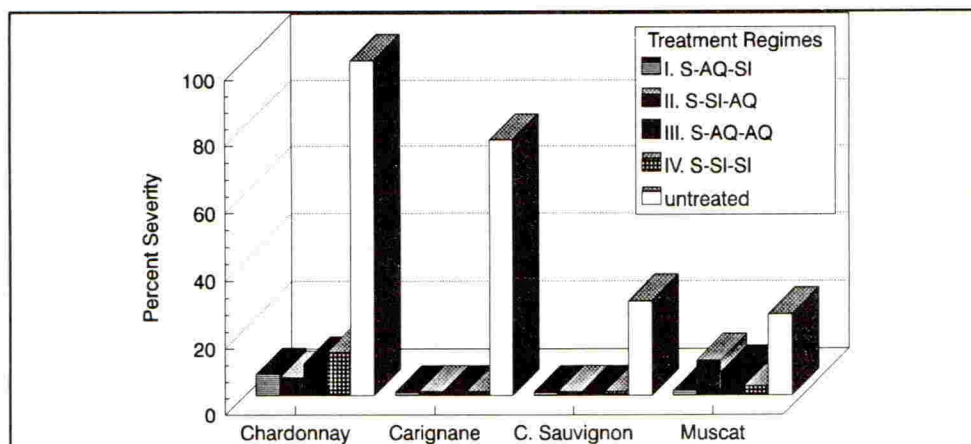
Figure 3: Control of powdery mildew on grapes, comparing several alternatives of IPM programs. The options are I: 2-3 sulfur (S) sprays followed by 2-3 sprays with AQ_{10} and 2-3 sprays of sterol inhibitors (SI). Other options are represented in II-IV.

In essence, it should be emphasized that the product has to be evaluated first as a stand-alone treatment of the pathogen. However, as soon as that part is accomplished, the biofungicide can be included in an IPM program that meets criteria of commercial acceptability of damage suppression. An IPM system is probably the best option for a comprehensive attack on pathogens as they represent a multifactorial event, primarily due to the fact that more than one pathogen develops simultaneously on the host.

Design of a comprehensive disease control program is the main challenge of those who intend to introduce biological pesticides as an attractive alternative or addition to existing control programs. The wisdom of the biopesticide industry resides in its capabilities in cost-effective fermentation and formulation of the product together with skillful incorporation into modern schemes of IPM systems.

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