MAIN SUBJECT 5

NON-CHEMICAL METHODS OF CROP PROTECTION

TOPIC 5A

BIOTIC AGENTS

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THE CURRENT STATUS AND POTENTIAL OF ENTOMOGENOUS FUNGI AS AGENTS OF PEST CONTROL

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ABSTRACT

Detailed research on seven entomogenous fungi or groups of fungi has led to commercial scale production of Beauveria bassiana and Metarhizium anisopliae which are used for pest control in national programmes in the USSR, China and Brazil and of Verticillium lecanii and Hirsutella thompsonii which are marketed and registered for use as microbial pesticides in Europe and the USA, respectively. The reliability of V. lecanii for aphid and whitefly control in glasshouses is well documented but evidence for that of other species, outdoors, is less convincing. Nevertheless there is broad scope for promoting control exerted by fungi by strain selection and by manipulating the ecosystem into which they are introduced.

INTRODUCTION

Concern that insect pests will continue to develop resistance to a growing number of chemical pesticides and an increase in public and grower awareness of the undesirable effects on the environment of the widespread use of chemicals have encouraged research into non-chemical methods of pest control. One among many of these is the use of entomogenous fungi. Representatives of these occur in all the major divisions of the fungi but those being considered seriously for pest control are Deuteromycetes and Phycomycetes (Entomophthorales). The development of fungi for pest control has been surveyed in recent detailed reviews (Ferron, 1978; Hall & Papierok, 1982). This paper summarises aspects of this development as background against which the efficacy of fungi already in commercial use or being developed for it and ways of improving efficacy are considered.

DEVELOPMENT OF FUNGI FOR PEST CONTROL

Production and storage

Many species of entomogenous fungi, especially Deuteromycetes, grow readily in standard mycological media and in commercially acceptable alternatives. The fungi may be harvested as mycelia, conidia, blastospores or, in the Entomophthorales, as resting spores. Blastospores, asexual spores formed by budding, are produced typically in agitated liquid culture and are less robust than the conidia that form aerially. Mycelia and the resting spores of the Entomophthorales form both in liquid and solid media. Fungal propagules for field use are produced in quantity by either liquid or solid substrate fermentation or in stages involving both. Liquid fermentation utilises a well established industrial technology. In solid substrate fermentation, the fungus is usually grown on a particulate substrate, often cereal grains to which nutrients are added. This provides a large surface area on which the conidia are produced.

A proportion of conidia of most entomogenous Deuteromycetes often survive in nature for many months, but bulk storage of propagules of most entomogenous fungi is a problem. Resting spores of certain of the Entomophthorales are an exception, surviving unformulated for up to several years. Most Deuteromycetes can be lyophilised and many fungi, including the Entomophtorales can be stored indefinitely in liquid nitrogen. However

such methods are expensive and unsuitable for the storage of the quantity of material needed for field use. Ways of improving storage by appropriate formulation of the product are being investigated: survival of spores is often greatly improved by mixing with refined clays and by refrigeration.

Safety for man, domestic animals and beneficial insects

The maximum temperature at which most entomogenous fungi survive is 37°C. They are therefore unlikely to infect mammals and birds since the body temperature of most of these is higher. Only Aspergillus spp. and Conidiobolus coronatus (Entomophthorales) occasionally infect mammals, including man; these are not being considered for insect control. Tests on the infectivity for rodents of candidate fungi and those now in use have all been negative. Beauveria bassiana produces allergic responses in some people although in China, where this fungus is used widely, it appears to cause little problem (Hussey & Tinsley 1981). There are no records of allergic reactions to other entomogenous fungi.

Predatory and parasitic insects which prey on pest species are themselves susceptible to infection by fungi. Fortunately these pathogens are much more selective than most chemical pesticides. Also many species comprise intraspecific strains each infective for a restricted range of hosts, so a strain applied for control of a certain pest is unlikely to affect beneficial organisms in the same environment.

Registration of pathogenic fungi for pest control

Until recently, microbial pesticides had to satisfy the requirements laid down for the registration of chemicals, but regulatory agencies in some countries, including the USA and the UK, now accept that a different set of safety criteria for microbial pesticides is required. Guidelines for the registration of entomogenous fungi as insecticides, including those for safety testing, have been drawn up recently by a study group of the International Organisation for Biological Control, WestPalaearctic Regional Section (Hall & Papierok, 1982).

EFFICACY OF FUNGI CURRENTLY IN COMMERCIAL USE OR BEING CONSIDERED FOR USE

Application approach

Two approaches in the use of fungi for pest control are recognised. The application can be made, like an insecticide, to produce a "once for all" high mortality in an existing, potentially damaging pest population. The alternative is to introduce an inoculum into the pest population, relying on subsequent multiplication and spread of the fungus to effect control. This latter approach has received most attention; fungi frequently take too long to kill their host to allow their use as an insecticide except when damaging populations can be forecast many days in advance.

Measuring efficacy

The efficacy of a fungus or any other biological agent in pest control cannot be measured simply by comparing its impact on the target pest with that of a chemical pesticide. A fungus will almost certainly not kill its host as quickly as a chemical; its value will lie more in its persistence, through reproduction, in the crop environment and in its compatibility with other biotic agents attacking both the target and non-target pests. Accordingly, damage to the crop and yield are the most important criteria of success. Nevertheless most examples from the literature stress the effect of the pathogen on the pest rather than the protection afforded to the crop.

For clarity, each species or group of entomogenous fungi is considered separately.

Beauveria spp.

Beauveria bassiana commonly infects insects of many orders particularly Coleoptera and Lepidoptera. It is produced on a large scale for field use only in the USSR and China. In the USSR the fungus is used as an insecticide, together with reduced doses of chemical insecticides, to control Colorado beetle (Leptinotarsa decemlineata) and codling moth (Cydia pomonella). When combined with chemicals, consistent results are obtained irrespective of the climate (Ferron, 1981). In China it is used mostly to control European corn borer (Ostrinia nubialis) (Hussey & Tinsley, 1981). In 1977, for example, 0.4 million ha of corn was treated. Control of 80% of first generation larvae was claimed and no application against the second generation was needed. Whilst such claims are not validated statistically one is left to speculate whether such effort would be expended if the organism were not effective. Elsewhere B. bassiana has been tested on a plot scale. For example, in 1978 in Normandy, France, it almost completely controlled 4th instar larvae within 13 days of application, though this result was achieved in a moist cool period particularly favourable to the action of the pathogen (Fargues, et al, 1980).

Metarhizium anisopliae

Metarhizium anisopliae is a common pathogen, chiefly of soil dwelling and pasture insects. It is produced on a large scale as 'Metaquino' in Brazil where it is used for the control of leaf hoppers (Hemiptera: Cercopidae). Production is by solid substrate fermentation on boiled rice in autoclavable polypropylene bags (Ferron, 1981). It was applied by air to 50,000 ha in one state alone in 1978 more cheaply than alternative chemical treatments, but there are few data on its efficacy (Ferron, 1981). This fungus is not used widely elsewhere but in recent field tests it was as or more successful than presently used chemicals in controlling the pasture cockchafer (Aphodius tasmaniae) in S. Australia (Coles & Pinnock, 1982). A high dose of conidia (10^{15} ha^{-1}) caused 55% mortality of the larvae of this univoltine beetle 4.5 months after application.

Nomuraea rileyi

Nomuraea rileyi attacks lepidopteran larvae including such serious pests as cabbage looper (Trichoplusia ni) and corn earworm (Heliothis zea). Although the fungus grows readily in vitro, commercially acceptable methods for its production have not been developed. Mohamed et al. (1978) applied it as an insecticide to sweet corn infested with H. zea in field cages. A mean of 65% of larvae was killed by the fungus following 3 applications, but even this number of applications failed to prevent economic damage. Similarly, the number of H. zea larvae on soybeans in field cages fell to 23% of that in untreated cages, after an application of 10^{13} conidia ha^{-1} (Ignoffo et al., 1978). The proportion of damaged seeds was significantly reduced but not sufficiently to be of commercial value. It would seem that when applied as an insecticide the fungus kills its host too slowly to prevent crop damage. The results of other experiments, however, suggest that its application might be a useful prophylactic treatment.

Hirsutella thompsonii

Hirsutella thompsonii infects only mites, Acarina, in tropical or subtropical regions. It is marketed in the USA as 'Mycar' and was first registered for use against citrus rust mite (Phyllocoptruta oleivora) on citrus crops in 1981. The product is formulated with nutrients to encourage saprophytic development in the field. More than 4500 kg of Mycar were sold in 1981 to treat over 2000 ha of citrus (McCoy, 1982). Inocula of an unformulated preparation of this fungus initiated epizootics on treated trees

earlier than on untreated ones giving good control of P. oleivora within 2-3 weeks of application but only when environmental conditions were suitable (McCoy et al.,1971). However there has been only one published report (McCoy & Couch, 1982) on the effect of the commercial product on mite populations. The results of the three field trials described are difficult to interpret. In the first, mite populations were significantly diminished by the treatment, but the proportion of fruit damaged was not significantly affected. In the second trial the fungus was applied in 1% oil and it was impossible to separate the effect due to the oil from that of the fungus. In the third trial Mycar kept numbers of mites significantly smaller than on the untreated trees but, unfortunately, figures for the proportion of damaged fruit in this trial are not given.

Verticillium lecanii

Verticillium lecanii most frequently attacks aphids and scale insects in the tropics and sub-tropics. It was registered for use in the UK in 1981 and marketed as 'Vertalec' for control of glasshouse aphids and subsequently (another isolate) as 'Mycotal' for whitefly control. Details of the commercial production of the fungus are not available but the product, a wettable powder, contains formulation ingredients that permit saprophytic growth to recommence after application. In this way up to forty times more spores are produced than are sprayed (Hall & Papierok, 1982). The efficacy of conidia and blastospores of V. lecanii in controlling Myzus persicae, the major pest on the ornamental crop, chrysanthemums, has been convincingly demonstrated in several glasshouse trials (Hall & Burges, 1979). One inoculum of unformulated blastospores eliminated or nearly eliminated even small populations of M. persicae in 2-3 weeks and maintained the population at a low level for the duration of the development of the crop. In further glasshouse trials, commercial substrate-containing spore formulations of this species virtually eliminated the cotton aphid (Aphis gossypii) on cucumbers in 28 days and maintained glasshouse whitefly (Trialeurodes vaporariorum) populations far below the level at which economic damage occurs (Hall, 1982).

Aschersonia aleyrodis

Aschersonia aleyrodis is a pathogen of whiteflies and scale insects (Hemiptera: Aleyrodoidea and Coccoidea). It is being considered for control of whiteflies in glasshouses in the Netherlands (Ramakers et al.,1982) and in the UK (R.A. Hall, personal communication). In the Netherlands the fungus was applied to cucumbers in combination with the release of the parasitic wasp Encarsia formosa. The combined treatments caused 85% mortality of T. vaporariorum compared with 49% due to the wasp alone. This fungus may have particular potential because it develops at lower humidities than most others.

Entomophthorales

There are more than 150 species of these fungi many of them infective for insects and mites though most of these are restricted to certain host groups. No member of the Entomophthorales is currently produced on a commercial scale for pest control. Procedures for the production of the resting spores of certain species infective for aphids have been developed but the products failed to provide control of the pests against which they have been tested. Even if resting spores can be induced to germinate they fail to do so synchronously, some taking as long as a month. Progress is now being made in producing and stabilising mycelia of strains of species of proven field efficacy including Zoophthora radicans and Erynia neoaphidis. The detailed results of applying such products in the field have not yet been published. However, two species, E. neoaphidis and Neozygites fresenii, were applied to plots of Vicia beans infested with black bean aphid (Aphis

fabae) by releasing aphids infected with the fungi (Wilding, 1981a). In two of four years the fungi significantly diminished the aphid population and in one year, when aphids were very numerous, yields from the treated plots were significantly greater than those from untreated plots. These results encourage the belief that Entomophthorales might be used to control pest populations if ways of producing an infective and stable inoculum could be found.

POTENTIAL FOR IMPROVING EFFICACY

The introduced fungus

The fungal strain, its formulation, dose and frequency and timing of application all affect the efficacy of the pathogen.

Intraspecific strains of these organisms differ greatly in their behaviour and infectivity. For example, the LC50 of strains of Conidiobolus obscurus for the pea aphid (Acyrthosiphon pisum) differed by a factor of up to sixteen (Papierok & Wilding, 1981). Strains of this species fell into two biological races distinguished by their infectivity and characteristics of their development in vitro and in vivo. Attempts to increase the virulence of strains of B. bassiana with mutagenic agents have so far been unsuccessful (Ferron, 1981). Different strains of few entomogenous fungi have been field tested, although a 'whitefly isolate' of V. lecanii controlled whitefly populations more effectively than an 'aphid isolate' and the whitefly isolate failed to control aphids satisfactorily (Hall, 1982).

Although unformulated suspensions of fungal propagules can be used to establish infection in the field, commercial preparations are usually formulated with one or more of a range of substances to enhance or prolong their field effectiveness. Inert carriers often improve the stability and homogeneity of distribution of a product. Alternatively a growth substrate mixed with the preparation may promote saprophytic development and spore production after application. The efficacy of V. lecanii in controlling A. gossypii on cucumbers was greatly improved in this way (Hall, 1982). In addition, wetting, sticking, spreading, U.V.-screening, hygroscopic or thixotropic agents may be added, either to the product or, if used in suspension, to the tank mix before application.

Recommendations concerning how much material should be applied, when and how frequently, are available only for the commercial preparations of V. lecanii. A single application achieved the best results on chrysanthemums (Hall & Burges, 1979), but 100-fold differences in the concentration of spores applied produced equally good control probably because the effect of the dose was quickly masked by the spread of the fungus after application (Hall, 1980). Similarly, ten-fold differences in the dose of E. neoaphidis had only a temporary effect on the infection of A. fabae on field beans (N. Wilding, unpublished data). Conversely, control of H. zea was progressively better the greater the number of applications of N. rileyi (Mohamed et al., 1978) and the larger the dose applied (Ignoffo et al., 1978). Entomogenous fungi are best applied in the evening to reduce their exposure to sunlight and to the usually relatively low daytime humidity.

Manipulation of the ecosystem

The crop ecosystem can be manipulated in ways that will improve the efficacy of introduced entomogenous fungi and those present naturally. The three components of the ecosystem amenable to modification are the crop climate, particularly humidity, and the host and fungus populations.

Crop climate

Humidity within the crop canopy is primarily affected by precipitation which can be supplemented by irrigation. The effect of irrigation on the infection of A. fabae by Entomophthorales was demonstrated in an experiment on Vicia beans at Rothamsted in 1979 (N. Wilding, unpublished data). Only 2 mm of rain fell between 26 June and 22 July and the proportion of infected aphids in plots given about 10 mm water twice weekly was significatly greater than in non-irrigated plots during the latter part of this period.

Secondary factors affecting humidity within the crop canopy, including plant density and the growth characteristics of the crop cultivar, can also be modified artificially. For example, a significantly greater proportion of larvae of Heliothis spp. were infected with N. rileyi in a closed canopy variety of cotton than in an open canopy one (Burleigh, 1975). The presence of weeds may also increase the humidity within a crop canopy. The effect of weeds on the infection of cereal aphids by Entomophthorales was investigated at Rothamsted in 1980 and 1981 (W. Powell, G.J.W. Dean, N. Wilding & A.M. Dewar, unpublished data). Significantly more Sitobion avenae were infected in untreated plots than in those treated conventionally, in February, with herbicides, probably due to the more humid conditions generated by the weeds.

Scope for the modification of physical conditions is greatest in protected crops (glasshouses and polyethylene tunnels); the control of Chrysanthemum aphids is enhanced by the humid conditions provided by the black polyethylene covers used to shorten daylength and so induce flowering of the crop (Hall & Burges, 1979).

Host population

There are no fully documented accounts of how host density affects the spread of an entomogenous fungus introduced for pest control. However, host density is known to influence the natural infection of insects by Entomophthorales (for references, see Wilding, 1981b), so its manipulation might improve the efficacy of an introduced fungus. Factors that affect pest density and that might be manipulated include the choice of a crop cultivar with a degree of insect resistance, the quantity of nitrogen fertilizer applied and the sowing date of the crop.

The natural fungus 'population'

Laboratory studies show that entomogenous fungi are very sensitive to most fungicides and to many other pesticides. However insects are seldom protected from infection by entomogenous fungi by fungicides applied against plant pathogenic species in the field. Of several chemicals tested in a laboratory study at Rothamsted (Wilding & Brobyn, 1980), mancozeb and benomyl completely inhibited growth in vitro and conidium germination of E. neoaphidis, and benomyl prevented the production of conidia from infected A. pisum. Further, mancozeb protected A. pisum from infection when applied 6 h before the aphids were inoculated with conidia of the fungus. A corresponding test with benomyl was impossible because the chemical killed the aphids. When these chemicals were applied each week, far more frequently than in commercial practice, to plots of field beans infested with A. fabae, the proportion of aphids infected with E. neoaphidis was unaffected by mancozeb but significantly diminished by benomyl (Wilding, 1982). The aphicidal effect of benomyl observed in the laboratory was not evident in the field.

There are other ways in which crop husbandry might be expected to affect fungus activity. For example, most fungal spores remain near the surface of undisturbed soil. Conventional ploughing would distribute the spores through a plough depth of soil but minimal cultivation would leave them on or near the surface where they would then be most likely to contact a susceptible host. Fungus infection of a pest in a crop might also be encouraged

by intercropping or underplanting with another crop liable to early infestation with a susceptible insect. Further, N. rileyi caused a greater mortality of larvae of Heliothis spp. in early- than late-sown soybeans (Sprenkel & Brooks, 1975). Ignoffo (1981) suggested, therefore, that an early epizootic of N. rileyi might be encouraged by alternating one early-planted row of soybeans with about 100 late-planted ones.

CONCLUSION

Recent results with V. lecanii in the UK have shown convincingly that this fungus is an effective agent of control of certain glasshouse pests. The use of entomogenous fungi in the glasshouse environment is encouraged not only because the humidity and other abiotic factors can be modified readily but also because the fungi are compatible with other systems of biological control now in widespread use in the glasshouse. Evidence for the successful use of fungi in the field is less convincing. Unfortunately, many of the published data, especially from the USSR, China and South America are unsupported statistically. However, there is some validated evidence for crop protection by the introduction of fungi and much more demonstrating important effects on pest populations in the field. Further, as described above, there is broad scope for developing ways of promoting control exerted by fungi. With preparations of at least two species now available commercially, and with experience in the production of commercial quantities of several others, the use of entomogenous fungi for crop protection should receive increasing attention.

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RECENT DEVELOPMENTS IN THE USE OF NEMATODES IN THE CONTROL OF INSECT PESTS

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ABSTRACT

Recent field trials with neoaplectanid and heterorhabditid nematodes have shown positive results that compare with standard chemical pesticides. The most efficient use of these nematodes is in soil or protected areas of plants where the parasites are protected from rapid desiccation.

The field of insect nematology has developed considerably over the past decade. There are now over 3,000 known associations between insects and nematodes, indicating the number of investigations that have occurred since the discovery of the first entomogenous nematode by Reamur in 1742.

Tabulations of insect nematode associations appear in the books of Shepard (1974) and Poinar (1975) and those nematodes having potential as biological control agents are discussed in a more recent book (Poinar, 1979).

Although at present, the neoaplectanids and heterorhabditids are by far the most promising of all entomogenous nematodes from the standpoint of control, a few words should be said about two other species that have been used successfully in biological control programs. The first is Deladenus siricidicola, parasite of siricid woodwasps. This nematode has been liberated throughout most of the Sirex noctilio infested areas in Victoria, Australia and has given a high degree of control (Bedding & Akhurst, 1974).

The second nemtatode that was intensively studied and used experimentally was the mosquito infecting mermithid, Romanomermis culicivorax. A system of mass rearing the parasite allowed a source of inoculum for extensive field tests (Petersen & Willis, 1972) and successful control was achieved in several instances (Poinar, 1979). However, due to various causes, neither of the above 2 nematodes was successfully commercialized and activity on them has decreased.

Within the past few years, the potential of the soil-inhabiting, insect infecting neoaplectanid and heterorhabditid nematodes have attracted more attention. Their system of carrying and releasing symbiotic bacteria allows them to parasitize an astounding number of insects from an assortment of different orders (Poinar, 1979), something that Deladenus and Romanomermis were incapable of doing. Their ability to be mass cultured either "in vivo" or "in vitro" makes production relatively easy. Possession of a resistant infective stage juvenile that is suitable for storage is another asset.

The absence of any adverse environmental effects, including toxicity to vertebrates, earthworms, plants and most non-insect invertebrates gives them an edge over chemicals.

There is also no record of the development of resistance to this nematode-bacterium complex.

These features are so attractive that four separate commercial organizations in the United States have now started developing programs for the production of these nematodes.

Successful field trials with these nematodes are shown in Table 1. The most successful results were achieved when the nematodes were applied to the soil (Bedding & Miller, 1981; Georgis et al., 1982; Simons, 1981; Poinar et al., 1982; Jourdheiul et al., 1974; Edwards & Oswald, 1982; Glaser, 1932) or in protected plant parts such as galleries of borers (Lindegren et al., 1981; Kovacs et al., 1980; Bedding & Miller, 1981).

Applying the nematodes on exposed plant surfaces may require the use of antidesiccants, surfactants or other substances which can keep the parasites on the leaf surface and protect them from drying out. Moisture is an essential factor for survival and infectivity of these as well as most nematodes.

The potential for these nematodes is great. It is up to the scientists and industry to work together so that the wide range of noxious insects that are killed by these nematodes in the laboratory can be destroyed under field conditions. It is a challenge but I believe it can be done.

TABLE 1
Successful field trials of *Neoaplectana* and *Heterorhabditis* against insect populations

Host	Nematode	Site	Dosage	Results	Location	Reference
Black vine weevil (*Otiorhynchus sulcatus*)	*Heterorhabditis heliothidis*	potted yew, raspberries, grapes, strawberries, cyclamens	20,000/pot; surface and injection	from 87-100% parasitism	Australia	Bedding & Miller, 1981b
Strawberry root weevil (*Otiorhynchus sulcatus*)	*H. bacteriophora* *H. glaseri* *N. carpocapsae* (Breton)	various plants in pots	5,000/pot	from 82-91% infection for all 3 species	California	Georgis *et al.*, 1982
Corn rootworm *Diabrotica* sp.	*N. carpocapsae* (Breton)	30 acres of corn	10,000/ linear meter	significant difference between nematode treated and control	Nebraska	Poinar *et al.*, 1982
Hylemia brassicae Cabbage root maggot	*N. carpocapsae* *H. bacteriophora*	brussel sprout seedlings in field	250 nemas per seedling on surface	good control	California	Georgis *et al.*, 1983
Root weevil *Nemocestes incomptus*	Mixtures of *N. carpocapsae* *N. glaseri*	strawberry raspberry in field	5×10^6 nemas in plots	62% control	California	Georgis (in Press)
Carpenter worm	*N. carpocapsae*	borings in California oak (11 trees)	not known	84% control	California	Georgis & Poinar, 1983
Currant borer (*Synanthedon tipuliformis*)	*N. bibionis*	spraying bundles of cuttings	30,000 nemas/ml	99% control	Australia	Bedding & Miller, 1981a

Table 1 continued

Host	Nematode	Site	Dosage	Results	Location	Reference
Altise beetle *Psylliodes chrysocephala*	*N. carpocapsae* (DD-136)	mustard field	1 x 10^6 per m^2	92% control	France	Jourdheiul *et al.*, 1974
Wireworms *Selatosomus reichardti*	*Neoaplectana* sp.	field	3 x 10^3 nemas per ml	60% mortality	USSR	Loktin & Ivanova, 1970
Wireworms *Agriotes lineatus* *Selatosomus aeneus*	*N. carpocapsae*	field	1.5 X 10^6 nemas/m^2	69-78% mortality	USSR	Danilov, 1974
Butterfly *Papilio demodocus*	*N. carpocapsae* (DD-136 strain)	trees	600,000 nemas/ tree	63-67% mortality	Australia	Srivastava, 1978
Wireworms *Agriotes* sp.	*N. carpocapsae* *H. bacteriophora*	corn field	19,000 nemas/ linear meter	significant reduction of insects with both nematodes	Italy	Kovacs *et al.*, 1980
Carpenterworm *Prionoxystus robiniae*	*N. carpocapsae* (Mexican strain)	borings in fig trees	9000 nemas per gallery	44-100% mortality	California	Lindegren *et al.*, 1981
Blackflies *Simulium* sp.	*N. carpocapsae*	stream	35 nemas/ml	50% mortality	New York	Gaugler & Molloy, 1981
Borer *Zeuzera pyrina*	*N. carpocapsae*	596 holes in 20 orchards	3-7 x 10^4 nemas per each gallery openings	85% mortality	Italy	Deseö, 1982

Table 1 continued

Host	Nematode	Site	Dosage	Results	Location	Reference
Grass grub *Costelytra zealandica*	*N. glaseri* *H. bacteriophora*	Soil in field	1000 million nemas per hectare	0-92% mortality	Australia	Jackson & Trought, 1982
Springtails (Collembola) various beetles	*N. carpocapsae*	plots in sugar beet field	250-250,000 nemas per linear meter	Significant reductions of springtails and beetles	England	Edwards & Oswald, 1982
Japanese beetles *Popillia japonica*	*N. glaseri*	Pasture turf	27,000 nemas per ft^2	High mortality	New Jersey	Glaser, 1932
Cabbageworm *Pieris brassicae*	*N. carpocapsae*	field	--	80-94% control	Poland	Sandner & Pezowicz, 1980
Colorado potato beetle *Leptinotarsa decemlineata*	*N. carpocapsae*	on surface of potato plants with antidesiccants	75-210 nemas/cm^2 of leaf surface	30-60% mortality	Colorado	MacVean *et al.*, 1982
Codling moth *Carpocapsae pomonella*	*N. carpocapsae*	trunks and branches of apple trees	--	60% mortality	Eastern U.S.	Dutky, 1959
Winter moth *Operophtera brumata*	*N. carpocapsae*	soil beneath apple trees	3.4×10^4 to 1.6×10^5 nemas	significant reduction of larvae & per ft^2 pupae	Canada	Jaques *et al.*, 1968
Cutworms	*N. carpocapsae*	rice fields	1×10^6 nemas to a 3.0 x 6.5 m^2 plot	Significant reduction of insects	India	Israel *et al.*, 1969

Table 1 continued

Host	Nematode	Site	Dosage	Results	Location	Reference
Southern Pine beetle *Dendroctonus frontalis*	*N. carpocapsae*	Pine bark	740 nemas per ft^2	40-50% mortality of adults & brood	North Carolina	Moore, 1970
Otiorhynchus sulcatus	*Heterorhabditis* sp.	Strawberry, primula and cyclamen in pots	100 nemas per cm^2	90-97% mortality	Holland	Simons, 1981
Hylemya spp.	*N. carpocapsae*	tobacco	38,200 nemas per plant	Control by nemas equal to that of Diazinon	Ontario	Cheng & Bucher, 1972
Pecan weevil *Curculio caryae*	*N. carpocapsae*	Pecan trees	703,000 nemas per inch	67% mortality of larvae	Georgia	Tedders *et al.*, 1973
Cutworm *Spodoptera frugiperda*	*N. carpocapsae*	corn	4,000 nemas per plant	50-60% mortality	Columbia	Landazabal *et al.*, 1973
Rice stem borer	*N. carpocapsae*	rice stubble	spray	100% mortality	Japan	Torii, 1975
Navel orangeworm *Paramyelois transitella*	*N. carpocapsae*	Almond grove	46-1,500 nemas per almond	24-100% mortality	California	Lindegren *et al.*, 1981

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BIOLOGICAL CONTROL OF WEEDS WITH PLANT PATHOGENS - STATUS AND PROSPECTS

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ABSTRACT

Increasing interest has recently been shown in using plant pathogens as biological control agents for weeds. The several studies in this field can be considered under two headings : i) the classical method - where the target is an introduced plant species and a search is made in the native habitat of the weed, to locate any host specific pathogen which might control it; ii) The bioherbicidal method - where both the host weed and the pathogen are endemic but the pathogen for one reason or another is not able to control it at the level required. In such a situation pathogens may be mass produced and applied as a herbicide. A brief account of current projects of both types is given.

INTRODUCTION

In recent years particular attention has been paid to the use of plant pathogens for the biological control of weeds (Cullen et al., 1973, Freeman et al.,1977, Hasan,1974a, 1974b, 1979, 1980, Oehrens & Gonzalez,1974, 1977, Templeton et al.,1979, Zettler & Freeman,1972).

There are two major ways plant pathogens can be used for the biological control of weeds : i) The classical method - where an introduced plant has become a weed in the absence of its associated natural enemies. A search is then made for effective pathogens in the native area of the weed or elsewhere where the weed occurs and, if suitable, the pathogens are introduced in the target area; ii) The bioherbicidal method - where the plant pathogens occur on weeds in their native ranges but in the absence of a suitable combination of factors they are not able to build up sufficient populations to depress their host's population to the required level. In such a situation more virulent strains of the known pathogens are searched for, or the level of infection is increased by applying them in larger amounts or at more suitable times, in a manner similar to chemical herbicides. A brief account of these methods along with current projects are discussed in this paper.

CLASSICAL METHOD

As success of control depends on the ability of the pathogen to perpetuate and disperse in the introduced country, special care is taken to select agents which are strongly pathogenic to the plant and are ecoclimatically suited to the target area. Also pathogens must be host specific to the weed species (or to its close relatives if they have no economic or other value). A brief account of the proceedings for these steps is as follows :

1. Selection of pathogens

All types of plant pathogens including viruses, bacteria and fungi could be used as biological control agents for weeds. So far fungi have received most attention, probably because they have a well defined

taxonomy, in many cases their host specialization is well known, and more is known of the mechanism and stability of their specialization. Also fungal pathogens are easier to handle than bacteria and viruses, they spread independently and can be introduced into new environments without considering possible vectors. However, it is important to select pathogens which are absent from the target region or are only represented by host specialized races which are ineffective in reducing their host's population. Races of pathogens which severely attack the target weed are therefore searched for and selected for further detailed study.

2. Climatic requirements

Like many other biological organisms plant pathogens are dependent on climatic factors. Thus spores of most fungal pathogens need the presence of free water on the host for germination whilst a few others require certain level of atmospheric humidity for infection of the host plant. Assessment is therefore made in the native environments of a candidate pathogen concerning its virulence, effectiveness and dispersal in the climatic conditions analogous to those existing in the proposed area of introduction.

3. Host specificity

This is one of the most important aspects in selecting agents for the biological control of weeds. An introduced weed species may have economically or otherwise important relatives, some of them even native to the target area. It is therefore essential to determine if the selected pathogen is likely to cause damage to plants other than the host weed. The host range of the pathogen is studied by exposing it to a wide range of important plants under optimum conditions of inoculation and incubation. However, it is clearly impossible to test each and every plant species so methods have been developed to select representative species. One of the most appropriate methods of selecting plants for testing is based on phylogenetic relationships to the target weed (Wapshere, 1974). The centrifugal phylogenetic testing method gives priority to close relatives, both wild and cultivated, of the target weed, i.e. other members of the same genus or same family as the weed. Other plants selected for testing are crops which belong to families phylogenetically related to the family of the target weed.

When determining host specificity of a fungal pathogen a visual examination of inoculated test plants is made to assess the external symptoms produced by the pathogen. Detailed microscopic observations have also to be made to determine if the plant is immune or resistant to the pathogen. In the latter case there is some development of a mycelium within the host tissue and this can vary according to the environment or physiological condition of the plant. Microscopic examination of cleared and stained leaves or stem sections showing spore germination, germ tube penetration and further development of the mycelium leads to more accurate conclusions concerning the host specificity of the fungal pathogens (Hasan, 1981a).

Most of the steps described above have been taken in several successful programs using plant pathogens in the biological control of weeds.

i. Skeleton weed (*Chondrilla juncea*) (Compositae) is an important pest of wheat in south-east Australia (McVean, 1966). The weed is of Mediterranean

and Middle-Eastern origin and has now spread to North and South America and Australia. The plant is not a problem weed in the Mediterranean area, to a great extent due to the presence of natural enemies (Wapshere et al., 1974). *C. juncea* is an apomict and in Australia it exists in three morphological forms which can be differentiated by leaf shape (forms A, B and C; Hull & Groves, 1973), known as narrow-, intermediate- and broad-leaf forms. The plant multiplies by both seeds and rosettes regenerating from a vertical rhizome which can be as deep as 4 m.

Investigations were carried out in the Mediterranean region to discover and assess biological control agents of skeleton weed. Among these the rust fungus, *Puccinia chondrillina*, was found to be the most damaging (Wapshere, 1970, Hasan, 1973, Hasan et al., 1973). This is a macrocyclic and autoecious rust which attacks all stages and all aerial parts of the plant. It remains active throughout the year and in the Mediterranean region it multiplies by the urediniospores only. On the whole the rust attack destroys the infected plants or reduces their reproductive capacity (Hasan & Wapshere, 1973).

The rust is important in the reduction of the skeleton weed populations in the Mediterranean region in situations similar to those of Australia. Thus observations, made at sites in climatic conditions analogous to those existing in Australia where skeleton weed is important, showed that there was a correlation between the increase in the rust infection and the reduction in the plant density. Also, infection by *P. chondrillina* reduced seedling survival to half or a third that of healthy plants (Hasan & Wapshere, 1973).

Several strains of *P. chondrillina* were collected during surveys in Europe and a strain collected at Vieste (S. Italy) was found to be most virulent to the narrow-leaf form (the most common form) of Australian skeleton weed. The host specificity of this strain of the rust was tested by inoculation of many cultivated and wild plants including a small group closely related to *Chondrilla*. Fifty-six species belonging to 30 families were found to be immune to the rust (Hasan, 1972).

From these studies it was evident that *P. chondrillina* possessed biological control potential and the strain from Vieste was introduced into Australia (Hasan, 1974b). Shortly after its release the rust became established, and widespread in the skeleton weed infestations, and steadily reduced the population of the narrow-leaf form of this weed. As this form of *C. juncea* began to disappear, the other two forms became more widespread, in places, replacing the narrow-leaf form (Burden et al., 1981). It therefore became necessary to discover strains of the rust affecting the other two weed forms. Further surveys in Europe and the Middle East discovered a few more strains virulent to the unaffected forms. Two more strains of the rust infecting the intermediate-leaf form of Australian *Chondrilla* have very recently been introduced into Australia, after confirmation of host specificity, and further searches are underway to discover a virulent strain of rust for the broad-leaf form of skeleton weed.

ii) Several wild blackberries (*Rubus fruticosus* agg.) of European origin have become serious weeds in Chile, Australia and other parts of the world. Their excessive growth forms impenetrable thickets in wastelands, forests, national parks, riverbanks, road sides and agricultural land.

The rust fungus, *Phragmidium violaceum*, has been found to be highly damaging to the blackberries in Europe (Oehrens & Gonzalez, 1974). It is a macrocyclic and autoecious rust multiplying mainly by urediniospores throughout the summer. Rust attack causes premature defoliation of blackberry and an important reduction in the vigour of the canes. It also reduces the height of the clumps and seed production.

P. violaceum has recently been introduced in Chile to control the two common weedy blackberries, *R. constrictus* and *R. ulmifolius*. *R. constrictus* was severely attacked by the rust and soon an important reduction in infestations was observed in several parts of Chile (Oehrens & Gonzalez, 1977). The attack was less damaging on *R. ulmifolius*, but no plants other than the two blackberries have been found to be infected by the rust fungus.

Encouraged by this success, a program of research is being conducted by Australia to consider the introduction of *P. violaceum* for the biological control of European blackberries, which are serious weeds in the south-eastern parts of the country. Studies have recently been completed, in Europe, on the host range of the fungus, in relation to cultivated and wild *Rubus* species and other members of Rosaceae existing in Australia (Bruzzese & Hasan, unpubl.). Recommendations will soon be made to the Australian Health Department for introduction of the rust into Australia.

iii) *Ageratina (Eupatorium) riparia* (Compositae), commonly known as hamakua pamakani, is an important weed of range and pastures in Hawaii. This plant of Mexican origin has no forage value. Recently, a leaf spot fungus *Cercosporella ageratinae* from Jamaica has been introduced into Hawaii for the biological control of *A. riparia* (Trujillo, 1976). The pathogen caused serious damage to the weed and, when exposed to more than 40 plants representing 29 families, was found to restrict its attack to pamakani weed (E.E. Trujillo, pers. comm.).

Among other projects, using the classical method of biological control, which have not yet reached maturity can be cited the possible use of the rust, *Uredo eichorniae*, from South America for the control of waterhyacinth in the U.S.A. (Charudattan et al., 1977).

Several pathogens are currently under investigation in Europe for their eventual introduction into Australia for the biological control of weeds of European origin (Hasan, 1979; 1981b). These include *Puccinia barbeyi* against onion weed (*Asphodelus fistulosus*), *Uromyces heliotropii* and *Cercospora* spp. against heliotrope (*Heliotropium europaeum*), and *Peronospora rumicis* and *Cercospora tripolitana* for the biological control of spiny emex (*Emex spinosa*) and doublegee (*Emex australis*).

BIOHERBICIDAL METHOD

Any plant pathogen causing disease in a weed can be used as a bioherbicide. Fungal pathogens used in this manner are termed mycoherbicides. Such fungi have to be applied frequently, so it is necessary to multiply them on artificial media to mass produce the most infective stage. Among other fungi, those Deuteromycetes (Fungi Imperfecti) which sporulate well on artificial media will be most suitable for this use. The pathogen has to be both highly virulent and host specific since it will be applied at high levels.

The safety of the pathogen is tested not only for host specificity against wild and crop plants related to the weed but also against animal species which live in the same area as the target weed and other plants growing in that region. As the pathogen has to be highly virulent a search is made for one or more strains most virulent to the local ecotypes of host weed.

The following are four major examples where fungal pathogens have been used as mycoherbicides :

i) Northern jointvetch (*Aeschynomene virginica*) is an important leguminous weed in Arkansas, USA, in rice fields, canals and waste areas and to a lesser extent in soybean fields (Smith & Shaw, 1968). The weed is competitive with rice and reduces its yield and quality. It is very probably native to the USA.

The fungal pathogen *Colletotrichum gloeosporioides* f. sp. *aeschynomene* causing the anthracnose of northern jointvetch has been isolated by Daniel et al. (1973). Under natural conditions this endemic disease occurs on all stages of the host from seedlings to fully grown plants but the level of infection does not seem to be sufficiently high to destroy plants or reduce vigour or seed production. During host specificity tests the fungus infected only Indian jointvetch (*A. indica*) whereas the other 165 crop and native plants tested remained unattacked.

In field trials to determine effectiveness of the fungus, spores produced on artificial medium were sprayed (concentration, 2 million spores/ml water; rate 94 l/h) on weed infested rice crops early and mid-way through the season. Ninety-nine percent of *A. virginica* plants were killed (Daniel et al., 1973). In other experiments it has been shown that *C. gloeosporoides* f. sp. *aeschynomene* is not infective or toxic to laboratory animals (Beaseley et al., 1975). The fungus is now being produced commercially for the control of northern jointvetch in rice fields (Templeton et al., 1977).

ii) Waterhyacinth (*Eichhornia crassipes*) is a serious problem in waterways in southern USA and other tropical and subtropical areas of the world. Recently a disease of this weed caused by the native *Cercospora rodmanii* has been discovered in Florida, USA (Conway, 1976). The fungus is responsible for the widespread decline of waterhyacinth in natural populations. It is cultured on artificial medium and when water suspensions of conidia and mycelium are sprayed on waterhyacinth plants in lakes, it causes considerable damage to the weed, demonstrating its effectiveness as a mycoherbicide. The specificity of the fungus to waterhyacinth was shown by exposing *C. rodmanii* to 80 varieties of economically and ecologically important plants phylogenetically related to *E. crassipes*. Commercial production of the pathogen as a biological control agent of waterhyacinth has now been patented and evaluation of the product is underway (Conway et al., 1978).

iii) Milkweed vine (*Morrenia odorata*) is a serious vine pest in the citrus groves of Florida. Ridings et al. (1977) have demonstrated that a strain of *Phytophthora citrophthora* isolated from diseased vines could be a successful mycoherbicide for control of milkweed. In field trials more than 80% of vines were killed when a water suspension of the fungus was

applied at the rate of 6.3 x 10^7 chlamydospores per acre. The authors also tested 58 representatives of 12 families by pre-emergence, post-emergence and foliage inoculation tests. Some plants from six of the families tested were found to be susceptible. However, the degree of susceptibility was low, and the rate necessary for infection was much higher than that required for milkweed vine.

iv) Spurred anoda (*Anoda cristata*) (Malvaceae) is a serious pest of cotton in various parts of the United States. An isolate of the fungus *Alternaria macrospora* causes considerable damage to its host. Young seedlings are particularly affected, and *A. macrospora* is considered to be a successful mycoherbicide (Walker & Sciumbato, 1979). During host range testing in greenhouses and growth chambers, the isolate produced negligible damage to other malvaceous plants including cotton, and spurred anoda was the most susceptible species tested. In field trials, foliar application of a spore suspension of *A. macrospora* with 5 x 10^5 spores/ml killed 75% of *A. cristata* young seedlings. The fungus was also effective when applied to the soil as a granular formulation of vermiculite, spores and mycelium (Walker, 1980).

Two other attempts have been made using fungal pathogens as mycoherbicides : Boyette & Templeton (1981) have successfully controlled Texas gourd (*Cucurbita texana*) in sandy loam soybean fields of south-western Arkansas by applying a strain of *Fusarium solani* and French & Schroeder (1969) have effectively used the oakwilt fungus, *Ceratocystis fagacearum*, as a selective sylvicide to eradicate undesirable oak trees in the forest of Minnesota.

DISCUSSION

These two approaches in the use of plant pathogens for biological control of weeds are now well defined and have been demonstrated by well established examples. The success achieved in the use of *P. chondrillina* as a biological control agent of *C. juncea* has been remarkable and has opened the way for further research using the classical method in microbiological control of weeds. This rust fungus quickly became established after only one field release, and damaged skeleton weed severely. Similarly, though no systematic evaluation was made after introduction of *P. violaceum* in Chile against blackberries, it seems that the fungus has become widespread in a short time and is found to attack *R. constrictus* populations throughout the country (Oehrens, pers. comm.).

The use of plant pathogens as bioherbicides is another important technique which can be used for both exotic and indigenous weeds. However, exotic weeds do not always have pathogens which have either followed them or have become adapted to them as a part of local flora. On the other hand, there is a better chance of finding a pathogen on endemic weed which lives in equilibrium with the pathogen, which requires the additional stress exerted by artificially increasing the pathogen to reduce its populations to acceptable levels. The application of *C. gloeosporioides* f. sp. *aeschynomene*, *C. rodmanii*, *P. citrophthora* and *A. macrospora* as mycoherbicides against northern jointvetch, waterhyacinth, milkweed vine and spurred anoda, respectively, are some of the most successful examples of the bioherbicidal technique.

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PROSPECTS OF BIOLOGICAL CONTROL OF PLANT PATHOGENS WITH FLUORESCENT *PSEUDOMONAS* SPP.

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ABSTRACT

Recently, bacteria of the *Pseudomonas fluorescens-putida* group have been detected which, after application to seed and seed tubers, promote plant growth and increase yields by displacement of deleterious elements of the natural root microflora. Long term rotational field experiments have demonstrated that such unidentified pathogenic elements of the root microflora are responsible for crop losses up to 30% in potato and other crops. The losses increase with high cropping frequency so that the maximum yield potential of plants in most agricultural systems is far from being realized. Application of the growth promoting bacteria, which aggressively colonize plant roots, seems to be especially successful in high frequency crop rotations. The mechanism of plant growth promotion and the prospects of biotechnical exploitation of these bacteria are discussed.

INTRODUCTION

The biotechnical exploitation of microbial antagonists to protect plants against pathogenic micro-organisms is successful in only relatively few cases. Failure in the application of microbial antagonists to control plant pathogens is generally ascribed to the difficulty in introducing the antagonist into a substrate which is usually already occupied by the natural microflora. This holds especially for the introduction of antagonists in soil and on the root surface to control soil-borne pathogens.

Recently this view has been altered with the detection of bacteria of the *Pseudomonas fluorescens-putida* group, which aggressively colonize plant roots and displace elements of the natural root microflora (Kloepper et al. 1980, Suslov and Schroth 1982). This paper is restricted to the discussion of the prospects of biological control using this promising group of bacteria.

Application to seed and seed tuber pieces of these bacteria, selected for their antagonism in vitro towards a variety of pathogenic and saprophytic root colonizing bacteria and fungi, has demonstrated consistent increases in plant growth and in yield of potato up to 33%, of sugar beet up to 50% and of radish ranging from 60 - 144% in field trials (Burr et al. 1978, Kloepper and Schroth 1978, Suslov and Schroth 1982).

MECHANISM OF PLANT GROWTH PROMOTION

The mechanism of this plant growth promotion has been demonstrated to be the displacement of deleterious components of the root microflora (Suslov and Schroth 1982). Growth promotion could not be demonstrated under gnotobiotic conditions. It is therefore unlikely that it is caused by growth promoting metabolites (Kloepper and Schroth 1981). Mutants of these plant growth promoting rhizobacteria (PGPR) which have lost their antagonism in vitro, also lost their growth promoting character but colonize the roots equally well as the wild type (Kloepper and Schroth 1981,

Kloepper and Schroth 1981). This means that their aggressive root colonization is not due to their special antagonistic properties, but must be based on some special bond to the plant root surface.

The aggressive root colonization of the PGPR has been studied with mutants resistant to the antibiotics rifampicine and naladixic acid (Kloepper and Schroth 1981, Suslov and Schroth 1982). Population densities on roots up to 10^5 colony forming units (cfu)/cm have been demonstrated 2 weeks after plant emergence following application on sugar beet seed (Suslov and Schroth 1982) and potato seed tuber (Kloepper and Schroth 1981) and averaged 10^3 cfu/cm throughout the season. Similar results were obtained with wheat by Weller and Cook (Weller and Cook 1983) in Washington State and by Geels and Schippers (unpublished data) in cooperation with the Research Station for Arable Farming and Field Production of Vegetables (PAGV) at Lelystad, in the Netherlands.

Suslov and Schroth (Suslov and Schroth 1982) have demonstrated that the PGPR reduce the number of fungi and G+ bacteria on the root surface of potato and sugar beet while no such effects could be obtained with mutants which lost their antagonistic ability. Several isolates of these microorganisms caused reduced seed germination, root malformation, root lesions, reduced elongation and increased root infection by fungi. Their numbers were reduced by PGPR on sugar beet roots in greenhouse tests. Schroth and Hancock (Schroth and Hancock 1982) conclude that these deleterious rhizobacteria are apparently toxigenic because they do not invade the root tissue and suggest that they represent an important group of bacterial pathogens which has been overlooked.

In detailed long term rotational experiments at the Experimental Farm 'De Schreef' (The Limit) and at the PAGV at Lelystad, accumulated evidence shows that such unidentified pathogenic elements of the root microflora, other than those causing major diseases, are responsible for crop losses up to 30% in potato and other crops. These losses increase with increasing cropping frequency (Hoekstra 1981, Lamers 1981, Schippers et al. in press). These experiments show that the maximum yield potential of plants in most agricultural practices is far from being realized because of these yet unidentified deleterious root microorganisms. Growth promotion by seed tuber bacterization with PGPR isolated and selected at the Phytopathological Laboratory 'Willie Commelin Scholten' in Baarn was especially obtained in narrow rotation soil (Geels and Schippers 1983b, Schippers et al. in press). Considerable increases in yield of glasshouse grown radish, resulting from seed treatments with these PGPR that were isolated from potato and wheat, also particularly occurred in soils with a history of high cropping frequency (Geels and Schippers, unpublished data). PGPR seem to have the ability to increase plant yields by protecting roots from deleterious root microorganisms that apparently increase with high cropping frequency.

MECHANISM OF ANTAGONISM

The main mechanism of antagonism in relation to plant growth promotion seems to be iron deprivation by Fe^{3+}-complexing chelates called siderophores that are only synthesized in Fe^{3+}-limited conditions as normally exist in soil (Kloepper et al. 1980). The antagonism in vitro by PGPR does not occur or occurs much less when the Fe^{3+}-deficient agar medium is enriched with 1 µg $FeCl_3$ (Geels and Schippers 1983a, Kloepper et al. 1980). If the antagonism in vitro does not or only partly disappears, then apparently antibiotics other than siderophores are produced. Many of these

probably belong to N-containing heterocycles (phenazines and pyrrolnitrins) which are secondary metabolites produced by many fluorescent pseudomonads (Leisinger and Margraff 1979). Plant growth promotion does not take place if the soil is amended with $Fe^{3+}[EDTA^{-}]$. The root colonization by the PGPR, however, is not restricted by this amendment. Addition to soil of pseudobactin, the purified siderophore of the American PGPR isolate B10, also increased plant growth but ferric-pseudobactin did not (Kloepper et al. 1980). These results indicate that siderophores produced by PGPR confer an advantage to these bacteria in competing for iron. Other microorganisms in the rhizosphere that produce less siderophores or siderophores with less affinity for Fe^{3+}, cannot obtain sufficient iron for their growth.

The crystal and molecular structure of the fluorescent siderophore ferric-pseudobactin in isolate B10 was identified by Teintze and co-workers (Teintze et al. 1980) and shown to consist of a linear hexapeptide of both L- and D-aminoacids linked to a fluophoric quinoline derivative. The iron chelating groups, a hydroxamate group, a hydroxy acid and an O-dihydroxy aromatic group distinguishes this siderophore from previously described siderophores (Teintze et al. 1980). The unusual alteration of L- and D-aminoacids in the pseudobactin sequence is supposed to be a characteristic that protects pseudobactin against microbial degradation (Schroth and Hancock 1982). A variety of differently structured siderophores seems to be formed within the fluorescent pseudomonad group which differ greatly in their affinity to Fe^{3+} and in their persistence in natural environments.

Recently, evidence was presented that siderophore producing pseudomonads also have a key function in disease suppressiveness that develops in soil against take all of wheat caused by *Gaeumannomyces graminis* var. *tritici* after continuous cropping of wheat, and in certain soils suppressive against flax wilt caused by *Fusarium oxysporum* f. sp *lini* (Kloepper et al. 1980). Disease suppressiveness of these soils could be eliminated by the addition of $Fe^{3+}[EDTA^{-}]$, while disease conducive soils could be turned into suppressive soils by treatment of seeds with PGPR B10 and by the addition to soil of pseudobactin, the purified siderophore produced by B10.

Other isolates of fluorescent pseudomonads were obtained from take all suppressive soils by Weller and Cook (Weller and Cook 1983) and by Geels and Schippers (unpublished data). When applied to seeds they significantly suppress take all in winter and summer wheat and increase yields in the field. Thus, fluorescent pseudomonads also have the potential to successfully control major diseases caused by well-known pathogens. This is also supported by the prevention of the development of Dutch elm disease obtained by injecting fluorescent pseudomonads in the vascular system of elms prior to their inoculation with the fungal pathogen *Ophiostoma ulmi*. These results were obtained in small scale field experiments with *Ps. syringae* (Strobel and Myers 1982) and with *Pseudomonas* isolates from potato periderm (Scheffer 1983) which had demonstrated growth promotion in potato and radish (Geels and Schippers 1983a, Geels and Schippers 1983b). Large scale field experiments involving over 15000 elms spread over The Netherlands were started in 1982 in a cooperation between the Phytopathological Laboratorium 'Willie Commelin Scholten' at Baarn, the Plant Protection Service (PD) and the State Forestry Service.

PROSPECTS

Experiences over the last 5 years have convincingly demonstrated that biotechnical manipulation of bacteria within the group of fluorescent pseudomonads has great potential for controlling plant health and for a considerable increase in plant production. This seems to be largely based on the suppression of deleterious rhizobacteria, the numbers of which apparently increase with high cropping frequency (Geels and Schippers 1983b, Hoekstra 1981, Lamers 1981, Schippers et al in press). This group of pathogens, responsible for yield depressions up to 30%, has been largely ignored by plant pathologists.

The stimulated plant growth and increase in yield has to be ascribed to increased root health and the consequential improved exploitation of soil minerals, added fertilizers and soil water.

Key factors in the biological control of the root pathogens are the aggressive colonization of plant roots and the production of siderophores with a high affinity to Fe^{3+} by the effective isolates of pseudomonads.

The soil water content and the soil water potential will certainly influence the effectiveness of the application to seed and seed tubers of effective isolates of pseudomonads.

The genetic and molecular characters that govern the aggressive colonization, the production of siderophores and the ability for optimal functioning or survival under varying environmental conditions are not yet understood.

Cooperative research between plant pathologists, microbial geneticists, microbiologists, molecular biologists, chemists, soil physicists and agronomists is essential to fully explore the potential of certain fluorescent pseudomonads as biocontrol agents.

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USEFULNESS OF ACERIA DRABAE IN BIOLOGICAL CONTROL OF HOARY CRESS

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Background and objectives

Hoary cress, Cardaria draba(L.)Desv.(Cruciferae) is considered to be one of the 50 most important weeds in the world. Being of Mediterranean origin, it became naturalized in the Palaearctic and Nearctic regions, growing in cultivated fields, grainfields, grasslands, waste places, meadows and roadsides. It is a perennial, reproducing by seeds and by horizontal creeping roots, a feature which makes it difficult to control.

In an extensive study on arthropods associated with cruciferous weeds, we have found on hoary cress 102 insect and mite species in Poland. Among them is an eriophyid mite, Aceria drabae(Nalepa) attacking flowers and causing sterility. Its possible use in biological control of the hoary cress was investigated.

Materials and Methods

The survey was conducted throughout Poland to find new locality records of A. drabae in addition to the one close to Kutno found in 1974. Observations on the population dynamics of A. drabae in a natural stand of C. draba were continued. Attempts to establish A. drabae in two other stands of C. draba were made. On microplots and in natural biotopes, observations and tests were carried out on the possibility that A. drabae is able to attack other cruciferous plants.

Results and conclusions

Since its discovery in 1974, A. drabae has persisted in the C. draba stand close to Kutno. No other additional natural locality records were found. However, the mite has been successfully established in two natural C. draba stands close to Poznań which indicates that it can be easily introduced into new areas.

At the original site, A. drabae causes permanent sterility of about 95% of C. draba plants. This stand consists of about 1,000,000 plants and does not show any increase in number as seed production is almost completely suppressed by A. drabae. The same situation was created in those stands of C. draba where A. drabae was introduced. Although A. drabae prevents increase in numbers of C. draba, it cannot destroy the host population as its ability to reproduce by horizontal creeping roots compensates for the destruction of seeds.

Observations conducted in natural biotopes and tests conducted in greenhouses proved that A. drabae infests only C. draba. The following cruciferous cultivated plants and weeds were not attacked: Armoracia lapathifolia Gilib., Berteroa incana L., Bunias erucago L., Brassica napus var. napus L., Capsella bursa-pastoris(L.), Diplotaxis tenuifolia Juslen, Erysimum cheiranthoides L., Lepidium latifolium L., Raphanus sativus var. radicula Pers., Sisymbrium austriacum Yacq. and Sisymbrium loeselii L. Further tests on the host specificity of this mite are being conducted.

Results of observations and experiments indicate that A. drabae is a monophagous mite with a clear potential for use in biological control of hoary cress. By eliminating the seed production by C. draba, it greatly limits its reproductive capacity while soil cultivation prevents reproduction by creeping roots.

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5A—R2

POTENTIAL FOR MYCOHERBICIDES IN AUSTRALIA

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Introduction

Biological control of weeds using imported fungal pathogens has already been demonstrated in Australia (Cullen et al. 1973). However there is some risk associated with this strategy. Puccinia xanthii, unintentionally introduced from the U.S.A. has been found on two sunflower cultivars, the garden marigold and Calendula officinalis, in Australia, although it is restricted to two weed genera Xanthium and Ambrosia in the U.S.A. Indigenous fungal pathogens with specificity to a weed species could be adapted for use in Australia as they have been in the U.S.A.

Advantages and limitations

Advantages include: no damage to non-target species; no environmental contamination; reduced spillover effects cf. classical biological control (i.e. technique is species and location specific).

Limitations include: specificity being undesirable from a commercial standpoint (Bowers, 1982); greater dependence on ambient environmental conditions than other control techniques.

Strategies for using mycoherbicides

There appear to be three main strategies for the use of mycoherbicides: (1) Use of high concentration (cf. naturally occurring) of inoculum (inundative strategy); (2) Application of inoculum at different times in the season and/or weed growth stage than normally occurs in the field; (3) combination of (1) and (2). In addition mycoherbicides could be combined with other biotic and chemical agents.

Criteria for use of mycoherbicides

The costs of applying mycoherbicides appear to be similar to those incurred using conventional herbicides. Thus to justify the cost of developing mycoherbicides one or more of the following criteria should be satisfied:
Weeds: (1) resistant to herbicides; (2) occur in situations where herbicides cannot be safely used; (3) occur in high value crop; (4) occur in several situations which preclude recommendation of a specific herbicide treatment.
Fungi: (1) readily grown in culture; (2) produce an abundance of propagules; (3) be host specific; (4) show high infectivity.

Potential candidates for mycoherbicides

A number of exotic weeds in Australia are attacked by native and exotic fungi but there have been few detailed studies of these interactions. Colletotrichum xanthii has been recorded on Xanthium weed species, causing anthracnose and seedling blight on Xanthium spinosum (Butler, 1951). C. xanthii satisfies the criteria for a fungus (above) and Xanthium spp. are widespread and important weeds in Australia. However the location of the weeds in extensive agricultural and rangeland situations may limit their potential for control by mycoherbicides on economic grounds.

Paspalum dilatatum and P. urvellei, two persistent weeds which do not respond well to herbicide treatments, occur in high value horticultural crops in humid coastal areas. Scolecosporiella spraguei, a leaf blighting fungus may have some potential for mycoherbicide development.

There are also two weed species in Australia, Echium plantagineum and Rubus fruticosus agg., for which classical biological control agents have been obtained but the importation of the agents is still awaiting approval. Both species are attacked by fungi which occur in Australia. These fungi could be investigated for mycoherbicide potential, should importation of the exotic agents be prevented, or, they could be used in an augmentative role should the introduction be of limited success.

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CONCERN FOR U.S. NATIVE PLANTS AFFECTS BIOLOGICAL CONTROL OF FIELD BINDWEED

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Background and objectives

There is increased concern that arthropods introduced into North America for weed control may damage non-target native plant species. This concern has led to the inclusion of U.S. native plants in preintroduction host specificity studies. For example, biological control candidates for field bindweed, Convolvulus arvensis, must be tested against North American Calystegia spp., which are close relatives of the target weed and minor members of some important plant communities in the western U.S. (Andres 1980). During 1982, the survival and development of larvae of Tyta luctuosa (Lepidoptera: Noctuidae) (Det. R.W. Poole, Systematic Entomology Laboratory, United States Department of Agriculture) was determined on 11 U.S. Calystegia spp. in Rome. The ability of an eriophyid mite (tentatively identified as Aceria sp. by Nuzzaci) to attack and gall the leaves and buds of Calystegia spp. was also investigated. Rosenthal & Buckingham (1982) felt that both T. luctuosa and Eriophyes (Aceria) spp. would be valuable biological control agents for field bindweed.

Materials and Methods

T. luctuosa larvae used in the laboratory tests were the progeny of adults collected in Rome. Neonate larvae were transferred to containers (2/container) and supplied with bouquets of freshly excised foliage from potted test plants grown out-of-doors. Larval mortality was recorded when the bouquets were changed three or four times/week. A varying number of larvae (10 to 111) were tested on each plant species.

Aceria sp. was collected from a site near Rome. C. arvensis stems bearing heavily galled leaves were collected just a few hours before the outdoor tests were begun. Stems with 5-10 galled leaves were attached to healthy, rapidly-growing test plants with strands of thin wire. Three to 13 plants/species were exposed to galls in this manner. These potted plants were randomly arranged on a shaded porch; 30-40 cm apart. Infested plants were inspected weekly for 3 weeks or more. After 3 weeks some foliage from each plant was examined microscopically to determine the presence of living mites, spermatophores, and eggs.

Results and conclusions

A total of 90.9% of the T. luctuosa larvae completed development and pupated on the control plant (C. arvensis). Less than 34% of the larvae completed development on Calystegia stebbinsii (rare U.S. native), C. fulcrata, and C. polymorpha. Pupae were formed by 56.3% to 90% of the larvae on C. macrostegia, C. purpurata, C. subacaulis, C. occidentalis, C. longipes, and C. malacophylla. Larvae failed to develop on C. atriplicifolia and C. collina. Aceria sp. caused severe leaf distortion on the control and on 5 Calystegia spp. (C. macrostegia, C. purpurata, C. occidentalis, C. stebbinsii, C. longipes). Galls were formed to a lesser extent on C. fulcrata, C. polymorpha, and C. subacaulis. Galls were not formed on C. collina and C. malacophylla. Four months after the study was started mites were recovered from bud and leaf galls on field bindweed, C. longipes, C. stebbinsii, and C. macrostegia.

Thus we conclude that Italian populations of these two candidates are capable of damaging U.S. Calystegia spp. in the laboratory or when grown as potted plants. As both Calystegia spp. and C. arvensis occur along roadsides and in other disturbed habitats they are often found in close proximity. Unless Italian T. luctuosa and Aceria sp. can be shown unable to damage these Calystegia spp. in nature the risk of releasing them as biological control agents in North America is too great.

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5A—R4

EXOTIC PLANT PATHOGENS FOR BIOCONTROL OF MUSK THISTLE IN THE UNITED STATES

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Background and objectives

The situation regarding musk thistle (Carduus nutans) in the United States is representative for plant species which have become noxious after introduction without natural enemies. Musk thistle infesting ranges and pastures is not consumed by livestock because of its spiny nature. Presently, over 80,000 hectares are infested by C. nutans in the continental United States. Low economic returns from this land precludes use of conventional weed control strategies. Some reduction in musk thistle stands with the release of insects has been reported. Recently, a search for plant pathogens damaging to musk thistle has been initiated to complement insects. The evaluation of a rust of C. nutans (Puccinia carduorum) will illustrate the procedure followed in the United States for the introduction of plant pathogens for biological control of weeds.

Guidelines written by the Working Group on Biological Control of Weeds (WGBCW) provide the basic framework within which exotic organisms are introduced into the United States. The Plant Disease Research Laboratory (PDRL) is the only USDA facility designated by the federal government for plant pathogen quarantine and containment, and it is the only national laboratory where exotic plant pathogens can be evaluated for use in weed biocontrol.

Materials and Methods

Six isolates of P. carduorum collected in 1978 from Turkey, Bulgaria, and Romania were increased in containment at PDRL and stored until testing was initiated in 1981. Research proceeded with two objectives: (1) to determine the suitability of this rust in stressing its host, and (2) to determine the specificity and safety of this rust regarding other plant (and especially crop) species. Much of this information was obtained through the use of a host range study with two basic parts: (1) plant species related to the weed host, based on the centrifugal phyllogenetic testing method described by Wapshere, and (2) plant species of economic importance to U.S. agriculture. Additional environmental information was obtained by inoculating plants and incubating them over a range of temperatures in dew chambers. All research has been conducted in the containment greenhouse at PDRL.

Results and conclusions

Puccinia carduorum shows promise as a biocontrol agent of C. nutans since several isolates are aggressive on most (23 of 27) collections of C. nutans. Resistance in some collections may reflect the presence of Carduus taxons either not clearly separated from C. nutans or not properly documented in the U.S. Host range studies revealed that three additional members of the tribe Cardueae can be infected under greenhouse conditions. Of these three, Carduus tenuiflorus was not very susceptible, and infection of the other two, Cynara cardunculus and C.scolymus (artichoke), usually was limited to older leaf tissues. Symptoms on these species included very small pustules usually surrounded by a chlorotic or large necrotic halo. Numbers of pustules per unit leaf area were always much less on artichoke than on musk thistle inoculated with the same number of uredospores. None of ten other species in the tribe Cardueae, or 22 species representing the remaining 12 tribes of Compositae were susceptible. None of eight economically important U.S. crop species tested to date have been found susceptible.

Favorable moisture and temperature conditions in the United States and the general susceptibility of C. nutans indicate that P. carduorum can infect C. nutans and proliferate in North American stands of musk thistle. A better understanding of the stress of artichoke from infection by P. carduorum is required. Field studies now being conducted in Europe should yield valuable information on the effect of the rust on artichoke. When we are confident P. carduorum is suitable and safe for biocontrol, a proposal for field studies in the U.S. will be submitted to the WGBCW. If release of the pathogen is deemed appropriate, field studies in the U.S. will be required to provide more definitive information on the suitability of P. carduorum for controlling musk thistle.

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BIOLOGICAL CONTROL OF INSECT PESTS WITH INDIGENOUS NATURAL ENEMIES IN THE PEOPLE'S REPUBLIC OF CHINA

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Although introduction of exotic natural enemies in biological control of pests has been emphasized in many parts of the world, the use of indigenous species to regulate pest populations often received more attention in the People's Republic of China. Mass rearing and release of native parasites such as Trichogramma spp. Anastatus sp. and Dibrachys cavus to control agricultural pests have been successful and practiced on a considerable scale. However, mass production is feasible only in a very few beneficial species. Injurious insects are generally kept under control by a multitude of natural enemies in the field. Conservation of biocontrol agents is of upmost importance in a pest management scheme. In recent years more effort has been devoted to the manipulation of environment to encourage natural enemies. Some examples are discussed in this paper.

Rice hoppers(Nilaparvata lugens, Nephotettix spp.) have become increasingly devastating in recent years. The spiders Erigonidium graminicolum, Oedothorax insecticeps, Lycosa pseudoannulata, Pirata subpiraticus, and P. japonica were found to play a very important role in regulating the hopper populations. Population of spiders fluctuated greatly due to various cultural activities and often reduced to very low levels after insecticidal treatment, irrigation, and harvesting of the crop.

A conservation programme of spiders was initiated in Hunan, 1975 on a 4-acre rice field and has expanded to 500,000 acres by 1981 in this province alone. Some of the measures adopted to manipulate spider population consisted of (1) planting winter crops or provide hibernation quarters for the spiders; (2) transferring egg masses of spiders to newly planted rice crop; (3) using straw bundles to collect spiders when rice field is under irrigation; (4) digging protective refuges around rice field during harvest or planting soybean on borders of rice field, and (5) judicious use of pesticides.

The programme was primarily designed to conserve spider populations, but as a result many other natural enemies were also saved. In years of moderate rice hopper infestation the complex of natural enemies would keep rice hoppers in check and no chemical control was necessary.

Cotton aphid, Aphis gossypii, and cotton bollworm, Heliothis armigera, are the two main pests of cotton in northern China. Chemicals have been employed extensively against these pests since 1950. Control cotton aphid with insecticides early in the season was found to be devastating to the natural enemies of cotton aphid and often induced cotton bollworm outbreaks.

Wheat fields serve as reservoir for many entomophagous insects. Lady beetles, spiders, and lacewings, preying on aphids of the wheat crop, increasing in numbers, migrate later into the cotton fields and become important natural control agents of cotton aphid.

Interplanting or strip cropping cotton with wheat or rape has been found favorable to natural enemies. The rape, often infested with aphids and had a more rapid and vigorous growth than cotton at its early stage, attracted more natural enemies. When rape was cut and used as green manure, the predators migrated into the cotton rows and kept cotton aphid and the second brood bollworm at low levels.

When carbofuran was incorporated recently in the integrated control programme, cotton crop was found to be protected from aphid damage till the end of June with no additional spray.

The citrus red mite, Panonychus citri, is one of the key pests of citrus in southern China. In recent years 6-8 applications of pesticides have been used annually in combating this pest. Amblyseius newsami was found to be a potent predaceous mite of the citrus red mite along the coastal region of Guangdong province, where the maximum temperature in summer rarely exceed 35°C, and fog and dew are common. But in the more interior parts of this province summer temperature is high and fog and dew rare, a condition which is not favorable to the predaceous mite. By growing a wild plant, Ageratum conyzoides, in the citrus groves as a cover crop, the temperature above the canopy of the trees could be lowered from 40-45°C to 35°C and relative humidity increased. This plant also provides pollen as food for the predaceous mite, suppresses the growth of weeds and can be used as a green manure. In those groves where A. conyzoides was grown, the citrus red mite population was kept low by the predaceous mite.

5A—R6

THE ROLE OF POLYPHAGOUS PREDATORS IN THE CONTROL OF CEREAL APHIDS

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Background and objectives

Polyphagous predators may be important in controlling cereal aphid populations and many of these predators overwinter in hedgerows. Hedge removal could therefore have important repercussions on the frequency of cereal aphid outbreaks. In this study the information from overwintering surveys of polyphagous predators and their dispersal to cereal crops in the spring were used as a basis for a simulation study to investigate their effect on cereal aphid population growth.

Materials and Methods

Most of the potentially important polyphagous predators, Agonum dorsale, Demetrias atricapillus and Tachyporus spp., overwinter in hedgerows. Catches in pitfall traps, placed in cereal fields, showed that species such as A. dorsale moved into the crops in mid-May and were present 200 m away from the hedgerows by early June. An absolute measure of densities of polyphagous predators was obtained from surface searching in June using 0.25 m^2 quadrats.

A simulation model describing aphid population growth, crop development and predation has been developed. Logistic population growth of the two major cereal aphid species, Sitobion avenae and Metopolophium dirhodum, is temperature-dependent and for S. avenae is also dependent on the developmental stage of the crop. Crop development is calculated from a two-factor polynomial equation with day degrees, above a threshold of 6°C, as the independent variable. Predation is calculated from the observed numbers of predators and their predation rates at various temperatures and aphid densities.

Simulations were carried out using 1981 temperature data, starting with realistic aphid densities in the presence and absence of polyphagous predators. As it is unlikely that all predators will be found by surface searching the observed numbers were multiplied by two and four to estimate the sensitivity of the system to sampling accuracy. Immigration of aphids at a low and a high rate, within the range of observed results, was incorporated to study its influence on the effectiveness of polyphagous predators.

Results and discussion

Tachyporus adults were the most numerous group with densities of over 2 m^{-2} while A. dorsale and D. atricapillus were also present.

In the absence of predation the simulation model indicates that cereal aphids can reach outbreak levels even when initial densities are very low. In the presence of polyphagous predators the simulation model shows that the peak aphid population reached is sensitive to changes in predator density and the effects of aphid immigration. With a low aphid starting density and little immigration an outbreak can be prevented by the action of polyphagous predators alone.

Thus, the balance between predator density, initial aphid density and immigration is crucial in determining the likelihood of an outbreak occurring. In the event of a large immigration of aphids into cereal crops it is unlikely that polyphagous predators alone could prevent rapid aphid population development, although they may still play an important part in combination with other aphid-specific predators, parasitoids and fungal diseases. Hedgerows provide overwintering sites for polyphagous predators so their removal, in combination with other agricultural practices, such as increased herbicide and fungicide use, could increase the frequency of cereal aphid outbreaks.

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MASS RELEASE OF PREDATORS FOR THE POSSIBLE CONTROL OF MYZUS PERSICAE SULZER ON POTATOES

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Background and objectives

In natural aphid populations at Adelaide and Milang, South Australia, aphid predators have little impact upon huge increase in aphid numbers which occur each autumn. However, the results of a predator-exclusion study (Hussein, 1982) showed that the hemerobiid predator, Micromus tasmaniae may possibly be used as a biological control agent to control M. persicae on potatoes in autumn. Elsewhere, the hemerobiids have been shown to have great potential for biological control and have been suggested for controlling early season aphid infestations when prey numbers are still low (Neuenschwander, 1975; Syrett and Penman, 1981; Neuenschwander and Hagen, 1980). This small-plot field experiment has been carried out to test the hypothesis that when large numbers of M. tasmaniae eggs are periodically released to the potato crops in late March to coincide with the period of migration of alate M. persicae, an early suppression of the developing initial aphid populations may be achieved, thus maintaining the aphid vector population at a very low level and preventing the incidence and spread of potato leaf roll virus infection.

Materials and methods

Certificate potato seeds were planted on Feb. 10, 1981 following the commercial planting requirements. Plots were arranged 2 x 3 completely randomized design with two treatments, i.e. 1) Sprayed with eggs, and 2) Control. Each treatment was replicated three times. A 5 m alley of bare ground was maintained between columns and rows of plots. The plots were furrow irrigated one day prior to spraying and subsequent irrigation was made when necessary. The first spraying of eggs was made when the first alate aphid was found in a yellow-pan water trap placed in the center of the plots. The initial 900-1000 eggs sprayed to each treatment plot was based on the expected final density of 3 eggs per plant (Hussein, 1982). Eggs were sprayed using a specially designed compressed-air sprayer equipped with a hollow cone-type nozzle at 2.06 kg/cm^2 pressure. Spraying was repeated twice weekly for a period of 4½ weeks using the same rate.

Thirty leaves randomly sampled weekly from each replicate in both treatment and control plots. Total number of aphids and their predators were counted directly. Yield of fresh tubers was also taken from both treatment and control plots.

Results and conclusions

There were obvious differences in the aphid numbers in the treated as opposed to the control plants. The peak number of aphids in the sprayed plots was reduced by 70% ($P > .05$). Counts of M. tasmaniae eggs verified the increased in the eggs and larval populations in the sprayed plots. An overall reduction of aphid population due to predator's activity clearly demonstrated the potential of periodic releases of M. tasmaniae for vector control. The releases also increased the yield of tubers by 38% ($P > .05$). However, practical application of periodic release of M. tasmaniae for bio-control of M. persicae in commercial plantings requires further research.

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5A—R8

MANIPULATION OF CEREAL APHID NATURAL ENEMIES

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Background and objectives

In autumn, cereal aphids colonise winter cereals sown in September and early October and in mild winters survive until spring. A preference for winter cereals at the expense of spring varieties and earlier sowing of winter wheat have provided more crops and sites suitable for aphid overwintering. Levels of parasitism as high as 35% have been recorded in Sitobion avenae populations in spring following mild winters in Britain. Further, both parasitoids and entomophthoraceous fungi occur in overwintering aphid populations on cereals in north-west France. Recent mild winters have allowed survival of overwintering aphids and subsequent summer infestations have been small. The role of the overwintering aphid populations in helping to prevent large summer infestations by providing a reservoir of parasitoids and pathogenic fungi and by attracting predators to cereal crops is being investigated.

Materials and Methods

In March and April 1982 the cereal and grass aphid, Metopolophium festucae, was released at a total density of 35 aphids/m^2 onto ryegrass which was either undersown or sown as strips in plots of winter wheat (48 m x 48 m). Before release the aphids were exposed to the parasitoid, Aphidius uzbekistanicus (1 female : 300-400 aphids), which is the principal species recorded in overwintering cereal aphid populations. The fungal pathogen, Erynia neoaphidis was distributed over the ryegrass areas, on two dates in May, by broadcasting pea aphids (Acyrthosiphon pisum) which had been killed by the fungus in the laboratory. The wheat was sown after mid-October and contained very few aphids before the releases.

Aphids were sampled weekly using an insect vacuum net and by counting those present on four groups of ten tillers in each plot. Parasitoids and fungal pathogens were monitored by collecting aphids and rearing them on leaf segments at 18°C for twelve days. Predators were monitored using pitfall traps and the vacuum net.

Results and conclusions

Populations of M. festucae and A. uzbekistanicus were larger in the undersown plots than in the control plots during May, indicating successful establishment. Other parasitoids were rarely caught in the experimental plots at this time. As M. festucae declined at the end of May S. avenae colonised the plots and began to increase. The S. avenae population remained lower in the undersown plots than in control plots throughout the summer, but peaked on the same dates in all treatments.

Of the predators, more staphylinid adults and staphylinid and carabid larvae were caught in both pitfall traps and suction net samples, in the undersown plots than in control plots. Slightly fewer adult carabids were caught in pitfall traps in undersown plots than in control plots but this may have been the result of reduced activity rather than reduced abundance. More spiders were caught in the suction net in the undersown plots than in control plots.

There were no significant differences between strip plots and control plots for any of the insects sampled, apart from M. festucae which were slightly more numerous in strip plots during May.

In this experiment cereal aphid parasitoids and fungal pathogens were manipulated by releasing them with a carrier population of M. festucae, whilst predators were manipulated by undersowing in the same plots. This resulted in a reduction in numbers of S. avenae in the treated plots. In 1983 the two types of manipulation are being used separately to elucidate the relative importance of each.

Cereal aphid populations overwintering anholocyclicly probably play a significant part in restricting the development of summer infestations by providing reservoirs of parasitoids and fungal pathogens, and by stimulating the build-up of predator populations.

BACILLUS THURINGIENSIS AS A BIOLOGICAL CONTROL AGENT VS. COTTON PESTS IN EGYPT

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Background and objectives

The increase in pesticidal application for the control of cotton pests in Egypt has necessitated the search for other safer control measures to protect cotton crop. In this concern, bacterial insecticides can be used with success, but their effectiveness must be made predictable and reproducible. The present work was carried out to provide information concerning the susceptibility of the major cotton pests, Spodoptera littoralis, Spodoptera exigua and Heliothis armigera to the endotoxins of the pathogen Bacillus thuringiensis. The possible use of various agricultural and industrial byproducts for reducing the production costs of these microbial insecticides, together with enhancing their potency by using feeding stimulants have been considered and were made use of in field application.

Materials and Methods

Cultures of Bacillus were grown for endotoxin production (Salama et al. 1981). The byproducts were used as protein source in a defined base medium(Salama et al. 1983). Extraction of favourable host plants of target insects was made with water and different solvents and the extract was incorporated with Bacillus in the insect diet for bioassay. The assay procedure proposed by Dulmage et al. (1071) was adopted. Experimental insects were taken from laboratory cultures maintained on artificial diet (Salama 1970). Field experiments were carried out on cotton plants (Gossypium barbadense) and water suspensions of the tested formulations with or without feeding stimulants were sprayed. The mortality of the larvae fed on the leaves was determined at different intervals after spraying, together with spore viability.

Results and Conclusions

Twenty nine cultures of B. thuringiensis belonging to 15 serotypes were screened for for their activity vs. target insects. Varieties kurstaki, aizawaii had high activity vs. H. armigera, while var. entomocidus was highly potent vs. S. littoralis and S. exigua.

Highly promising results with respect to sporulation and insecticidal activities were obtained on using the byproducts cotton seed meal and fodder yeast as components in the fermentation media of endotoxin production. Also the utilization of low priced leguminous seeds as a protein source in the media resulted in high yield of spore endotoxin with high activity.

Investigations on feeding stimulants show that petroleum ether extracts of cotton plants contain volatile and non volatile fractions that are attractive to the larvae of target insects. Some components of the volatile oil fraction increased the potency of B. thuringiensis entomocidus vs. S. littoralis. Cottonseed flour or soybean flour can be used in baits with other additives.

Based on the results, 14 field tests were conducted during 2 cotton seasons to determine the efficacy of some selected Bacillus formulations when applied to cotton. The potency of formulations vs. target insects and persistence of spores showed insignificant changes during 24 h after spraying. Some feeding stimulants increased the potency of the tested formulations, i.e., B. thuringiensis galleriae HD-129 and entomocidus HD-635.

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5A—R10

BIOLOGICAL CONTROL OF THE MEALYBUG *Phenacoccus manihoti* AND THE GREEN SPIDER MITE COMPLEX *Mononychellus spp.* ON CASSAVA *Manihot esculenta* IN AFRICA

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Background and objectives

Cassava is the staple food of 200 million Africans. In the cassava belt, more than 50% of the calories consumed come from cassava roots. In many countries the leaves also are eaten as a high protein diet complement. The total area under cassava cultivation is estimated at 7.5 million ha.

The cassava mealybug (CM) and green spider mite complex (GSM), first reported from Africa in 1973 and 1971, respectively, have since spread over 4.5 million ha of cassava and are causing yield losses estimated at US$ 1.8 billion per year. These losses lead to an exodus from rural areas into already overcrowded cities, an extension of the cropping area, and an increasing need for food imports.

The two pests were accidentally introduced from Latin America, their area of origin where they are under natural control. Their exotic nature makes them prime candidates for classical biological control. IITA, in cooperation with CIBC, CIAT and EMBRAPA[1)], is now undertaking an Africa-Wide Biological Control Programme against the two pests for the benefit of small farmers.

Research progress[1)]

The exploration work in South America yielded 11 species of predators and parasitoids against CM, of which 8 are now under study and in mass culture at IITA. For the CM, *Apoanagyrus ?lopezi* and *Diomus* sp. have proven to be very effective in experimental releases made in the 1981-82 dry season at IITA. They brought the CM population under control within 2 months and kept it at the usual low rainy season level of 5 to 15 mealybugs per terminal shoot throughout the following dry season. From a one spot release of 3000 adults made in Abeokuta, Nigeria, in November 1982 *A. ?lopezi* multiplied, spread and colonised an area of about 30,000 km^2 within five months. Releases of CM natural enemies reared at IITA are also in progress in other ecological zones. Against the GSM, an exotic phytoseid mite predator has been identified and is now being reared and released at IITA.

Results of the bionomic studies of *A. ?lopezi, Diomus* sp., and other predators are covered in detail in IITA's annual reports for 1981 and 1982. *A. ?lopezi* has a life cycle of only 12 days at 27°C, which gives it an edge over the CM. This, together with its good dispersal behavior, is the key to successful control.

Conclusion and outlook

Research for the Africa-Wide Programm includes intensified foreign exploration in the Americas, bionomic studies of the beneficial insects and mites discovered, development of new and efficient techniques of mass culture, release and follow-up, and development of optimization models for the cassava agro-ecosystem. Over a period of five years, all infested cassava areas will be inoculated with beneficial insects and mites that have been reared in a centrally located laboratory and released from the ground and air. Once theparasitoids and predators are established, control of the pests is expected to occur gradually over a period of two to three years. In addition, biological control specialists will be trained and biological control facilities set up in Africa.

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[1)] The project has been financed by the International Fund for Agricultural Development, Rome; the Directorate for Technical Development and Humanitarian Aid, Switzerland; the German Agency for Technical Cooperation and the International Development Research Centre, Canada. CIBC: Commonwealth Institute of Biological Control; CIAT: Centro International de Agricultura Tropical, Cali, Colombia; EMBRAPA: Empresa Brasileira de Pesquisa Agropecuaria.

NATIVE BIOLOGICAL AGENTS IN CONTROLLING VAPOURER MOTH/*ORGYIA ANTIQUA*/ AND THEIR EXPLOITATION IN INTEGRATED PROGRAMME OF PESTS CONTROL

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Background and objectives

Vapourer moth, *Orgyia antiqua*, had no economic importance in Poland until after 1965 when its population started to increase; within 3-4 years the moth reached the level of gradation throughout the country. Usually this pest only causes econimically important damage in large, sprayed orchards. Field and laboratory experiments were done to explain this phenomenon and to develop an integrated method of controlling this pest.

Materials and Methods

Egg masses of *O. antiqua* were collected from 37 apple orchards in different parts of Poland in 1976-1979 during autumn and early spring. The egg masses were examined in the laboratory for evidence parasitism and the number of eclosed larvae.

In the laboratory parasitized eggs were immersed in aqueous solutions of different pesticides and the number of parasites that emerged from treated eggs was determined. Some of the insecticides also were applied in three apple orchards.

Results and conclusions

It was found that parasites significantly supressed populations of *O. atniqua*. The eggs of the pest were parasitized by 2 species (*Trichogramma cacoeciae, Telenomus dalmani)*, the larva by 9 species (*Apanteles solitarius, A. vitripennis, A. lacteicolor, Hyposoter trinctus, H. vulgaris, Campoplex rufifermus, C. qeniculata, Cosinaria vidua, Eulephus larvarum)* and pupae by 4 species (*Apechthis compuctor, Iseropus storcorator, Pimpla instigator, Habrocytus seriotus)* of parasites.

The number of parasitized eggs in different orchard varied from 1 to 87 %, the number of „dead"eggs (eggs from which neither pest larva nor parasites emerged) varied from 2 to 47 %, and the number of eggs, from which *O. antiqua* hatched, varied from 0 to 82 %. In 21 % of the orchards, less than 7 % of the pest eggs contained caterpillars.

The parasitism of eggs in an orchard increased with the increase in the moth population, the number of years the moth occured in the orchard, and the age of the apple trees. Parasitism was also higher at the edges of orchards. Thirty percent of the larva and from 2 to 30 % of the pupae were parasitised.

In the laboratory it was observed that the majority of pesticides tested were toxic to eggs and larva of the pest; however DNOC, metoxychlor, decamethrin, bioresmethrin, carbaryl, phosalon, pirimicarb, vamidothion, tetrachlorvinphos, dipterex, fenitrothion, orthocide, zineb, wettable sulphur, dodine, thiuram, copper oxychloride, mancozeb, pyrazophos and bacterial preparation „Dipel" showed little or no toxicity to parasites developing in *O. antiqua* eggs. „Pirimor", even when applied at half the normal concentration, not only showed selective toxicity but also effectively controlled aphids in apple orchards.

High selectivity of DNOC, dipterex and fenitrothion was confirmed in experiment in commercial orchards.

The insecticides: diazinon, malathion and methylparathion were very toxic to parasites inside the pest eggs, and they should be not used in orchards infested with *O. antiqua*.

These results indicate, that by using insecticides that are harmless to parasites and toxic to the larva and eggs of *O. antiqua*, this pest may be effectively controlled biologically and chemically.

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5A—R12

SURVIVAL OF THE CODLING MOTH *LASPEYRESIA POMONELLA* L. REARED ON ARTIFICIAL DIET WITH CHEMICALS ADDED TO CONTROL A MICROSPORIDIAN *NOSEMA CARPOCAPSAE* PAILLOT

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Background and objectives

Mass rearing of codling moth *(Laspeyresia pomonella)* is frequently needed for the purpose of conducting bioassays of insecticides, for studies on entomopathogens and especially for genetic control attempts. In order to have a maximum productivity of rearing it is necessary to prevent and to control pathogens that may attack the codling moth. A microsporidian *Nosema carpocapsae* Paillot is a very common pathogen of codling moth occurring in its natural populations (Lipa; Ziemnicka 1982) as well as in laboratory rearing. The purpose of this research was to evaluate the effectiveness of one antibiotic and two fungicides in inhibiting the development of *N. carpocapsae.*

Materials and Methods

The codling moth *(L. pomonella)* was reared on wheat-germ diet for several generations (Badowska-Czubik, 1983). An antibiotic Fumagillin DCH and fungicides Benlate (benomyl 50 %) and Bavistin (MBC 25 %) suspended in water were added to diet at 50°C. The following concentrations were used: Fumagillin 200, 400 and 800 ppm; Benlate 10, 100 and 1000 ppm; Bavistin 100, 1000 and 10000 ppm. Three generations of codling moth were maintained on the diet containing Fumagillin and Benlate, and one generation on diet containing Bavistin. Weight, longevity and fecundity of moths were checked, as well as percentage of fertile females and larval hatch from eggs. Dead larvae and pupae were microscopically examined and the effectiveness of used compounds was evaluated on the basis of percentage of their infection by *Nosema carpocapsae.*

Results and conclusions

None of the compounds added to the diet had the detrimental effects on the development and survival of the codling moth. The weight of adults, their fecundity and longevity, and the percentage of hatching larvae were practically the same on the diets with various doses of the chemicals as on the normal diet

Incidence of microsporidian infection on diet without studied chemicals was on the level of 37.0 - 52.2 %. Even the highest doses of Benlate and Bavistin did not decrease the level of microsporidian infection. However, addition of Fumagillin to the diet effectively reduced the per cent of infection among insects. When doses of 200 ppm and 400 ppm of Fumagillin were added to the diet the level of infection among insects reared was only 11.3 % and 1.9, respectively. Fumagillin at the dose of 800 ppm completely prevented the infection and no infected insects were observed during the three consecutive generations of the codling moth reared on such diet.

Results of these studies clearly indicate that Fumagillin prevents and controls *Nosema* infection in the mass rearing of the codling moth. On the contrary, Benlate did not have such effect, although some authors found it effective in controlling microsporidian diseases of other insects.

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EVALUATION OF CODLING MOTH GRANULOSIS VIRUS IN THE UNITED KINGDOM

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Background and objectives

Field trials in a number of countries have shown that codling moth granulosis virus (Cp GV) can give good control of Cydia pomonella. We have investigated the effectiveness of Cp GV in the United Kingdom, in particular, the relationship between virus dose and reduction in damage by C. pomonella. Because Cp GV is often applied in a mixture with 1.0% skimmed milk powder, we have also tested the effectiveness of this additive. One of the potential advantages of the virus is that it could be used as a selective control agent within an integrated pest management system. We have therefore examined the effects of Cp GV on other tortrix moths (leaf rollers) and predators of Panonychus ulmi, all of which are usually killed by the conventional insecticides used against C. pomonella. In a further, long-term experiment the effects of the virus are compared with the use of diflubenzuron.

Materials and Methods

Cp GV was cultured in C. pomonella larvae reared on artificial diet in the laboratory. Before use in field trials, the virus was purified to remove insect debris and contaminating micro-organisms, then bioassayed by incorporation into artificial diet fed to first-instar C. pomonella larvae.

In field trials from 1978-1981, one or two sprays were applied to small plots of 1-4 apple trees, replicated 5-8 times. In 1981 and 1982 two sprays of virus or diflubenzuron were applied to two adjacent 0.5 ha plots. Except when evaluating its usefulness, 1% skimmed milk powder was added to all virus sprays, which were applied by hand-lance to run-off. Sprays were timed by reference to moth catches in pheromone traps. In measuring damage to apple fruits, stings (shallow damage by young larvae) were assessed in samples of harvested fruit only, whereas both windfall and harvested fruit were examined for deep damage (by older larvae). Virus persistence in the field was measured initially by rearing first-instar larvae on leaves from treated trees, then from 1980 onwards by washing virus off leaves and incorporation into artificial diet fed to first-instar larvae.

Results and conclusions

The average yield of purified virus (9×10^9 capsules/fifth instar larva) was increased by 78% by addition of methoprene (a juvenile hormone analogue) to diet. Initial field trials showed that virus at a concentration of 7×10^{10} capsules/litre was as effective as azinphos-methyl in reducing the incidence of damaged fruit and mature larvae. A comparison of 5 concentrations from 10^8 - 10^{11} capsules/litre showed that efficacy was directly related to virus concentration. Whilst the lowest concentration reduced deep damage, it was not effective against all commercial damage, because of a high incidence of stings. At concentrations greater than 6×10^8 capsules/litre damage decreased progressively but slowly with increasing virus concentration. A 90% reduction in deep damage required only 4×10^9 capsules/litre, whereas an equivalent reduction in all commercial damage required 1×10^{11} capsules/litre.

Following application, Cp GV infectivity was reduced by half in about 3 days, but some activity persisted 4-8 wk at least. The addition of 1.0% skimmed milk powder improved efficacy, but resulted in increased growth by the leaf roller Archips podana. Cp GV had no effect on leaf rollers, P. ulmi or its predators, whereas azinphos-methyl reduced leaf roller damage but induced outbreaks of P. ulmi by killing its predators. In trials carried out in 1981 and 1982, the numbers of damaged fruits and mature larvae were reduced by 90-98% on both virus and diflubenzuron-treated plots. Leaf roller damage was highest on the virus-treated plot but was also unacceptably high on trees treated with diflubenzuron. Neither virus nor diflubenzuron interfered with the biological control of phytophagous mites, but the incidence of wooly apple aphid was lowest on virus-treated trees.

We conclude that Cp GV effectively controls numbers of and damage by C. pomonella but other effective selective agents are needed to control leaf rollers if Cp GV is to be used in an IPM programme on apples in the U.K.

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5A—R14

ENHANCING THE USE OF ENTOMOGENOUS NEMATODES IN CONTROLLING INSECT PESTS

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Introduction and Objectives

Nematodes of the family Steinernematidae are used in Australia and the United States as valuable components of integrated pest management programmes (Webster, 1982). These nematodes and their symbiotic bacteria (Xenorhabdus spp.) are exempted the necessity for Environmental Protection Agency licence in the United States. Since no one species/strain of this group of nematodes is equally effective against all species of insect pest the acquisition, mass culture and maintenance of many species/strains of Steinernematids is essential to provide the optimum control agent for each pest situation (Bedding et al., 1983). Long-term maintenance and the economic mass culture of these nematodes requires techniques for the rapid isolation and purification of the symbiotic bacterium in its primary (1^{o}) form for each nematode species/strains. These isolated 1^{o} forms are used to inoculate new nematode cultures. We have developed a new technique in order to help facilitate the culture and use of particular nematodes in controlling certain pest insects in British Columbia, Canada.

Materials and Methods

Fifteen strains of Heterorhabditis heliothidis, one of Steinernema (syn. Neoaplectana) glaseri, three of S. feltiae and one of S. bibionis were obtained. Infective juveniles of these species were stored as described by Bedding (1983). Each strain was passaged through Galleria mellonella larvae every 6 months to ensure a continuous supply of viable infectives.

Primary form Xenorhabdus spp. were isolated from infective juveniles using the following technique: about 100 freshly emerged, infective juveniles were centrifuged in a 30 ml tube containing 0.1% Thimerosal (Sigma) for 1 h, allowed to settle, tansferred via pipette to distilled water for one minute, and then in one or two drops of water to a plate of Tergitol-7 agar containing 0.04% tetrazolium trichloride. The mass of larvae were then cut randomly with a pair of micro scissors (sterilized in 70% ethanol). Mixed colonies of 1^{o} and 2^{o} bacterial appeared after 2 days @ 27°C and they were restreaked onto fresh Tergitol-7 plates to facilitate isolation of pure 1^{o} form colonies. The bacteria were characterized as either 1^{o} or 2^{o} by the method of Akhurst (1980). Once the pure 1^{o} form bacteria was isolated it was used in initiating mass culture of the nematodes based on the methods of Wouts (1981) or Bedding (1983).

Results and Discussion

This method enables the rapid isolation of the 1^{o} form of the bacteria, even when only 1% or 2% of the infective juveniles contain such forms. Primary forms were isolated from a strain of S. glaseri for the first time and have been used to initiate new cultures of the S. glaseri strain. Initial in vitro culture tests using bacteria isolates by the above technique have shown that the 1^{o} forms from Steinernema spp. support the growth and development of all the nematode species tested within the genus but not of Heterorhabditis. The 1^{o} isolates from Heterorhabditis spp. do not support the growth of Steinernema spp.

It is anticipated that trials against pest insects using S. glaseri reared on selected 1^{o} form bacteria will show enhanced effectiveness over previous trials. This technique, therefore increases the pathogenicity of various strains of these nematodes against selected pest insects.

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THE USE OF VERTICILLIUM LECANII, AN ENTOMOPATHOGENIC FUNGUS, TO CONTROL GLASSHOUSE WHITEFLY (TRIALEURODES VAPORARIORUM)

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Background and objectives

The fungus Verticillium lecanii is an entomopathogen commonly observed on the glasshouse whitefly, Trialeurodes vaporariorum, and which occasionally controls populations under glass by natural infection. One strain of this fungus, isolated from a dead whitefly taken from a commercial cucumber crop, has been developed by Tate & Lyle as Mycotal microbial insecticide. The objective of the present study was to assess field performance against whitefly in a tomato crop.

Materials and Methods

Mycotal was applied at 2.5g/l of water (equivalent to 5x10" spores/ha). Application was made late in the afternoon and spray directed to the undersurface of the leaves and the growing points. Humidity conditions within the treated crops were monitored following application and were generally recorded as being greater than 85% rh for at least 10h/day.

Numbers of insects and % infection were recorded at four day intervals and were a mean of 10 plants selected at random.

Results and conclusions

Initial infection was seen on scales after 4 days but significant control was not observed until 16 days after application. By day 20 only one live adult whitefly was seen on 10 plants and no live scales were found. An inspection of the crop 2½ months after application revealed no whitefly adults or scales on any of 10 plants used for the assessment. Adults infected with V. lecanii were however seen on other plants.

Mycotal gave similar good control of whitefly on tomatoes and cucumbers in 90-95% of trials at at least 70 sites in 11 European countries during 1981-2. Several commercial trials resulted in 4½ months continuous control of whitefly populations.

5A—R16

STUDIES ON *Verticillium lecanii* FOR THE BIOLOGICAL CONTROL OF APHIDS

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Background and objectives

Verticillium lecanii (Zimm.) is a well documented and widespread entomopathogenic fungus. Its mass production and potential as a microbial insecticide under glasshouse and field conditions have been studied by various workers. Media, pH of the medium and temperature are important factors in mass production of fungi. The present studies are concerned with the effect of these factors on mycelial growth and sporulation and the effectiveness of *V. lecanii* against various aphid species.

Materials and Methods

To determine the effect of various media (carrot, cellulose, Czapeak-dox, malt, malt-glucose, malt-lactose-peptone, meal, potato and sabouraud's "A" and "B") on radial growth, 20 ml of each medium in petri dishes was inoculated in the centre with the spore suspension. The dishes were incubated at 25°C and 100% r.h. and colonies were measured after 5,10,15,20,25 and 30 days. To measure the effect of media on mycelial dry weight and sporulation, 50 ml of each fluid medium in 250 ml flasks was inoculated with 2 ml spore suspension. Mycelial dry weight was determined after the flasks were incubated at 25°C for 30 days. For sporulation, the flasks were agitated for 96 h and blastospores harvested and counted. The effect of 17 different levels of pH (3-11) on mycelial development and sporulation was determined by the above methods. Similarly, the effect of temperature 5°, 10°, 15°, 20°, 25° and 30°C on the fungus was also studied.

For testing the effectiveness of *V. lecanii*, 400 potted sugar beet and 3,000 cucumber plants heavily infested with aphids were sprayed with the blastospore suspension (concn. 10^8 spore/ml) in glasshouses. The mean numbers of live aphids per plant were recorded one day before and 5,10,15,20,25,30 and 35 days after spraying.

Results and conclusions

V. lecanii grew on all the agar media and culture solutions used, except cellulose where growth was abnormally thin or nil. The results showed that complex media like malt-lactose-peptone and sabouraud's "A" and "B", pH 5-7 and temp. 20°-25°C are optimum for mass production. The maximum radial growth and mycelial dry weight obtained under these conditions were 78-83 mm and 1144-1498 mg, respectively, after 30 days and spore production 23-27 x 10^8 spores/ml after 96 h. The fungus could not produce blastospores in cellulose cultural solution, probably due to the sedimentation of the ingredients.

Glasshouse experiments revealed that *V. lecanii* is highly effective against *Aphis fabae*, *Brachycaudus helichrysi*, *Macrosiphoniela sanborni* and *Myzus persicae* and could be used against them in biological and integrated control programmes. Complete control of *A. fabae* on sugar beet was obtained after 14 days while the same level of mortality in *M. persicae*, *M. sanborni* and *B. helichrysi* on cucumbers was reached after 25, 30 and 35 days, respectively. Ecological and behavioural factors were probably responsible for the differential control. The fungus, also tested in field conditions (at low r.h.) failed to control *A. fabae* on sugar beet and broad beans.

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THE USE OF ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR QUANTIFICATION OF VIRUS SPRAY DEPOSITS

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Background and objectives

The accurate placement of an insect virus ensures that all the virus is available for ingestion, minimising the amount necessary for effective control. Assessment of application requires the quantification of virus on the target. Enzyme-linked immunosorbent assay (ELISA) has proved to be a rapid and sensitive method for detection and quantification of animal and plant viruses (Clark and Adams, 1977; Kilton et al, 1981). This technique results in a colour reaction, the intensity of which is related to the amount of virus present. In this study we assess the potential of ELISA for quantification of virus spray deposits.

Materials and Methods

The nuclear polyhedrosis virus (NPV) of the Egyptian cotton leafworm, Spodoptera littoralis and the double antibody sandwich ELISA method, described by Clarke and Adams (1977) were used. The antibody, kindly provided by Dr.N Crook of the Glasshouse Crops Research Institute, was raised in rabbits against the purified polyhedral protein of S.littoralis NPV. Tests were performed in polystyrene microtitre plates. In all experiments the coating gamma-globulin was diluted in carbonate/bicarbonate buffer to a concentration of 4 µg protein per ml. The alkaline phosphatase conjugated gamma-globulin was diluted 1:800 in phosphate buffered saline (PBS) containing 0.05% Tween 20 2% (wt/vol) polyvinylpyrrolidone and 0.2% (wt/vol) bovine albumin. Prior to the addition of the test suspension the virus polyhedra were dissolved in a solution of 0.5M Na_2CO_3 and 0.5M NaCl containg 0.05% (vol/vol) Tween 20. After addition of HCl to return the pH to neutral the samples were diluted in PBS/Tween to give a three fold dilution series. Samples of virus alone, virus washed from cotton leaves and virus plus spray additives (Teepol, Tinopal and molasses) were tested.

Results and Conclusions

Concentration-effect curves showed an increase in colour intensity up to a maximum at a virus concentration of between $1x10^4$ and $5x10^4$ polyhedral inclusion bodies per ml (PIB/ml) followed by a decrease in intensity at higher virus concentrations. Within a microtitre plate the peak occured at a dilution that was proportional to the concentration of virus in the sample. For example, a sample initially containing $1x10^6$ PIB/ml peaked at a dilution factor of 0.03, whereas a sample containing $1x10^5$ PIB/ml peaked at a dilution factor of 0.3. Formulation additives and leaf washings alone did not produce an effect, but when added to virus suspensions the positions and heights of the peaks were altered. However, providing the nature and concentration of these constituents were held constant the relationship between virus concentration and peak position remained the same.

In conclusion, unknown suspensions of S.littoralis NPV can be quantified using ELISA by comparison with a suspension of known concentration and containing the same additives. These comparisons must occur within a single microtreiplate. Using this technique samples containing as little as $2x10^4$ PIB/ml can be quantified.

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5A—R18

COMBINED EFFECTS OF DIFLUBENZURON AND THE ENTOMOPATHOGENIC FUNGUS METARHIZIUM ANISOPLIAE ON THE TOBACCO HORNWORM, MANDUCA SEXTA

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Background and objectives

Synergism between entomopathogenic fungi and insecticides has been demonstrated many times (Benz, 1971; Ferron, 1978). However, combinations have usually been chosen empirically and co-operation between fungus and insecticide is likely to be gratuitous. When synergism occurs it is often a result of the chemical acting as a general stressor which predisposes the insect to disease (Benz, 1971). By contrast, diflubenzuron, an inhibitor of chitin synthesis in insects (Post and Vincent, 1973), could, a priori, act as a "true synergist", facilitating entry of pathogenic fungi by weakening insect cuticle. The present study was done to test this hypothesis, using the pathogenicity of the green muscardine fungus (Metarhizium anisopliae) for the tobacco hornworm moth (Manduca sexta).

Materials and Methods

Insects were reared to 2nd instar on an artificial diet, then fed on tomato leaves for the duration of the experiment. Simultaneous applications of fungus and insecticide were performed by dipping larvae in aqueous suspensions containing 6.25 mg/l diflubenzuron (LC3 - contact dose) and appropriate concentrations of conidia of the green muscardine. Separate treatment was achieved by dipping the food in suspensions of diflubenzuron (6.25 mg/l diflubenzuron, LC50 - ingested dose) and the larvae in suspensions of conidia. The concentration of insecticide used did not apparently affect germination or growth of the fungus. Treated larvae were maintained at 25°C and 100% RH under a 12h light : 12h dark photoperiod. Mortality was assessed after 5 days and cadavers were transferred to 27°C and 100% RH for 2 days. Those individuals which became mummified by green muscardine were assumed to have died from mycosis. The procedure and terminology of Benz (1971) were adopted for determining the degree of synergism.

Results and conclusions

Dual applications of diflubenzuron and green muscardine had a synergistic action against larvae of M. sexta, whether the agents were applied separately or simultaneously. The extent of the synergism depended on the age of the insect at the start of the treatment. Although newly moulted 2nd instar insects (6h ± 6h old) were the most susceptible to the pathogen, the highest level of synergism ("supplemental synergism", see Benz, 1971) occurred in 24h old insects. Mycosis was the predominant cause of death in the combined treatments. Observations made by transmission electron microscope suggest that enhanced penetrability of the fungus through diflubenzuron-weakened cuticle may be a particularly important aspect of the synergism (Hassan and Charnley, unpubl.).

Although conidia of green muscardine will not germinate in distilled water, a 20h period of "presoaking" synchronises and accelerates germination when a nutrient source is provided either artificially in vitro (nutrient broth) or in vivo by natural diffusion from insect cuticle (Dillon, Hassan and Charnley, unpubl.). Therefore, it is noteworthy that in the present study "presoaking" of conidia enhanced pathogenicity of green muscardine for 24h old larvae. This effect was particularly marked when a premium was set on speed of germination by interspersing two 15h periods of high humidity (90%) with a 9h period of low humidity (50%), a constraint likely to be frequently encountered by entomopathogenic fungi.

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THE INTERACTION OF NOSEMA WHITEI AND MALATHION ON TRIBOLIUM CASTANEUM

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The successful control of insects in stored products depends mainly on the use of insecticide. But many kinds of insects are increasingly found to be highly resistant to the chemicals. Our purpose was to solve the problem of resistance in T. castaneum.

In this project the toxicity of malathion and Nosema whitei were measured by feeding. One type of combination used a constant pathogen dose with a variable insecticide dose. Eggs were collected from treated females to study the fecundity and fertility.

Data showed that the normal strain of T. castaneum was more susceptible to malathion (LD50 2.4ppm) than the malathion resistant strain (LD50 6.1ppm), while the LD50 of microsporidian was $3.4X10^{6}$ sp./g.

The mortality in the treatment of 0.5ppm malathion plus $3.2X10^{5}$ sp./g. of pathogen on normal strain was nearly the same effect of 1.0ppm malathion plus $3.2X10^{5}$ sp./g. of N. whitei on resistant strain.

The number of eggs of T. castaneum laid by treated females in all the treatments was less than the number of eggs in the control. The lowest number was in the treatment of malathion plus pathogen on the normal strain.

The number of hatched eggs was less than the control, but the percentage of fertility of eggs in insecticidal treatments was higher than the percentage in the combined treatments. The lowest percentage fertility was in the treatment of malathion plus pathogen on normal insects.

The lowest dose of insecticide produced a synergistic effect when applied with the dose of microsporidia.

5A—R20

PROTEIN AND RNA SYNTHESIS IN TOMATO PLANTS INFECTED WITH ATTENUATED STRAIN OF TOBACCO MOSAIC VIRUS : AUTOREGULATION AND INTERFERENCE

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Background and objectives

Attenuated strain of tobacco mosaic virus (TMV : $L_{11}A$) has shown interfering activity against wild parent strain (L). Thus in Japan, $L_{11}A$ strain has been using for the protection of tomato plants in a greenhouse. In order to know the mechanism of interference by $L_{11}A$, we studied a synthesis of protein and RNA in plants infected with $L_{11}A$ or L.

Materials and Methods

Tomato plants, whose leaves were directly inoculated with $L_{11}A$ or L at 250 ug/ml were kept at 28°C. Three grams of infected leaf tissues were labelled with radioisotope in Hoagland medium at 28°C for 16 h. TMV was purified and its optical density at 260 nm and radioactivity was determined. RNA synthesis was studied with $L_{11}A$ or L infected tissue that was labelled with ^{3}H-adenosine. RNA was prepared by phenol extraction, and was fractionated into replicative intermediate (RI), replicative form (RF), TMV RNA and ribosomal RNA by a gel electrophoresis. $L_{11}A$ or L infected tissues were labelled with ^{3}H- or ^{14}C-histidine, respectively. They were homogenized and fractionated into cytoplasm, mitochondria, chloroplast and nucleus fractions. The analysis of non-coat protein synthesis in each fraction was performed by SDS-polyacrylamide gel electrophoresis. (Histidine is absent in a coat protein of TMV.)

Results and conclusion

Infection with either $L_{11}A$ or L resulted in virus multiplication. After 4 d of post-infection, rate of multiplication of $L_{11}A$ was drastically reduced (autoregulation) as compared to the constant rate of multiplication of L. By either coinfection or preinfection of plant with $L_{11}A$, however, the rate of multiplication of L in the plants was greatly reduced to the same extent of the rate of $L_{11}A$ (interference). In single infection, multiplication of L was not inhibited even 10 d after inoculation. Results suggest that $L_{11}A$ induces inhibitory mechanism to a virus multiplication at 4 d after inoculation.

Five peaks of radioactive RNA were observed with a sample prepared from the plant of 4 d postinoculation with L. These peaks are corresponding to ribosomal RNAs (two peaks), TMV RNA, RF and RI, respectively. In $L_{11}A$ infection, however, synthesis of TMV RNA and RI were not detected. It suggests that a complimentary minus strand to TMV RNA can be formed but not progeny RNA.

Synthesis of protein other than viral coat protein was studied by the incorporation of radioactive histidine into subcellular fraction derived from 4 d postinoculated leaves with L or $L_{11}A$. Different patterns between two strains on a protein synthesis were noted. At least 5 proteins were predominantly synthesized in $L_{11}A$ infected plant. One of them was observed in mitochondria fraction and had high molecular wt. This protein can be viral coded 165K protein. Other 4 proteins were observed in chloroplast and cytoplasm fractions. Their molecular wt were relatively low. Kinetics of a formation of these proteins were studied after virus inoculation. Preferential synthesis of 165K protein was observed from the first day of $L_{11}A$ infection. In contrast, preferential synthesis of other 4 proteins were observed at 4th d of $L_{11}A$ infection.

These results suggest that unique nature of attenuated virus $L_{11}A$, autoregulation and interference, resulted by the inhibitory mechanism of viral RNA synthesis. This mechanism might be induced in infected plant after several rounds of infection cycle of $L_{11}A$ (4 d after inoculation). Analysis on nucleotide sequence of $L_{11}A$ RNA has supported this hypothesis. Whether the preferential synthesis of 165K protein and other 4 proteins were involved in this inhibition is now under investigation.

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A SOIL ASSAY FOR SCREENING POTENTIAL ANTAGONISTS OF *PYRENOCHAETA LYCOPERSICI*

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Background and objectives

Brown or corky root rot (BRR) caused by the fungus *Pyrenochaeta lycopersici* Schneider & Gerlach is an important root disease of tomatoes (Fletcher, 1975). The disease can be controlled by efficient soil sterilisation using steam or methyl bromide. However, these methods are costly and with methyl bromide application is limited by the danger of bromide residues in subsequent crops. The aim of this project was to investigate the potential of biological methods for the control of BRR. A method was required for testing potential microbial antagonists against *P. lycopersici* and assessing their ability to suppress BRR. No universally applicable technique for the screening of antagonists to soil-borne plant pathogens exists. Due to the slow growth rate of *P. lycopersici* and the concern over the use of presumptive culture studies (Baker & Cook, 1974), a method was developed whereby potential antagonists are screened against BRR infested soils, collected from commercial crops.

Materials and Methods

Brown root rot infested soil was diluted with sterilised compost to give a range of BRR concentrations. Tomato seeds were sown, in a layer of perlite, over each soil dilution. The quantity of tomato seed and the number of replicates per treatment were varied. Plants were harvested after three weeks and the number of lesions per root recorded. The number of lesions per gram dry weight of root was calculated.

Results and Discussion

All treatments demonstrated a direct relationship between lesion number per gram dry weight of root and the concentration of BRR in each soil. A large variation in lesion numbers were observed in treatments containing three and five tomato seedlings per pot. One seedling per pot, ten replicates per treatment produced the least variable results.

The development of an assay for the detection of BRR has provided a technique whereby antagonists can be tested for their ability to suppress BRR in a soil environment. The technique eliminates the necessity for presumptive culture studies so often used in preliminary antagonist screening programmes. Current work includes the use of this technique for testing the ability of fungi, with known antagonistic properties, to suppress BRR. The procedure can be modified to investigate application rate, timing, delivery etc. of the antagonists to the BRR infested soil. Results of these tests will be displayed on the poster.

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5A—R22

ANTAGONISTIC ACTIVITY OF TRICHODERMA SPP. AGAINST RHIZOCTONIA SOLANI IN FIELD SOIL

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Background and objectives

Rhizoctonia solani is a common widespread and destructive soil-borne plant pathogen in Taiwan. This pathogen is able to infect a wide range of important crops growing in Taiwan (Wu, 1978). Several species of Trichoderma have been found to be able to destroy this pathogen and increase the emergence of soybean (Wu, 1982). Although antagonistic activity by Penicillium oxalicum has been observed in the spermosphere of pea (Windels, 1981), the antagonistic effect of Trichoderma spp. has not been investigated in the soil. Therefore the mechanism by which Trichoderma spp. protects crops from infection by R. solani was studied further in order to screen and apply the most suitable antagonists to control this pathogen in the soil.

Materials and Methods

Sandy loam field soil (pH=3.8) was collected and mixed with one of the three test species of Trichoderma ($2.7\text{-}4.8 \times 10^6$ conidia/g. of soil) and placed in a petri dish (9 cm in diam.). R. solani was placed on the surface of the soil in the centre of the dish and incubated at 28°C for 5 days before examination under AO differential interference contrast microscope by incident light (DICV). So as to study the antagonistic activity of Trichoderma spp. in soil, a 8 cm cellophane sheet with an inoculum of R. solani was placed on the surface of 40 g. of soil which was infested with Trichoderma spp. ($1.5\text{-}3.5 \times 10^6$ conidia/g. of soil). The soil was placed in a petri dish. Then another 40 g. of the same Trichoderma sp.-infested soil was added on the top of the first layer of soil and incubated at 28°C for 3-5 days. In order to study the antagonistic effect of Trichoderma spp. in the rhizosphere, cellophane was placed beside the soybean seed which was sown in Trichoderma sp.-infested soil ($2.7\text{-}5.4 \times 10^6$ conidia/g. of soil). Sixty soybeans were planted in 2 kg. of soil in a box (30 x 22 x 3 cm., L x W x H) and incubated in a greenhouse (28 ± 2°C) for 7 days. 200 g. of R. solani which was cultured in a corn meal-sand (50/50,v/v) medium was placed along one side of the box. The antagonistic effect of Trichoderma spp. on cellophane in soil or in rhizosphere was observed under both light and scanning electron microscope (Hitachi S 550).

Results and conclusions

The hyphae of T. koningii, T. pseudokoningii and T. viride were all able to coil around the hyphae of R. solani either on the surface of soil, in the soil, or in the soybean rhizosphere. The hyphae of Trichoderma spp. constricted and depressed the hyphae of R. solani. Appressorium-like structures were occasionally initiated from the Trichoderma spp. to press on the hyphae of R. solani. The hyphae of R. solani with lysed holes or cracks was also present in Trichoderma sp.-infested soil.

These hyperparasitic effects produced by Trichoderma spp. were reflected in the fact that there was significantly less disease on soybean grown from antagonistic Trichoderma sp.- infested soil than from untreated field soil.

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THE USE OF STREPTOMYCES SP. AS A BIOLOGICAL CONTROL AGENT

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Background and objectives

Light-coloured Sphagnum fuscum peat, which has properties that make it an almost ideal substrate for plants, is the most common substrate used in greenhouse cultivation in Finland. In addition, fresh peat is free from all plant pathogens and pests. However, peat differs from artificial substrates in that sense that it has its own natural microbial fauna. Certain peat lots have been found to have a strong suppressive effect on seed and soil-borne fungal pathogens. The fact that the inhibitory effect is almost completely lost when the peat is steam sterilised, indicates that it is of microbiological origin. Addition of fresh peat to the disinfected peat results in the recovery of the inhibitory effect. Trichoderma viride and Streptomyces spp. are the strongest antagonists of the micro-organisms most frequently found in peat. Streptomyces spp. has given promising results in the control of plant pathogens on peat in the greenhouse. The aim of the study has been to develop a biological method for controlling the pathogens encountered in peat cultivation using a preparation of Streptomyces isolates obtained from peat.

Materials and Methods

A large number of antagonistic Streptomyces isolations were obtained from Finnish peat lots and the most effective ones then tested in control experiments against seed and soil-borne fungal pathogens. Preparations containing a known number of spores were prepared by making liquid cultures of the chosen isolates and then centrifuging the suspensions. The preparations were then diluted and used for coating the seeds, spraying the surface of the substrate and dipping the roots of the cuttings as a control measure against damping-off, cucumber root diseases, Fusarium wilt on carnations and Botrytis rot on lettuce.

Results and conclusions

Treating the cabbage seeds with the Streptomyces preparation afforded complete control against seed-borne damping-off caused by Alternaria brassicicola. Treating the seeds alone resulted in 80-90 % control against soil-borne Rhizoctonia solani. When, in addition to treating the seeds, the seeding layer was also sprayed with the Streptomyces preparation, the results obtained with some of the dilutions deteriorated significantly. Treating the soil against Alternaria damping-off also gave a poorer result.

Spraying the surface of the substrate with different dilutions of the Streptomyces preparation gave satisfactory protection against root diseases on cucumber (Fusarium spp., Phomopsis spp., Pythium spp.) and wilt disease on carnations (Fusarium oxysporum f. sp. diathi). The yield increase obtained in trials carried out on a practical scale with cucumbers has been over 10 % and it has been possible to maintain productive stands up until the end of the growing season without having to carry out replanting. The spread of wilt disease on carnations has been checked to the extent that the area of destroyed plants in two-year cultivations treated with Streptomyces has been less than 10%, the figure for untreated cultivations being 30-40%. The experiments have been repeated a number of times with similar results.

Treating lettuce seedling pots before planting has given promising results against Botrytis cinerea.

The microbial preparations made from Streptomyces isolates obtained from peat appear to be promising for the biological control of plant pathogens in greenhouse cultivation.

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5A—R24

BIOLOGICAL CONTROL OF CROWN-GALL IN GREENHOUSE AND THE FIELD

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Background and objectives

Crown gall, caused by Agrobacterium tumefaciens (Smith & Townsend) Conn affects a wide range of host plants and causes serious economic losses especially of fruit trees in nursery stock. Attempts to control the disease by chemical means have met with limited success. In 1972 (New & Kerr, 1972) published the first report of biological control of crown gall by using strain K 84 of Agrobacterium radiobacter which produces a bacteriocin that inhibits sensitive strains of A. tumefaciens. The method has given excellent control of the disease on stone fruits. Tumor induction by A. tumefaciens occurs only after the bacterium attaches to specific sites in a plant wound (Lippincott & Lippincott, 1969). The present study was designed to determine: 1. The timing of application of the controlling organism (K 84) on bean leaves; 2. The number of sensitive and resistant strains at various times after the inoculation of the antagonistic strain on bean leaves; 3. Biological control of crown gall in the field.

Materials and Methods

Strain K 84 of A. radiobacter was kindly supplied by Professor A. Kerr, Australia. Strain O is a wild type agrocin sensitive strain isolated from grapevine in Hungary. Strain 103 is an agrocin resistant virulent derivative of strain O (Süle & Kado, 1980). Bean plants were sown in sterile sand in a glasshouse and 7-9 days after sowing were selected for uniformity and transplanted to 60 mm pots on the day before use. For inoculation of bean leaves, the method of Lippincott & Heberlein (1965) was used. Bacteria on leaves were counted by obtaining antibiotic resistant/500 ppm of chloramphenicol and streptomycin using the replica plates method for counting the developed colonies. In the field experiment, cherry, raspberry, apple and pear seedlings were dipped in a suspension of K 84 (10^{9} cells/ml) and planted. Control samples (without dipping in K 84 suspension) were used. All seedings were harvested after one year and scored for galling.

Results and conclusions

Crown-gall tumors caused by A. tumefaciens can be controlled biologically on primary bean leaves by A. radiobacter 84. In the case of the agrocin-sensitive strain, no galls developed on leaves treated with K 84 30 min. after inoculation of the pathogenic strain, but galls appeared on leaves treated 24 hrs. after inoculation. Tumor formation was also reduced when strain 103 was inoculated simultaneously with or after K 84. This type of inhibition seems to be independent of agrocin sensitivity. In the field, peach, apricot and cherry seedlings were subjected to biological control, however apple and pear were not.

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THE CONTROL OF FUSARIUM CROWN ROT IN TOMATOES BY LETTUCE INTERCROPPING

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Background and objectives

Crown and root rot caused by Fusarium oxysporum f. sp. radicis-lycopersici (FORL) is a devastating disease of tomatoes in N. America, Japan and Israel, because of the rapidity of reinvasion of sterilized soil from airborne microconidia. A survey of management practices in an area of densely-sited glasshouses (81 ha) in southwest Ontario suggested that poor or no soil sterilization reduced the incidence of the disease, and preliminary experiments showed that amending sterilized, FORL-reinfested soil, with non-sterile peat or field soil also reduced the disease. Mindful of the risks of introducing other pathogens, particularly with field soil, we investigated the feasibility of inducing FORL-suppressiveness in sterilized glasshouse soil by green manure crops, an easy and even profitable modification of management practice for the grower.

Materials and Methods

In pot tests, steam-sterilized soil was reinfested by FORL microconidia, 25 cm^{-2}, and seeded with leaf lettuce, mustard greens, spinach or pepper cress, or it was left fallow but moist. After 30 d, the plants were chopped into the soil, and 9 d later a 30-d-old tomato plant, cv MR13, was transplanted into each treated and check pot, and grown to fruiting for a further 60 d. The tests were repeated over 2 yr. In groundbed tests in a glasshouse previously bearing a severely-diseased crop, the groundbed soil was either a) steam-sterilized, infested with a FORL-suppressive field soil and sown to a 60-d lettuce crop, whose residues were returned to the soil; or b) left fallow for 70 d, then steam-sterilized. Both soils were then reinfested with FORL and planted to a 6-mo crop of tomatoes, cv MR13. Following the removal of this crop, the two treatments were repeated, except for the field-soil amendment, and another crop raised to maturity. The incidence of rot at the base of each tomato stem and in the seminal root system was assessed on an arbitrary scale, 0 = no visible symptom and 5 = complete stem girdling, and, or, total loss of seminal roots.

Results and conclusions

In the pot tests, the mean disease rating in tomatoes following lettuce was 0.17, mustard greens 0.98, pepper cress 1.00, spinach 1.43, and fallow 2.07.

In the first groundbed test, 53% of the tomato plants that followed the normal tomato-fallow-sterilize-tomato procedure were affected by FORL, with a mean disease rating of 0.83; this compares with only 12% plants affected, and a mean disease rating of 0.57 in the tomato-to-sterilize-lettuce-tomato procedure. In the second test 100% plants were affected, with a mean disease rating 2.66 in the tomato-fallow-sterilize-tomato procedure, as against 88% plants affected, mean disease rating 0.85, in the tomato-sterilize-lettuce-tomato procedure.

In situations where other diseases and nematodes do not warrant soil sterilization, it would seem that lettuce as a catch crop could provide a sufficient, economic and even profitable control for Fusarium crown rot, and where sterilization is necessary for other diseases, lettuce provides adequate control for what is a severe disease in freshly sterilized soil.

Its mode of action is unclear. Joint work with Dr. T. Arnason of the University of Ottawa centres on its allelopathic role. The Chichorieae, which includes lettuce, produce a variety of biologically-active materials, and extracts from soil containing lettuce inhibit the activity of FORL. At the same time, populations of saprophytic Fusaria are enhanced and may play a competitive role against the saprophytism of FORL in the soil.

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5A—R26

ON THE EFFICIENCY AND RATIONAL USE OF CRYPTOLAEMUS MONTROUZIERI AGAINST PLANT PESTS IN THE GEORGIAN SSR

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The predatory coccinellid beetle Cryptolaemus montrouzieri Muls., introduced into Georgia from Egypt in 1933, is used for biological control of mealybugs and Lecaniine scales (Homoptera, Coccoidea) on tea, grapes, citrus and other subtropical crops as well as on ornamental plants. During the 50 years since it was introduced, a cold-resistant population has evolved in some regions. On the Black Sea coast of the Caucasus - in Abkhazia (Sukhumi, Gagra, etc.) - it overwinters and can tolerate a momentary fall in temperature to -11°C, but in Adjaria (Batumi, etc.), because of high humidity and scarcity of food in autumn and winter, overwinter survival is poor. In the east and in the mountainous regions of Georgia, it has not become established; although it breeds well in summer, it dies out in winter. However, even where conditions are favourable, the number of insects in spring and the first half of summer is not high. Therefore, C. montrouzieri is artificially propogated in laboratories and released in plantations at the beginning of the pest oviposition period.

This method of control, called seasonal colonization, permits a reduction or complete avoidance of pesticide applications which is important for such crops as tea and grapes at harvest when chemical treatment must be limited or is inadmissable. According to economic indexes, biological control is more profitable than chemical control when long-term effects are taken into account. Thus, annual releases in one and the same plantation are not needed.

At present, the main use of C. montrouzieri is in tea plantations, where the principal pest is Chloropulvinaria floccifera Westw. The yield of heavily attacked tea plants can be reduced to 40% and the quality of the tea leaf is also reduced. Releases of 3,000-5,000 beetles per hectare gives an 80-85% reduction of the pest and costs half as much as organophosphate pesticide applications. The density of C. floccifera on the tea plants is reduced to 0.8-1.0 in autumn and does not reach the economic threshold during the following 2-3 years. Besides the beneficial activity of native entomophagous insects and mites is noticeably enhanced.

The timing of releases with due regard to ecological conditions is recommended to increase the effectiveness of C. montrouzieri. Pest phenology, differential rates of development in mountain regions and the level of infestation have to be taken into account. Only the release of adult beetles is recommended as the larvae suffer high mortality during transportation and only feed for a short time so that the effectiveness of the control method is halved.

C. floccifera is widespread on forest and ornamental plants which provide a reservoir of this pest. Sometimes it causes serious injury to citrus and other sub-tropical crops.

In citrus plantations, C. montrouzieri is used mainly for the control of Pulvinaria spp. because citrophilus mealybug, Pseudococcus gahani Green and grape mealybug, Pseudococcus obscurus Ehrh., are now of no economic importance since the successful acclimatization of the introduced parasitic insects, Coccophagus gurneyi Comp. and Pseudaphycus maculipennis Merc.

In vineyards, C. montrouzieri is used for control of Planococcus citri Risso and Neopulvinaria imeritina Hadz. In the regions of East Georgia, characterized by low humidity and high insolation, the effectiveness of C. montrouzieri against these pests is considerably lower than in the West coastal regions.

On ornamental plants and in plantations of bamboo, fig, etc., C. montrouzieri is the only means of control.

BIOLOGICAL CONTROL OF SCLEROTIUM ROLFSII BY TRICHODERMA HARZIANUM IN SUGAR BEET

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Background and objectives

Biocontrol of soilborne plant pathogenic fungi by incorporation of antagonistic microorganisms to the soil is a potential non-chemical means for plant disease control. Among the many potentially antagonistic soil inhabitants, Trichoderma harzianum has recently gained considerable importance(Elad et al, 1980). In the present investigation, an antagonistic strain of T. harzianum, capable of controlling Sclerotium rolfsii was isolated from naturally infested sugar beet field soil and its efficacy was studied under laboratory, glasshouse and field conditions.

Materials and Methods

T. harzianum was tested for its antagonistic activity in vitro. Glasshouse experiment was carried out in a loamy sand soil artificially infested with S. rolfsii inoculum (grown on autoclaved sorghum seeds) @ 2 g/kg soil. T. harzianum was grown in 250 ml Erlenmeyer flasks on sorghum grains pre-soaked in 2% sucrose solution overnight and autoclaved for 30 min. at 121°C. This was mixed at various concentrations with the pot soil after being grown for 15 days at 30°C in illuminated chamber. Each pot was seeded with 20 seeds of sugar beet Cv. Ramonskaya and treatments in all experiments were replicated 4 times in randomized blocks. Observation on seedling mortality was recorded 45 days after planting. Seedlings were harvested after observation and each pot was reseeded in the same manner for second growth cycle. Survival of Trichoderma in the infested soil at different intervals was monitored with the help of a selective medium (Elad et al, 1981). For field experiment, a block naturally infested with S. rolfsii (with the previous history of sugar beet crop) was selected. The T. harzianum preparation at 2 concentrations (30 g and 60 g/sq m) was mixed with the ridge soil to a depth 5-6 cm with a hand hoe. Four litres water suspension of PCNB (2 g) was drenched on ridges in each plot (5 rows of 5 m each) either alone or in combination with application of Trichoderma preparation. Observations were recorded on per cent root rot and root and top yield.

Results and conclusions

T. harzianum antagonised S. rolfsii by coiling around the hyphae, penetrating into the hyphae and digesting the protoplasmic content and lysing the hyphae by production of toxic metabolic products. Incorporation of sorghum culture preparation of T. harzianum to S. rolfsii infested soil in the glasshouse effectively controlled the disease. The antagonist remained active (as evident from selective isolation from pot soil) and protected sugar beet seedlings from infection for 2 growth cycles of 45 days each. Such applications resulted in 19 to 76 and 14 to 88 per cent control of seedling mortality due to S. rolfsii in first and second growth cycles respectively. The degree of disease control achieved was positively correlated with the amount of antagonist preparation added to soil. An integrated control of S. rolfsii was tried by using a combination of Trichoderma preparation and the fungicide PCNB at sublethal dose under field conditions. Results indicated a synergistic interaction between Trichoderma and PCNB in controlling the disease, with increased yield and decreased disease incidence.

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