SESSION 3A

ADVANCES IN BIOLOGICAL CONTROL

Chairman:	Dr R GreatRex *Syngenta Bioline, Little Clacton, UK*
Session Organiser:	Dr K F Walters *CSL, York, UK*
Papers:	3A-1 to 3A-4

Initial testing of potential fungal biological control agents for potato cyst nematodes

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ABSTRACT

The potato cyst nematodes (PCN), *Globodera rostochiensis* (Wollenweber) and *G. pallida* (Stone) are serious potato pests causing yield losses valued annually at 300 M ECU in the European Community. Integrated approaches to PCN management using resistant cultivars and nematicides, in addition to crop rotation are encouraged. The development of fungal biological control agents (BCAs) to manage nematodes may become an important alternative to chemical control, especially as restrictions on some leading nematicides have occurred in a number of European countries. Here, we report on an investigation into three potential fungal BCAs in controlling PCN populations within pot trials. The presence of all three fungi had a significant affect on the decline of viable eggs, with the fungal populations remaining viable throughout the trial and for 5 months after harvest of the potato crop. The fungal densities remained similar to that of the initial inoculation rate throughout the trial.

INTRODUCTION

Understanding the factors that cause inconsistencies in the biological control of nematodes by nematophagous fungi has been hampered by a lack of useful techniques to determine the presence, abundance and viability of these fungi in the rhizosphere and soil. It is also important for regulatory purposes to develop methods to monitor the specific fungal BCAs after their release (Avis *et al.*, 2001). At present three fungal BCAs, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Plectosphaerella cucumerina* are being investigated at Rothamsted for their ability to control PCN populations. A wide range of techniques have recently been developed to monitor these fungi in the environment and to recover and enumerate them after release (Hirsch *et al.*, 2001; Atkins *et al.*, 2002a, b), increasing our understanding of their potential to act as BCAs. Selective media (Kerry *et al.*, 1993; Atkins *et al.*, 2002a; Atkins *et al.*, 2002b) enables enumeration of each fungus from root and soil samples. Baiting of soil with PCN cysts enables a quick method for assessing the level of the activity of isolates, and when combined with species specific PCR, detection of fungi from infected eggs enables a quick screening method for identifying BCAs after release (Atkins *et al.*, 2002a). Specific primers for the detection of *P. chlamydosporia* and *P. cucumerina* have been developed (Hirsch *et al.*, 2000; Atkins *et al.*, 2002b) and enabled detection of these fungi from root, soil and nematode samples. Further work is necessary to develop specific primers for the detection of *P. lilacinus* (Atkins *et al.*, 2002a)

All these techniques have been used for the detection of fungi from PCN suppressive soils (Atkins *et al.*, 2002a). Competitive PCR has enabled the quantification of *P. chlamydosporia* from root and soil samples (Mauchline *et al.*, 2002), and has demonstrated that there is a nematode host specificity that is highly relevant to the biological control efficacy of fungal isolates. Real-time PCR has been used for the detection and quantification of fungal propagules in the field (Cullen *et al.*, 2001) and has been used for the detection of *P. cucumerina* from seeded soil (Atkins *et al.*, 2002b). This work is ongoing but real-time PCR could be used to detect and quantify all three fungal cultures from soil samples.

The methods discussed have played an important role in the investigation of the three fungal BCAs and will continue to aid in their detection, monitoring and quantification of the cultures in future experiments and trials.

Here we report an experiment to assess the interactions between the fungal agents, host plant and PCN, and assess the survival and viability of the fungal isolates after harvest.

MATERIAL AND METHODS

All fungal cultures were isolated from PCN cysts obtained from infested soil in Jersey. *P. chlamydosporia* isolate 280, *P. cucumerina* 380408 and *P. lilacinus* 1 were maintained on potato dextrose agar (PDA-Oxoid, UK) plates at 25 °C and stored at 4 °C on PDA plates, or at –80 °C in 15 % glycerol stocks.

Spores for soil inoculation were collected from plates of *P. cucumerina* and *P. lilacinus* by washing plates in sterile distilled water, and counted using a haemocytometer. Spores from *P. chlamydosporia* were collected as described (Hidalgo-Diaz, 2000). The spores were partially dried, counted using a haemocytometer and mixed 1:10 with fine sterile sand.

All fungi were added to soil at an inoculation rate of 5000 spores g^{-1} soil. Spores of *P. lilacinus* and *P. cucumerina* were resuspended in sterile water and mixed with sandy loam soil, for *P. chlamydosporia* a chlamydospore/sand dry mix was added to the soil and mixed. Soil was contained in 12 cm pots, and a potato chit, cv. Maris Piper, added to each pot. The pots were arranged randomly in a glasshouse kept at 18 °C with 12 replicates for each treatment. Controls of potato chits un-inoculated with fungi were set up as above.

After 4 weeks of growth, half of the replicates were inoculated with 5000 *G. pallida* juveniles. Plants were harvested at 7, 10 and 13 weeks, after 13 weeks a quantity of the soil was left with light watering in the glasshouse for a further 21 weeks.

Soil CFU levels were estimated for all three fungi at 4, 7, 10, 13, 21, 25 and 34 weeks after inoculation with the fungi using selective media for enumeration of *P. chlamydosporia* (Kerry *et al.*, 1993), *P. cucumerina* (Atkins *et al.*, 2002b) and *P. lilacinus* (Atkins *et al.*, 2002a). Root CFU levels for all three fungi were estimated at 7, 10 and 13 weeks after inoculation with the fungi. The nematode life stages were investigated in plant roots harvested after 3 and 6 weeks (7^{th} and 10^{th} weeks) after the addition of *G. pallida* J2 by staining roots with acid fuchsin (Bridge *et al.*, 1982). Root, shoot and tuber

weights were recorded at each harvest. Cysts were extracted from 200 g soil 10, 13 and 34 weeks after inoculation with *G. pallida* juveniles. From these cysts, eggs per cyst, eggs per g soil and egg infection levels were recorded using standard techniques.

At 32 weeks pots were baited with 25 *G. pallida* cysts contained within a nylon mesh (Atkins *et al.*, 2002a). After 2 weeks these baits were removed and the level of infection calculated by plating the crushed cysts onto water agar (8 g technical agar per l + 50 mg streptomycin, 50 mg chloramphenicol, 50 mg chlortetracycline) and counting the number infected after 2 days incubation at 24 °C.

After 34 weeks in soil, cysts were combined from the replicates and the effect of the presence of the fungus on hatch was investigated by adding 5 replicates of 10 cysts to a small hatching dish immersed in potato root exudate, and counting the juveniles that emerged over a course of 73 days.

All data was subjected to analysis using one way analysis of variance (ANOVA) using the Genstat programme (Genstat 5 Committee, 1993).

RESULTS

The presence of the nematode or the fungus did not significantly affect root and shoot weights 7, 10 and 13 weeks after planting (data not shown). There was also no significant differences in the nematode life stages in roots from all fungal treatments compared to the control, neither was there any significant difference in the number of cysts in the soil. There was, however, a significant increase in the number of eggs per cyst in the treatment inoculated with *P. lilacinus* compared to the control. Mean numbers of eggs per cyst (± SE); *P. lilacinus* 152 ± 15 (F. p = 0.039) compared to 87 ± 21 for the control. All fungi were found to colonize the roots but at low levels, and no significant differences were seen in fungal treatments with and without nematodes. The soil colony forming unit (CFU) count for all treatments remained relatively constant at around the level of inoculation (Figure 1). There was no significant difference in the level of fungi in treatments with and without nematodes. The percentage egg infection increased in all fungal treatments with time (Figure 2), and on all sampling occasions was significantly greater than the control. A greater number of juveniles were seen to hatch from the cysts extracted from the control treatments compared to the fungal treatments but this trend was not significant. The level of egg infection in the baits added after 8 months since the treatments were inoculated with fungus was similar to that recorded for the egg infection at 34 weeks in the cysts extracted from the treatments (Figure 3).

DISCUSSION

PCN is an increasingly important problem in UK agriculture and control of the pest is problematic. The use of biological control agents to reduce infestations to densities below the economic threshold before a susceptible crop is planted may be a practical option, especially where chemical control is not feasible, i.e. organic farming, and when combined with other farming practices such as rotation in an integrated pest management strategy.

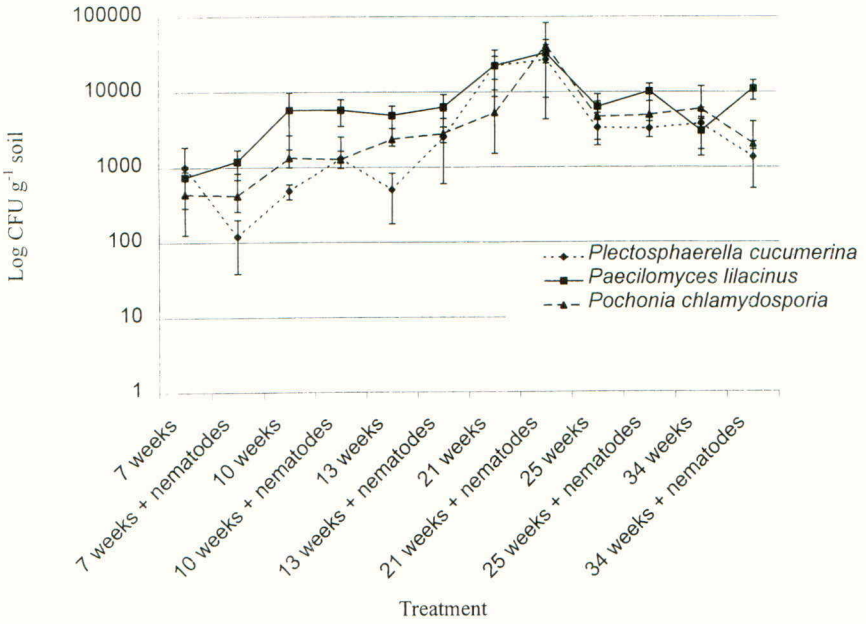

Figure 1: Log Soil CFU per gram soil for each treatment over time

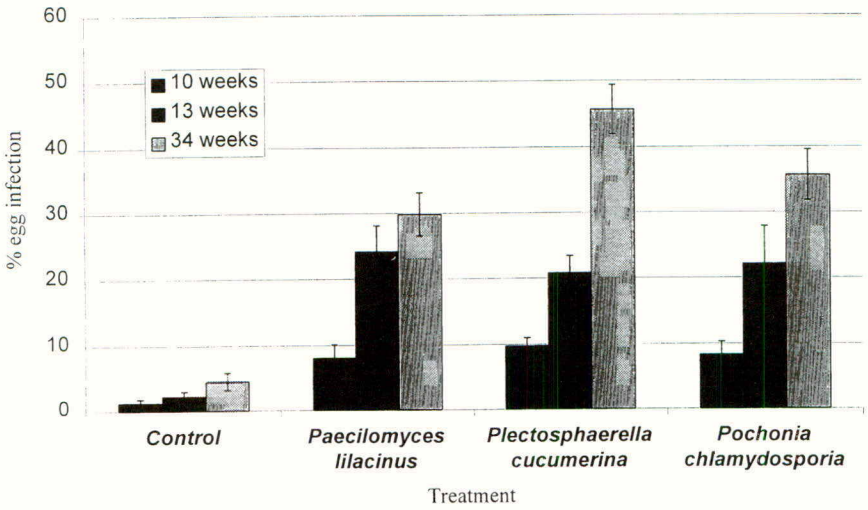

Figure 2: Percentage egg infection over time in treatments

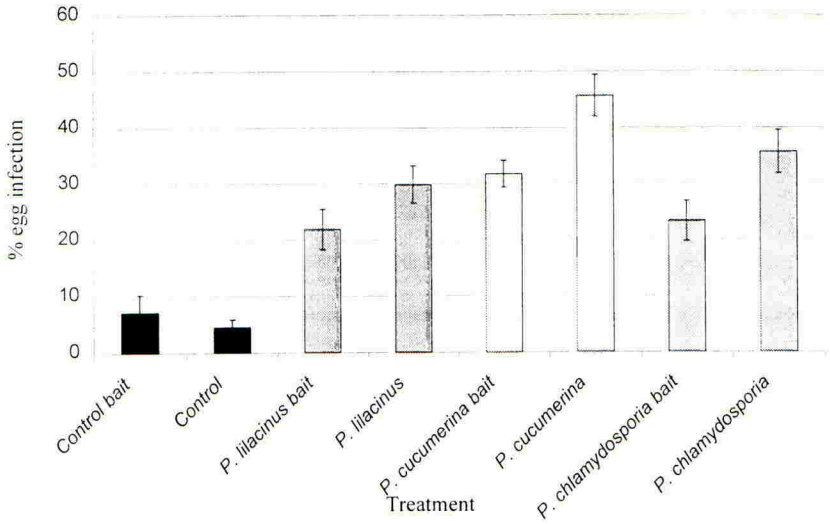

Figure 3: Comparison of percentage egg infection in cysts after 34 weeks and baited cysts in treatments

A wide range of techniques have been developed for monitoring, enumeration and identification of the three BCAs investigated in this paper. The fungi have been shown to significantly reduce the number of viable eggs and reduce the hatch rate, and would have a significant impact on future generations of the pest nematode. The fungi had no detrimental effect on the health or yield of the host crop and only attacked the egg stage in the nematode life cycle. The experiment outlined above demonstrated that the fungi remained in a viable state after harvest of the crop, and at densities in the soil similar to those added at the beginning of the experiment. When the soil was baited with fresh cysts there was a significant increase in the level of egg infection in the baits compared to the control in the presence of the fungi. This significant infection of baits may indicate that the fungus could be added to soil between potato crops, especially when a crop that favours fungal proliferation is grown. Several applications of the fungus to soil during the crop rotation could significantly increase the rate of decline of the pest nematode, therefore, reducing the time scale between planting of susceptible potato crops. To investigate this theory a number of plot trials have been set up. The demonstration of the presence of *P. lilacinus* significantly increasing the number of eggs produced per cyst is a concern although the viability of these eggs has not been tested. Further investigation of this anomaly is needed.

ACKNOWLEDGMENTS

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Antifungal activity of *Pseudomonas oryzihabitans* a bacterium symbiotically associated with *Steinernema abbasi* towards *Fusarium oxysporum* and *Rhizoctonia solani*

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ABSTRACT

Pseudomonas oryzihabitans (=*Flavimonas oryzihabitans*), a symbiont of the entomopathogenic nematode *Steinernema abbasi* significantly inhibited the mycelium growth of *Fusarium oxysporum* and *Rhizoctonia solani in vitro*. Antifungal compounds diffused from *P. oryzihabitans* exhibited chemotaxis toward *Fusarium* and *Rhizoctonia* mycelia in soft (0.2%) agar. Activity occurred at 15 - 28 °C but was strongest at the higher temperature. *P. oryzihabitans* cells were also tested for their root colonization and biocontrol abilities. A polyclonal antibody confirmed the bacterium had colonized roots. *P. oryzihabitans* induced soil suppressiveness against *Fusarium oxysporum*.

INTRODUCTION

A new species of an entomopathogenic nematode *Steinernema abbasi* isolated from soil in The Sultanate of Oman (Elawad *et al.*, 1999), has been shown to be a vector of the bacterium *Pseudomonas oryzihabitans*. The bacterium causes a rapid breakdown of the tissues of host insects on which the developing nematode *S. abbasi* feed. *P. oryzihabitans* was shown to produce unidentified freely diffusible compounds with fungistatic activity *in vitro*. The amounts of these compounds were correlated with the nutrients supplied in the culture medium. Bacterial filtrates confirmed the fungistatic effects of the cell-free solutions. The bacterial cells also affected the fungi and induced soil suppressiveness against *Fusarium oxysporum* and *Rhizoctonia solani* (Vagelas *et al.*, 2000; 2001; 2002). The objective of this work was to study the movement of bacterial cells and to visualise their presence in soil and in the rhizosphere. In addition, an attempt was made to induce suppressiveness against these soil borne pathogens.

MATERIALS AND METHODS

Microbial cultures

The pathogenic fungi used in this study were isolates of *F. oxysporum* f. sp. *lycopersici* (IMI 194417) and *R. solani* (obtained from Dr. R.T.V. Fox, The University of Reading, U.K.). The bacterium *P. oryzihabitans*, a symbiont of the entomopathogenic nematode *S. abbasi* was isolated from the haemolymph of infected wax moth larvae (*Galleria mellonella*) using the method described by Akhurst (1983). Pure colonies were multiplied in Nutrient Broth No2 (Oxoid; 30g/L), the suspension was centrifuged and the (bacteria) pellets were diluted with sterile tap water. Bacteria concentrations were determined using a spectrophotometer adjusted to the 600nm wavelength.

Bioassay

Petri dish tests: Inhibition of *F. oxysporum* f. sp. *lycopersici* and *R. solani* mycelia *by P. oryzihabitans* was assayed *in vitro* on nutrient agar (NA) plates. The bacteria were applied to the middle of a membrane placed on the NA plates at concentrations of 10^2, 10^4 and 10^6 cells/ml in 100μl. The control was 100μl of sterile distilled water (SDW). The toxic component that diffused through the membranes was recorded after 144 h by assessment of the extent of mycelium growth. Each treatment was replicated five times and the experiment was repeated once.

Motility tests: The motility of *P. oryzihabitans* was determined on semi-solid plates, containing 0.2% NA, and amended with Nutrient Both No2 (12g/L) to provide more nutrients (Issa & Wood, 1993). The bacterium was grown in Nutrient Broth No2 and diluted in SDW (1ml of 24 h broth into 10 ml SDW) before transferring them on to the plates. A drop (50μl) was spotted on the surface of each agar plate. All the plates were incubate at 28 °C and the swarming radius was recorded at intervals during the incubation time. Ten replicates per treatment were used and the experiment was repeated once.

Chemotaxis tests: *P. oryzihabitans* was tested for chemotactic response and degradation ability against the chitin-walled fungus *R. solani* and against *F. oxysporum* f. sp. *lycopersici*. The response was recorded using the semi-solid plate assay and the bacterium activity was recorded at 24 and 48 h. Replication was 20 fold.

Soil chemotaxis: Chemotaxis towards *F. oxysporum* in a sieved (2 mm) sand and loam 1:3 v:v (pH: 7,2) soil was assessed. Soil amended with 10^4 cfu/g of *F. oxysporum* spores plus fresh mycelia was placed into a 9cm Petri dish. Soil in the center ring of the Petri dish was removed with a sterile borer 5 mm in diameter, and the space thus left was filled with inoculated soil with bacteria (10^7 cells/ml) which had the same water potential as the surrounding soil (in order to eliminate any possibility for bacteria to move by water flow). Their lids were covered and the weights of dishes were recorded. At intervals SDW was added to bring the soil to the initial water potential. Total soil was approximately 100g/plate and was adjusted to field capacity (-0.03 MPa) by adding the appropriate amount of SDW (18g water/100g oven-dried soil). Soil samples were taken

from 3 points just 20mm distance from the inside ring and in the center of the outer ring, by vertically inserting a narrow spatula into the plate and withdrawing 1g soil (after mixture of 3 samples) into 9 ml 0,1 M buffer phosphate. Samples were taken from fifteen replicate plates incubated at 15, 20, 25 and 28 °C after 5 days incubation.

Colonization

Root colonization: *P. oryzihabitans* in soil and on rhizosphere were determined using a selective medium developed for this purpose, based on NA amended with antibiotics, 50μg/ml of ampicillin, 50 μg/ml of spectinomycin and 100ppm of benzimidazole.

Immunofluorescence: Serological techniques were used to observe *P. oryzihabitans* colonization using a polyclonal antibody developed for this purpose.

Pathogenicity tests: The pathogenicity of *P. oryzihabitans* from soil and roots was further tested in Petri dish bioassays and by injecting bacterium cells into *G. mellonella* larvae.

Impact of *P. oryzihabitans* to *Fusarium* propagules in soil

Trays assays were used to assess the fungistatic activity of the bacterium in soil. The inoculum density of *F. oxysporum* f. sp. *lycopersici* in the soil was estimated 10, 15, 20 and 35 days after application of treatments in trays with tomato seedlings. Soil samples (10-15 g) from the top 2-3 cm of soil beneath the canopy were taken from each treatment-replicate. Soil was dried and 1g of dry soil was used to quantity the fungus propagules by the dilution plate technique on malt extract agar and pentachloronitrobenzene (PCNB) based agar (Burgess *et al.*, 1994).

Statistical analysis

Analyses were performed using the SPSS 10.1 statistical programme. ANOVA and multiple range tests (Tukey's multiple comparisons) were applied to assess differences between treatments and identify statistical differences between means, respectively.

RESULTS

Bioassay

Petri dish tests: The mycelial growth of *F. oxysporum* f. sp. *lycopersici* and *R. solani* was significantly inhibited by the production of the inhibitory compounds of *P. oryzihabitans* that diffused through membranes on the agar at all concentrations (Table 1).

Motility tests: Results showed that *P. oryzihabitans* grew and "swarmed" in this medium. The bacterium was observed to produce visible rings during the swarming movement. The average rate of movement of *P. oryzihabitans* cells *in vitro* was 0.967 mm h^{-1} in the first 6 h and increased with time up to 1.63 mm h^{-1} after 22 h.

Table 1. Effect of bioactive compounds produced by *P. oryzihabitans* on mycelial growth (cm) of *F. oxysporum* and *R. solani*

Treatment	Control	10^2cells/ml	10^4cells/ml	10^6cells/ml	LSD $_{0.05}$
F. oxysporum	8.5 c	1.52 b	1.32a	1.3 a	0.988
R. solani	5.11 c	1.25 b	0 a	0 a	0.68

Chemotaxis tests: After 24 h mycelial exudates of *F. oxysporum* f. sp. *lycopersici* and *R. solani* (strongly) attracted the cells of *P. oryzihabitans* (Table 2). After 48 h *P. oryzihabitans* caused lysis of the mycelial structure of *R. solani*, which appeared as a thick layer around the fungi plugs. Rapid lysis of *F. oxysporum* was also observed without colonization of the plugs (in most cases).

Table 2. Chemotactic response and degradation ability of *P. oryzihabitans*

Treatment	*P. oryzihabitans* chemotactic response (mm)	Fungus mycelium response (24 h)	Fungus mycelium response after 48 h
P. oryzihabitans (alone)	28.0 a	-	-
-with *F. o. lycopersici*	41.0 b	No wall growth	Inhibition zones (1-2 mm)
-with *R. solani*	41.6 c	No wall growth	Fungus death
LSD $_{0.05}$	1.64	-	-

Soil chemotaxis: Population densities of *P. oryzihabitans* increased from $2.4x10^7 \pm 1.19x10^6$ up to $1.1x10^8 \pm 2.76x10^6$ cells/g soil after addition to the soil at all temperatures. Strong visible fungus limitation on chemotactic attraction by *P. oryzihabitans* was observed in all treatments. The chemotactic response was also present at 15 °C and *P. oryzihabitans* increased population densities from $2.4x10^7 \pm 1.19x10^6$ in untreated soil up to $4x10^7 \pm 2.09x10^6$ (treated with *F. oxysporum*) cells/g soil.

Colonization

Root colonization: When added to soil *P. oryzihabitans* survived well (log4.3 ± 0.098 cfu/g). A significantly high number of *P. oryzihabitans* cells were recovered immediately from root (log7.1 ± 0.054 cfu/g) and soil (log7.17 ± 0.044 cfu/g) after addition of *S. abbasi* to the soil. High bacterium populations confirmed the hypothesis that the bacterium was able in multiply on the root surface and was detected in all cases in the last sample (65^{th} day) suggesting that the bacterium multiplied in soil (log5.2 ± 0.12 cfu/g) and in rhizosphere (log6 ± 0.018 cfu/g) and possibly log5 up to log6 cfu/g will be the limit of the bacterium in soil and in rhizosphere respectively.

Immunofluorescence: Serological methods as a dot immunobinding assay (IDA) and an immunofluorescence colony (IFC) procedure using the specific polyclonal antibody (PC 451#2) against *P. oryzihabitans* showed that bacterium cells on the root surface increased in population size and produced more patches.

Pathogenicity tests: *P. oryzihabitans* isolated from soil showed fungistatic effects when used to challenge with *F. oxysporum* plugs *in vitro*. Cells isolated from roots initially did not control *F. oxysporum* growth but colonized and killed *F. oxysporum* hyphae after 96 h *in vitro*. *P. oryzihabitans* isolated from soil killed *G. mellonella* larvae after 14 h at 28°C. Cells isolated from roots showed variable rates of infection of *G. mellonella* causing death or paralysis after 18 h, but in some cases larvae remained active.

Impact of *P. oryzihabitans* to *Fusarium* propagules in soil

Fewer propagules of *F. oxysporum* were recovered in treatments with *P. oryzihabitans* (Figure 1). This shows that the bacteria cells are able to reduce the amounts of the pathogen in either the pathogenic growth or in the dormant phases.

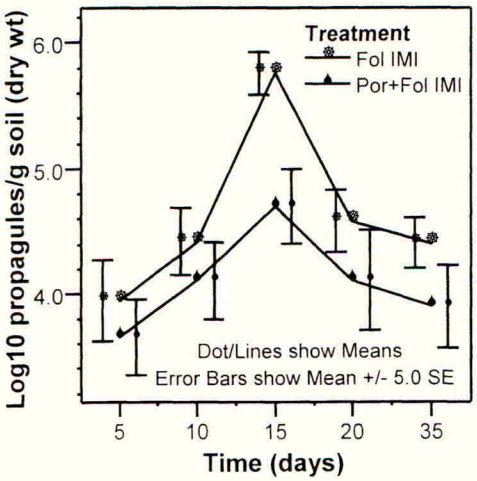

Figure 1. Effect of *P. oryzihabitans* (Por), cells on the survival of *Fusarium oxysporum* (Fol) in the soil.

DISCUSSION

This study shows that a small number of cells are able to have a very high competitive ability though the toxin(s) that defuse into the agar, supporting the conclusions of

Vagelas *et al.*, (2000). The motility of the bacterium was also demonstrated. *P. oryzihabitans* is able to multiply faster and exhibits stronger chemotaxis against fungi at high incubation temperatures (e.g. 25 or 28 °C). The numbers of cells recovered at 28 °C were significantly different from those at 15 and 20 °C (in the *F. oxysporum* treatment) and significant higher than in the bacterium only treatment (without added *F. oxysporum*) at the same temperature (28 °C). *P. oryzihabitans* was found to survive both in soil and rhizosphere for 65 days. Immunoflurescence techniques confirmed that the bacterium could multiply outside its nematode "host" and that this case probably is not one of classic mutualism (Elawad *et al.*, 1999). Moreover, there is less fungus survival in the bacterium treatment possibly through the effects of secondary metabolites and/or the toxin(s). It is confirmed that the bacterium induces soil suppressiveness against *F. oxysporum* and *R. solani* (Vagelas *et al.*, 2001, 2002).

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Possibilities and constraints of agro-ecosystem diversification as a pest management strategy: a simulation approach

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ABSTRACT

There is a rapidly increasing interest in using agroecosystem diversification as a pest management strategy. Using this strategy pest-disturbing and/or natural enemy-enhancing plants are intercropped with the crop, with the aim of decreasing the pest density. However, increasing the vegetational diversity of agroecosystems can have variable results depending on the species of herbivores, natural enemies and vegetation involved. There is an urgent need to develop a mechanistic framework to understand and predict the response of herbivores and natural enemies to spatial arrangements of vegetation in agricultural systems. In this paper we investigate the response of herbivore species to spatial arrangement of vegetation in agroecosystems by using an individual-based simulation model that includes behavioural based stochasticity, and spatial structures based on vegetation composition and structure. The model is used to determine optimal diversification strategy sets and hence generate guidance to establish an environmental benign control strategy in the field. In addition, it indicates which aspects of the ecology of the plants and insects involved are the determining factors to enhance the successful employment of habitat diversification.

INTRODUCTION

In recent years there has been increasing interest in integrating agroecosystem diversification with integrated pest management (Wood & Lenné, 1999). As a pest management strategy it relies on adding specific pest-disrupting or natural enemy enhancing vegetation to the agroecosystem, with the aim of decreasing pest density and damage in the main crop. However, field studies of herbivore population response to diversified environments show an enormous variation in the level of population regulation (Risch *et al.*, 1983; Andow, 1991). For example, in a review of diversification field studies, Andow (1991) reported that the population density of herbivores in polycultures compared to monocultures was lower in 52% of the studies, higher in 15%, equal in 13%, and in 20% inconsistent in repeated studies. As only herbivore densities were measured in most of these studies, successful pest regulation below economic damage thresholds is probably far below 50%. To account for this variability there is an urgent need to develop a mechanistic framework to understand and predict the response of herbivores and natural enemies to spatial arrangements of vegetation in agricultural systems. In this paper we introduce a simulation framework with which we investigate how insect behavioural ecology and agroecosystem composition influences the control efficacy of a diversified agroecosytem. Although there are a few examples showing that natural enemies can have an enhanced impact on herbivore populations in polycultures (Landis *et al.*, 2000), the response of herbivores to diversification is more likely to be related to a direct effect on the herbivore population, than to an indirect effect through enhancement of natural enemies of the

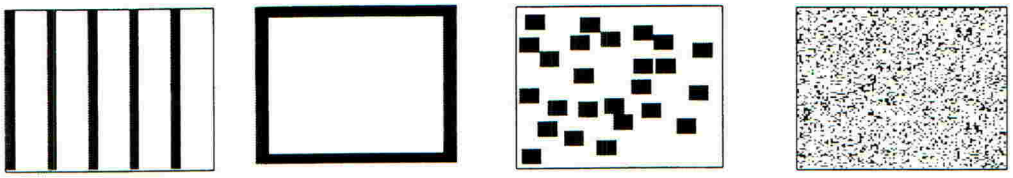

Figure 1. Examples of spatial structures used in simulations (based on 25% trapcrop coverage). (a) Intercrop 5x 5 rows (b) Border 7 rows (c) 25 Patches 10x10 (d) seedmix 25%

herbivore (Risch *et al.*, 1983; Andow, 1991). Therefore, in this paper we concentrated our simulation study on the direct response of herbivorous populations to heterogeneous environments.

The behavioural and chemical ecology of herbivorous insects and their natural enemies is well studied in laboratory and small-scale field experiments (Bernays & Chapman, 1994; Vet & Dicke, 1992). However, due to the small size of the insects involved further investigation into how such insect-specific ecological information can be used to understand insect population dynamics in open heterogeneous field systems is still needed. By using an individual-based spatially explicit modelling approach (DeAngelis & Gross, 1992) we can extrapolate our knowledge on individual behaviour of insects to population dynamics in the field and predict how particular herbivore species may respond to different agroecosystem diversification strategies (Cook *et al.*, 2002).

MODEL STRUCTURE

The models used in this study involve an individual-based simulation framework that includes behavioural-based stochasticity, and spatial structures related to vegetation composition and structure. A detailed description of the individual-based spatially explicit simulation framework will be published elsewhere, but the following gives a brief description of the model structure. The simulations are driven by assigning behavioural rule sets to individuals in the simulation environment. These behavioural rules (i.e. decisions) enable individuals to respond to stimuli in the computational environment in ways that emulate behaviour observed under laboratory or field experiments. Fundamentally, the individual-based model keeps track of the age, movement and position of each individual in the population. Individual movement decisions are based on the plant type and state (i.e. damage level) of the current position, which generally result in arrestment responses on preferred habitat types and displacement responses on less or non-preferred habitat types

The spatial environment is represented by a square lattice of 100x100 individual cells. The model is initialised with a specified fraction and spatial arrangement of two habitat types (see Figure 1 for examples). The vegetation types in the environment are based on species-specific preference ranking of habitat types. A typical simulation environment consists of 75% crop vegetation and 25% non-crop vegetation, which can be either a trapcrop (highly preferred host-plant) or repellent crop (unsuitable host-plant). The preference or non-preference for particular

habitat types is simulated by setting habitat-specific movement tendencies for the herbivore species in the system. In the simulations presented here a population of adult herbivores was initialised at the start of a model run (t=1) at randomly chosen habitat cells on the virtual landscape, simulating airborne colonisation. The population was allowed to disperse for a fixed amount of time and at each time-step a state-dependent movement was generated for each animal in the population. The movement tendency (stay or move), and movement length (hop to neighbouring cell or initiate flight), was dependent on the type and state of the currently occupied cell and calculated using randomly generated numbers and habitat-specific movement parameters. A non-preferred habitat type was typified by setting a high probability of an induced flight response and a preferred habitat type was typified by setting a high detection rate and low leaving tendency. The direction of movement was random. In the simulations presented here, animals were equipped with a sensory capacity to recognise preferred plant types while in-flight (i.e. olfactory guided upwind flight). In the model it is assumed that animals can always recognise their preferred habitat type at their current position and that they will interrupt a flight upon detection of the preferred habitat type within the generated flight path. Insects were assumed not to utilise (i.e. land on) repellent vegetation and respond to this type of vegetation by having an elevated wind borne emigration rate upon encounter of a repellent site. Animals can leave the simulation environment by age-specific natural mortality, by emigration to the air column or by crossing the grid edge. For each time-step the model records the animal density and cumulative number of herbivore days in each site. Mean herbivore-induced crop damage for a particular simulation scenario was estimated as the mean number of herbivore days in a crop cell and was used as the main output parameter to compare simulation runs.

RESULTS & DISCUSSION

Trap cropping strategy

To investigate the population regulatory effect of adding trapcrop vegetation to an agroecosystem, population dynamics of a hypothetical herbivore species was simulated in an environment with a fixed amount of trapcrop (25%) in three different spatial arrangements. The herbivore damage levels in the main crop were estimated for agroecosystems with or without an additional insecticide treatment to the trap crop (Figure 2). The addition of an attractive trapcrop to the agroecosystem significantly reduces the herbivore damage in the crop without any insecticide treatment (black bars in Figure 2). This effect of the attractive trapcrop is dependent on the spatial arrangement of the trapcrop vegetation. A trapcrop employed as a border around the main crop reduces the damage by a factor of 0.2, compared to the damage in a monocrop, whereas the trapcrop arranged in 25 patches or 5 intercropped rows reduces the damage by a factor of 0.46 and 0.52 respectively.

Using the same simulation framework, Potting *et al.* (unpublished) investigated several factors affecting the control mechanism of trap cropping in detail. They showed that successful employment of a trapcrop in an agroecosystem is dependent on the relative strength of attraction *and* retention of the herbivores by the trapcrop plants. The spatial arrangement and density of non-crop vegetation in relation to the perceptual range and mobility rate of the herbivore affects the population response to the diversified environment and hence determines the control efficacy of polyculture. Small arthropod herbivores, such as mites, thrips, aphids and whiteflies, that have an airborne colonisation pattern, limited host detection ability and

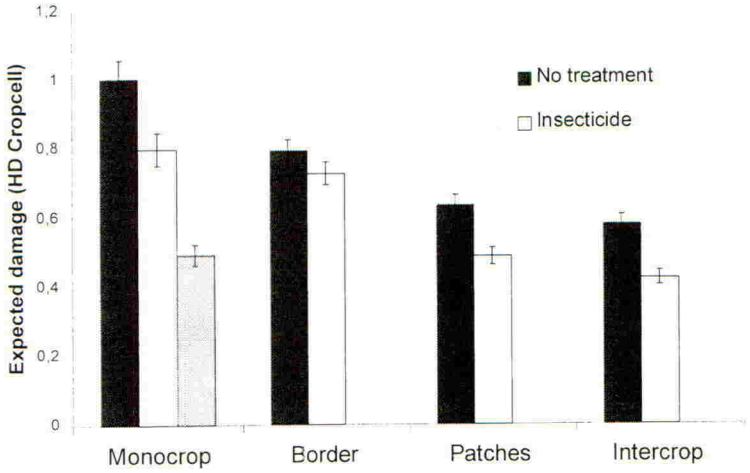

Figure 2. Expected damage levels (± SD) for crops in agroecosystems with trapcrops (25% of crop area) employed in three different spatial arrangements, without (black bars) or with (white bars) an insecticide treatment of the trapcrop area. The grey bar indicates whole field insecticide treatment to a monocrop.

slow displacement speed, are difficult to control with a trap cropping strategy. In contrast to small insects, also referred to as aerial plankton, bigger insects, such as beetles, butterflies and moths are generally highly mobile, have directed flight and good sensory abilities that enable oriented movements. Simulation results show that good control effects of diversification can be achieved for these types of herbivores, if plants with strong behaviour-altering characteristics are chosen and employed in an optimal spatial arrangement.

Insects perceive a trapcrop environment as favourable and stay longer due to a reduced fraction of emigration events. For example, in our simulation environment a typical population (500 animals for 50 timesteps, displacement speed 10) spent 10,667 ± 571 Herbivore Days (HD) in the monocrop, whereas in an intercrop system the total mean number of HD was 13,516 ± 454. The increased activity of a herbivore population in a trapcrop system generally does not lead to increased damage levels in the crop due to the majority of the population being retained in the trap crop. However, for herbivore species that preferentially colonise agroecosystems containing attractive trapcrops, the population pressure on this host can become too high, resulting in an overflow of herbivores into the main crop. One way to circumvent this problem has been to treat the trap crop with an insecticide (Hokkanen, 1991). Simulation results in this study show that an insecticide treatment of the trapcrop enhances the control efficacy (Figure 2). One localised treatment to the trapcrop area with a knockdown insecticide active for a 5 day period further reduced the expected damage by a factor of 0.23 for patches, 0.27 for intercrop, 0.08 for border and 0.20 for monocrop, compared to expected damage in untreated agroecosystems (Figure 2). The control efficacy of an insecticide treated trapcrop in the patches and intercrop arrangement was as effective as a whole field treatment of a monocrop

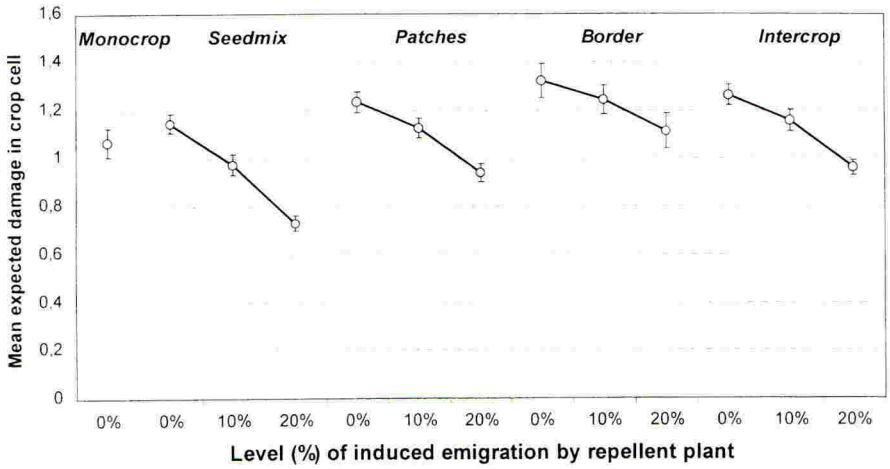

Figure 3 Expected damage levels (± SD) in crop with added repellent vegetation (25% of crop area), with varying repellent strength and four different spatial arrangements.

(indicated by the grey bar in Figure 2). Thus, the same control effect can be achieved with a 75% reduction in insecticide input. Whether or not an additional insecticide treatment of the trapcrop is an economically feasible strategy depends on the balance between the savings made by treating only a proportion of the field and the losses made by devoting part of the field to the trapcrop.

Repellent strategy

To investigate the effect of intercropping repellent vegetation on herbivore population response, the expected damage levels in simulated agroecosystems were estimated for repellent plants with varying levels of induced emigration (e) employed in four different spatial arrangements The results are summarised in Figure 3. The population control efficacy of repellent plants is dependent on the level of induced emigration imposed by the repellent plants. Intercropping non–crop vegetation with a neutral ($e_{repel} = e_{crop}$) or low ($e_{repel} = 2 \times e_{crop}$) effect on emigration rates actually increases the herbivore damage level in the crop. This increase in damage is caused by the herbivore population concentrating its activity in a smaller crop area (75% crop area, 25% non-used repellent area). Thus, a repellent-based intercropping strategy only works when the non-crop vegetation forces the population to emigrate from the agroecosystem. Repellent plants with a strong emigration-inducing effect on insect behaviour ($e_{repel} = 4$ to $8 \times e_{crop}$) can significantly reduce damage levels to the crop in the agroecosystem. Repellent vegetation applied as a seed mix resulted in the best control effect compared to the other spatial arrangements and the damage levels are significantly below the damage level in the monocrop. This improved control effect is due to the higher encounter probability when the repellent is distributed randomly. This is reflected in the mean number of animals (± SD) that emigrate from the field after encountering a repellent vegetation ($e_{repel} = 4$): 290.4±11.1 (out of

the initial 500) for a repellent applied as a seedmix, 203.4±11.0 for patch treatment, 201.3±12.6 for intercrop and 141.4±11.4 for border treatment.

CONCLUSIONS

The spatial arrangement and density of non-crop vegetation in relation to the perceptual range and mobility rate of the herbivore affects the population response to the diversified environment and hence determines the control efficacy of polyculture. Population regulation efficacy is further dependent on the strength of the behavioural effect of the added vegetation. Induced behaviours including attraction towards and retention within the trap crop vegetation can be utilised within a trapcrop control strategy, whereas a repellent-based strategy is largely dependent on the rate of emigration induced by the repellent vegetation. The wide variation in population responses of herbivores to diverse agroecosystems (Andow, 1991) is replicated in our simulation framework using different simulation scenarios by varying the behavioural features of the herbivore and ecological characteristics of the agroecosystem. Simulation results show that the population regulation effect of diversification can be positive, negative or negligible. Thus both the behavioural ecology of the target herbivore and the ecological characteristics of the agroecosystem influence the control effect of the vegetation in diverse agroecosystems.

ACKNOWLEDGEMENTS

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Alien pests: Opportunities and risks for biological control

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ABSTRACT

Quarantine controls operate to limit the introduction and spread of alien pest species. However, increasing international trade in plants and plant products provides opportunity for invasive species, including pests and diseases, to be dispersed to new regions of the world. Such accidental introductions of alien species can pose a significant threat to existing biological control systems, which are particularly well developed within protected cultivation, and challenge quarantine services to devise control measures that minimise disruption to existing pest management programmes. The use of biological control against alien pests in the UK is reviewed, with particular reference to the South American leafminer, *Liriomyza huidobrensis*. Opportunities and risks associated with the use of exotic biological control agents are discussed.

INTRODUCTION

Protected horticulture represents a key sector for the implementation of biological crop protection practices. However, the production of many protected crops, especially ornamentals, is heavily reliant on imported plants and propagation material from an increasing diversity of international sources. This has provided a pathway for a number of invasions of harmful insects in recent years (van Lenteren *et al.*, 1987). Crops grown under protection provide a favourable environment for the establishment of pests introduced with plants from tropical or sub-tropical climates, which then have the potential to disperse to new foci. Imports of propagation material have increased threefold in the UK, in the period from 1990-2000 (DEFRA, 2001), highlighting the increasing risk from this material alone.

Although quarantine services aim to prevent the introduction and spread of harmful organisms, both species listed in international quarantine legislation and many others that are not, continue to be introduced (Cannon *et al.*, 1999) presenting a continuing challenge in devising and maintaining effective eradication or containment strategies. This will be discussed with reference to both the use of existing biological control agents (BCAs) (i.e. those utilised against pest species already established in the UK) and the use of exotic BCAs.

BIOLOGICAL CONTROL AND QUARANTINE PESTS

Quarantine measures play an important role in minimising new pest incursions, which can cause substantial disruption to existing biological control programmes. However, the introduction of some invasive species, such as Western flower thrips (*Frankliniella occidentalis*) cannot be prevented and this species rapidly established and spread throughout

glasshouses in Western Europe (Baker *et al.*, 1993). Quarantine measures merely slowed the spread to enable management techniques to be developed. It remains one of the most damaging pests of protected cultivation in Western Europe and effective biological solutions are still being devised for a number of crops.

Eradication or complete elimination of a pest requires consideration and utilisation of a wide range of pest management techniques. Whilst physical measures such as destruction of affected plants/crops provide the most effective and rapid means of achieving eradication, on a large scale this can result in severe economic impacts that may prove socially and politically unacceptable. Thus, where effective treatment options are available, these can be offered as an alternative to plant destruction. Such options have traditionally relied on chemical measures, which are readily available, rapid, effective, and familiar to growers. Biological control solutions have not been considered appropriate for quarantine campaigns, largely because they have not been available, or where available, they have proved more complex, variable in efficacy, too slow, or dependent on the specific circumstances under which they are deployed. However, quarantine treatments must be compatible with existing crop management practices, and thus, pesticides remain the dominate treatment in most ornamental crops. In contrast, biological control has become the dominant pest management technique within edible crops, in response to consumer concerns over pesticide residues, and withdrawals of products and uses resulting from national and European Community reviews of plant protection compounds under Directive 91/414/EC.

Inundative introductions of parasitoids, have been deployed to assist in eradication and containment programmes of *L. huidobrensis* (using *Dacnusa sibirica* and *Diglyphus isiae,* in crops such as tomatoes) and *Bemisia tabaci* (using *Encarsia formosa* in poinsettia crops). These have been based on existing programmes developed for control of closely related pest species already established in these crops, but usually employing higher rates than are commonly used against established species. Sustaining high parasitoid introduction rates is often expensive and further work is needed to optimise application methodology for quarantine use. Quarantine programmes require integration with the use of selective insecticides, such as nicotine against adult stages, or buprofezin against larval *B. tabaci.* Complete eradication of the invasive pest is usually only achieved by crop clearance and glasshouse sterilisation at the end of the cropping season.

Case study: biological control strategies for *L. huidobrensis*

When *L. huidobrensis* was first found in the UK in 1989, a treatment programme was devised that integrated a range of control measures including physical and cultural techniques, but was reliant on chemicals rather than biological agents (Cheek *et al.*, 1993). This proved successful in the early years of the campaign but multiple annual outbreaks resulted in repeated pesticide applications, encouraging the development of resistance and exacerbating control difficulties. Official policy for dealing with outbreaks of *L. huidobrensis* at plant production sites not registered for propagation, changed from one of complete eradication, to pest suppression and containment. Eradication is nevertheless achieved on most production nurseries, and there has been a steady decrease in the incidence of outbreaks of *L. huidobrensis* in England since 1995.

Problems experienced with control in leafy salad crops, together with the decreasing availability of effective insecticides, stimulated the development of an alternative biological strategy for management of this quarantine species, using the entomopathogenic nematode, *S. feltiae*. In the foliar environment high humidity levels (>90%) are critical to success, but only need to be maintained for 8-10 hours (at 15-25°C) to enable the entry of the nematode into the oviposition puncture or leaf tear (Williams & Macdonald, 1995). Such conditions are readily maintained overnight without adversely affecting crop production, and enabled a successful treatment programme to be developed for commercial use in leafy salad crops (Head *et al.*, 2002; Williams & Walters, 2000). Higher leaf miner mortalities (up to 97% at the high rate of 5.4 x 10^5 *S. feltiae* per m^2) were achieved using *S. feltiae*, than conventional chemical treatments, for example with abamectin (65% mortality) (Figure 1). In addition to the high levels of efficacy achieved, *S. feltiae* has a broad target host range, is not subject to regulation or registration in the UK, and therefore can be adopted for use as a biological pesticide against other quarantine pests. This example illustrates the potential for achieving the goals of quarantine policy (i.e. eradication) through the integration of the full range of control options, including legislative and biological methods.

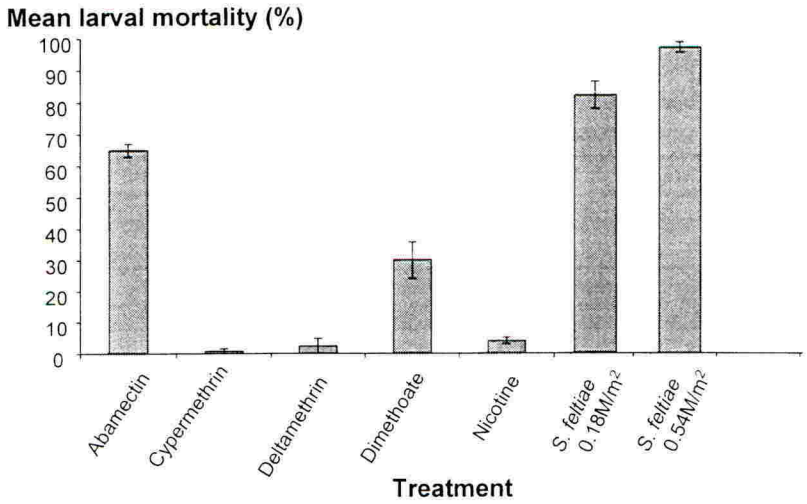

Figure 1. Larval mortalities of *L. huidobrensis on Brassica rapa "chinensis"* from foliar applications of the entomopathogenic nematode *S. feltiae* in glasshouse trials vs. insecticide efficacy bioassays (after Head *et al.*, 2002)

CHALLENGES FOR BIOLOGICAL CONTROL

Invasive alien species are currently the subject of much international debate, as a result of increasing concerns over national and regional biodiversity (Huber *et al.*, 2002). Whilst the practice of biological control has been highly successful in providing an economically

viable, sustainable and a more environmentally attractive alternative to pesticide usage in many crops (Hokkanen, 1999), the use of exotic BCAs, as a solution to exotic pest incursions has come under increasing scrutiny. For example, concerns are expressed by environmentalists and others that alien species pose particular threats to the conservation of biodiversity, via processes of competition, predation, habitat alteration, disease and genetic effects (hybridisation) involving native species.

Although it is suggested that there are few documented instances of damage to non-target organisms, or the environment, as a result of the release of exotic species for biocontrol purposes (Simberloff & Stirling, 1996), evidence for negative indirect and non-target effects of such programmes is accumulating (Cottrell & Yeargan, 1998). The majority of work has focused on classical introductions against invasive weeds, but more recently the impact of introductions within protected crops has been studied (Loomans *et al.*, 2002). The existence of such negative impacts should not in itself be used to prevent such introductions, but highlights the need for thorough risk assessment prior to release. For proponents of biological control, it is imperative to support mechanisms that increase public confidence in biological control as a pest management strategy.

A number of international organisations have produced recommendations to improve regulatory oversight in this area, particularly in the light of increasing commercial interest in importation for biological control purposes. The Secretariat of the International Plant Protection Convention (IPPC) facilitates the development of internationally agreed standards for the application of phytosanitary measures to prevent and control the spread of plant pests by the international plant trade. One such standard, the "Code of Conduct for the Import and Release of Exotic Biological Control Agents (1995)" sets out the responsibilities of government authorities, importers and exporters. It is intended to facilitate the safe import, export and release of exotic BCAs by introducing internationally acceptable procedures for all public and private bodies involved, particularly where national legislation does not exist or is inadequate. These standards are recognised by the World Trade Organisation (WTO) "Agreement on the Application of Sanitary and Phytosanitary Measures" (SPS Agreement). Within Europe, this Code of Conduct was perceived as too prescriptive, particularly for countries with little or no environmental legislation, or insufficient infrastructure to enable implementation. The European and Mediterranean Plant Protection Organisation (EPPO), has therefore developed its own "guidelines for safe use of BCAs". These specify minimum requirements for the import of exotic BCAs for research under contained conditions (EPPO, 1999) and for import and release of exotic BCAs (EPPO, 2001). A standard, outlining recommendations for environmental risk analysis for BCAs, is under development. A list of agents widely used in the EPPO region has been compiled, which can be used to facilitate the assessment of the suitability of proposed introductions into the same or similar ecological regions.

The OECD is also developing harmonised data requirements for countries considering registration of invertebrate BCAs, as part of national plant protection products legislation (although it is not currently being considered by the EU under Directive EC/91/414). Although there are no proposals for registration of 'macro' invertebrates in the UK, an increasing number of countries are doing so. Developing an appropriate level of regulation to improve confidence in the biological control industry, whilst not stifling further development, is the key challenge facing regulators and the biological control industry. The

experience of product development within the microbial pesticide sector, particularly the lack of registration of new products, is not encouraging. The development of more selective pesticides and compounds with improved environmental profile is particularly welcomed by quarantine services, to increase options, but also represent further challenges to increasing the use of BCAs in a competitive market.

A CO-ORDINATED APPROACH

New pest incursions provide both opportunities and risks for biological control. Compatible quarantine controls can protect biological programmes already used for existing pest complexes, and new strategies can be developed to manage new pests. These may include the use of non-native species of BCAs, for which more effective regulatory mechanisms need to be developed to facilitate adequate and transparent consideration of the risks and benefits of any introduction. Control strategies may also incorporate other, non-chemical components, such as the use of pheromones and attractants. These alternative methods may be threatened by inappropriate regulation under 91/414/EC, which would represent a retrograde step in the implementation of national programmes to encourage the development of more sustainable food production and pesticide reduction.

Whilst phytosanitary interests have been largely directed towards protecting commercial agriculture, horticulture and forestry, risks to the wider environment are also now being considered. Internationally, the interests and activities of the IPPC, and the Convention for Biodiversity (CBD) are converging. The original purpose of the IPPC was to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control. The CBD similarly requests contracting parties to "prevent the introduction, control or eradicate those alien species which threaten ecosystems, habitats or species" (Article 8h). It has agreed to adopt a number of guiding principles on how to develop effective strategies to minimise the spread and impact of these invasive alien species. In the UK, the formation of the Department for Environment, Food & Rural Affairs (DEFRA), replacing the former MAFF offers opportunity to integrate plant health and environmental protection regulations and to develop a more co-ordinated approach to risk assessment procedures for exotic BCAs.

This paper has dealt primarily with the protected crop environment, a sector at particular risk from pests introduced via international trade. However, the broadening of the concept of what constitutes a "pest", and the potential inclusion of environmental damage, could lead to greater impetus for biological control programmes to be implemented, in amenity and other areas. When faced with a first introduction of a new pest species, eradication measures must utilise the most effective measures available, and those that can be rapidly deployed. However, the utilisation of biological methods is a key component in the development of longer-term solutions, particularly in edible crops and amenity areas.

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