POSTER SESSION 4E

POST-GRADUATE STUDENT RESEARCH

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Poster Papers: 4E-1 to 4E-14

The phenology and host plant preferences of *Lygus rugulipennis*, a pest of glasshouse cucumber crops

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ABSTRACT

An increase in the reported damage symptoms to cucumbers grown in commercial glasshouses, caused by *Lygus rugulipennis*, has occurred throughout the 1990s. Studies on the field population dynamics of *L. rugulipennis*, movement into cucumber crops, and host plant preferences are in progress. Adult populations peaked in early October following an August peak in numbers of nymphs. Choice tests revealed that females exhibited a preference for cucumber leaves over nettle.

INTRODUCTION

Following the increase in biological control against glasshouse pests such as whitefly (*Trialeurodes vaporariorum*), the consequent reduction in use of broad spectrum chemicals has allowed capsids (Heteroptera: Miridae) such as *Lygus rugulipennis* to survive in the crops (Jacobson, 1997). *L. rugulipennis* causes damage to the fruits and growing tips of cucumber plants by feeding and oviposition (Jacobson 1999).

The work presented is part of a study of the ecology and biology of *L. rugulipennis* which will be used, in conjunction with other current work, to design and implement an integrated pest management (IPM) strategy for capsids on protected cucumber crops. Three questions are currently being addressed in this study: what is the phenology of adult capsids in the field; when do they move into the glasshouse crops; and do they show a preference for these crops over their wild hosts? Results of investigations into field population dynamics and host plant choice are presented along with the method that will be employed to investigate the timing and direction of movement of the bugs into the glasshouse crop.

MATERIALS AND METHODS

Field population dynamics

Sweep net sampling was carried out at Close House Field Station (University of Newcastle (NZ 1265)) from April 1999 to determine populations of *L. rugulipennis* throughout the year. Standardised sampling at five sites began in June 1999 when occurrences of *L. rugulipennis*

became more frequent than occasional detection of individual adults. A sample consisted of ten 90° sweeps taken whilst walking at an even pace through the vegetation. Two samples were taken at each site and the results pooled. A mixed clover (*Trifolium repens*) and cereal plot (predominantly clover) of 750 square metres, sown in February 1999, was sampled from August 1999 when *L. rugulipennis* were discovered at the site, until November 1999. The plot was monitored through the winter and sampling recommenced on 27th April 2000.

Host plant preferences

Individual 5th instar *L. rugulipennis* were isolated from culture and kept in petri dishes lined with filter paper, and held at 21°C (± 2°C) until adult emergence. Each individual was provided with moist cotton wool and a fresh green bean each day throughout nymphal development. When the adult emerged, food was withdrawn for approximately 24 hours prior to preference tests. All adults were used in tests within 72 hours of emergence.

Individual adults were introduced to a rectangular choice chamber (225 mm x 120 mm x 85 mm) which was lined with paper towel (replaced after each trial). Single leaves of cucumber (*Cucumis sativa* var *sativa*) (crop host) and nettle (*Urtica dioica*) (wild host) were placed at either end of the chamber. Adults were introduced to a central dish (40 mm diameter, 50 mm height) and the whole arena was darkened with a blackout. Activity was recorded using a video camera (Baxall CD9242/IR, sensitive to low light levels and infra-red illumination from an array of light emitting diodes) and a Panasonic AG-6040 time-lapse video recorder. Each trial lasted one hour. Videos were analysed to establish the species of leaf first contacted, whether this contact involved feeding or not, cumulative time that the bugs spent in contact with each plant species, and number of separate contacts made.

RESULTS AND DISCUSSION

Field population dynamics

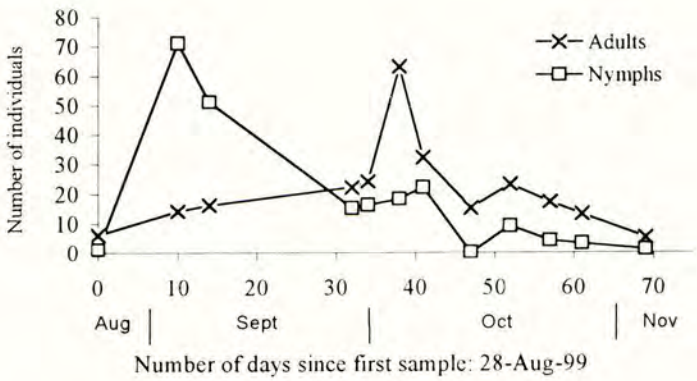

Figure 1. Abundance of *L. rugulipennis* in the field through the season: Close House field station – clover plot

Peak numbers of *L. rugulipennis* nymphs occurred in the clover plot in late August. Adult numbers peaked in early October (Figure 1). Numbers of *L. rugulipennis* were very low at all other sites throughout the sampling period, with no nymphs found. Occasional adults were found from early May at various sites near Close House. In Humberside, where *L. rugulipennis* have invaded commercial glasshouses, individuals have been found in the crops from early May.

Initial results indicate the pattern of occurrence in the field of *L. rugulipennis*. Further sampling in 2000 and 2001 will confirm the bionomics of this species in the north of England.

Host plant preferences

Initial laboratory studies reveal that males and females differed in their responses to the choice test with cucumber and nettle (Figure 2).

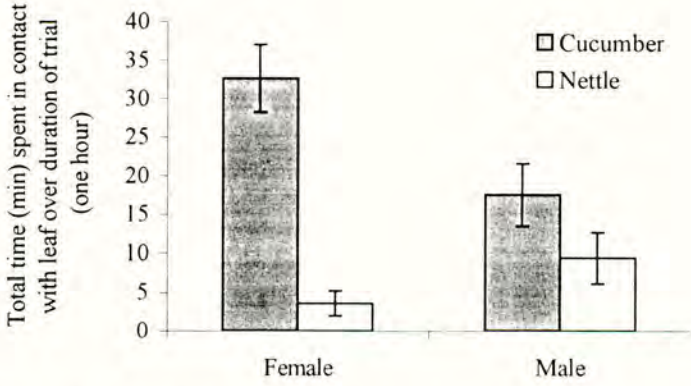

Figure 2. Mean time spent in contact with each host species during a trial of one hour duration. Bars indicate one standard error.

Female *L. rugulipennis* spent significantly more time in contact with cucumber leaves than with nettle leaves (Wilcoxon statistic = 195.0, $P \leq 0.001$, $n = 20$). Males did not show any significant preference.

Females made contact with cucumber as their first choice more often than nettle (although this contact did not always result in feeding) ($\chi^2 = 6.05$, $P < 0.05$, $n = 20$). Males showed no such preference in their first choice. Both male and female *L. rugulipennis* made fewer separate contacts with leaves when cucumber alone was chosen in a trial than when nettle or both species were chosen; i.e. when they made contact with a cucumber leaf they tended to stay there rather than move away (Mann Whitney U test: females - $W = 105.5$, $P < 0.0001$, $n = 20$; males - $W = 34.5$, $P < 0.01$, $n = 18$).

L. rugulipennis is a generalist species in terms of host selection for feeding (Holopainen & Varis, 1991). However, of the 51 host plants recorded from the British Isles, only 18 support

oviposition (Holopainen & Varis, 1991), suggesting that females may be limiting their choice of host plants to those which would maximise offspring survival. One possible explanation for females showing a preference towards cucumber plants may be that the species is a better quality host, particularly prior to egg maturation. Males do not exhibit this discrimination. Holopainen & Varis (1991) have suggested that nitrogen content of host plants may be the key factor in determining host choice of *L. rugulipennis*. Although females demonstrate a preference towards cucumber in choice tests it is not yet known whether they detect cucumber host plant volatiles in the field.

Further work: movement of *L. rugulipennis* into glasshouse cucumber crops

A small scale experimental system has been set up at the Close House field station to mimic the commercial glasshouse situation. Cucumber plants were established in a glasshouse twenty five metres from a plot of clover (*Trifolium repens*) known to contain high numbers of *L. rugulipennis* in 1999 (described above). Twenty sticky traps (clear plastic cylinders, 30cm high and 15 cm diameter, fixed 1.5 metres above the ground, painted with Tangle-trap © insect trap coating (The Tanglefoot Company, 314 Straight Avenue SW, Grand Rapids, MI 49504, USA)) were placed at five metre intervals around the perimeter of the clover plot. Twelve further traps were placed around the glasshouse. All traps were divided into sectors (north, south, east and west). Traps were checked every fortnight and the number and sex of *L. rugulipennis* individuals in each sector noted. Use of directional sticky traps will determine the predominant direction of movement of capsids in the vicinity of the glasshouse. Interception traps (white fine mesh with strips of clear tape painted with insect trap coating) were used to cover the open vents of the glasshouse, and strips of acetate with insect trap coating were attached to the sides of the glasshouse structure. This work will continue throughout the summer. Movement of adults into the glasshouse crops will be analysed. Further analysis of sticky trap catches until adults are no longer caught will indicate movement patterns around the experimental site throughout the season and may also indicate the direction of adult movement prior to overwintering.

ACKNOWLEDGEMENTS

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Assessing the field performance of seed treatments for the control of Fusarium seedling blight of wheat using quantitative PCR

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ABSTRACT

Quantitative PCR assays were used to determine the amount of *Fusarium* spp. and *M. nivale* in three seed lots produced from inoculated field plots and also a commercially produced seed lot. Seed was treated with bitertanol plus fuberidiazole (375 + 23 g/l) or fludioxonil (24.3 g/l) at manufacturer' recommended dose rates, or left untreated. The germination of seed was improved at both 4°C and 18°C through the use of seed treatments. Seed was drilled at two trial sites, seedlings were removed from each plot at the third leaf stage and the amount of *Fusarium* spp. and *M. nivale* determined in order to assess fungicide performance. A significant reduction in pathogen DNA was observed through the use of both seed treatments. The dominant pathogen present was *M. nivale*. Differences between the performance of the seed treatments used were also observed.

INTRODUCTION

Fusarium seedling blight can be caused by a variety of *Fusarium* species or *Microdochium nivale*, however in the UK the dominant pathogens responsible for the disease are *M. nivale* and *Fusarium culmorum*. Seed borne contamination is considered to be the primary source of inoculum (Paveley *et al.*, 1996). The control of seedling blight in infected seed is primarily achieved through seed treatment. The threshold for treatment in the UK is 5% infected seed. In 1997, 94% and in 1998, 93% of seed samples tested for *M. nivale* by the Official Seed Testing Station, Edinburgh, exceeded the 5% threshold for sowing untreated seed (V. Cockerell pers. comm.). The performance of any particular treatment towards seedling blight is considered difficult to measure using traditional methods due to the number of pathogens able to cause infections. Advances in PCR-based detection of plant pathogens have allowed the quantification of specific pathogens associated with infections. The technique has been employed to assess the performance of fungicide spray treatments towards ear blight pathogens (Doohan *et al.*, 1999) and seed treatment applications for the control of eyespot infections (Gac *et al.*, 1999). The present investigation presents results for the detection and quantification of *Fusarium* spp. and *M. nivale* on seed and seedlings in fungicide seed treatment efficacy trials.

MATERIALS AND METHODS

Infected seed cv. Equinox was produced according to the method described by Edwards *et al.* (1998). A commercially produced seed lot (lot 1) and three seed lots from inoculated field plots (lots 2-4) were used in fungicide seed treatment trials. Seed was treated with Beret Gold (fludioxonil 5 g AI/100 kg seed) or Sibutol (bitertanol 56 g AI + fuberidazole 3.5 g AI/100 kg seed) according to the manufacturers' recommendations, untreated seed was used as a control.

Germination tests were performed on all untreated and treated seed lots. One hundred seeds were placed on moist filter paper and incubated in the dark at 18°C or 4°C for one or two weeks respectively, four replicates per treatment were used.

Seed was drilled into plots at a rate of 300 seeds/m^2 according to a randomised block design at trial sites at Torphins, Aberdeenshire (plots 1.5 x 5m) and Harper Adams, Shropshire (plots 1.75 x 12m) with four replicate treatments at each site. Thirty plants were removed from each plot when three true leaves had emerged for PCR analysis.

DNA was extracted from 10 g of seed and from 30 seedlings according to the method outlined by Edwards *et al.* (1998). Pellets were air dried overnight, dissolved in 0.2 ml TE buffer and incubated at 65°C for 1 h and at 20°C for 24 h. Total DNA was determined by absorbance at 260 nm and 280 nm, samples were diluted to 40 ng/µl in TE buffer.

Oligonucleotide primers specific to *Fusarium* spp. and *M. nivale* were obtained from Novartis Agribusiness Biotechnology Research Inc. Competitor fragments for quantitative PCR were constructed based on the method described by Förster (1994). Template DNA from test samples was amplified using a PTC-100 Thermal Cycler and a PCR programme comprising an initial denaturation step of 1 min. 15 s at 95°C followed by 35 cycles of 15 s at 95°C, 15 s at 60°C, 45 s at 72°C followed by a final extension step of 4 min. 15 s at 72°C.

The final concentration of pathogen DNA in infected samples was determined using the method described by Edwards *et al.* (1998) and the results were analysed using ANOVA with site, seed lot and seed treatment as factors.

RESULTS AND DISCUSSION

Germination was improved at both 4°C and 18°C (Table 1) through the application of either seed treatment, though was lower in the untreated at 4°C than 18°C for seed lots 1, 3 and 4. For seed lot 2 where *F. culmorum* was the dominant pathogen, the germination of the untreated was unaffected by reduced temperature. These results suggest that the ability of *M. nivale* to inhibit the germination of infected seed is dependent upon temperature, this does not appear to be the case for *F. culmorum*.

Of the three infected seed lots produced, seed lot 4 contained the most *M. nivale* DNA and seed lot 2 the highest amount of *Fusarium* spp. DNA (Figure 1). Quantitative PCR analysis (Figure 2) of seedlings from the Aberdeen and Harper Adams trials show that infections from *M. nivale* were controlled well at both sites by either seed treatment.

Table 1. Germination of treated seed lots.

Temp	Seed lot1			Seed lot 2			Seed lot 3			Seed lot 4		
	unt	b + f	flu	unt	b + f	flu	unt	b + f	flu	unt	b + f	flu
4°C	94	99	100	72	90	92	76	97	97	68	99	99
SED (47 df) 2.02; $P = <0.001$												
18°C	99	99	99	72	89	89	87	95	94	88	98	99
SED (47 df) 1.71; $P = <0.001$												

unt = untreated; b = bitertanol; f = fuberidazole; flu = fludioxinil

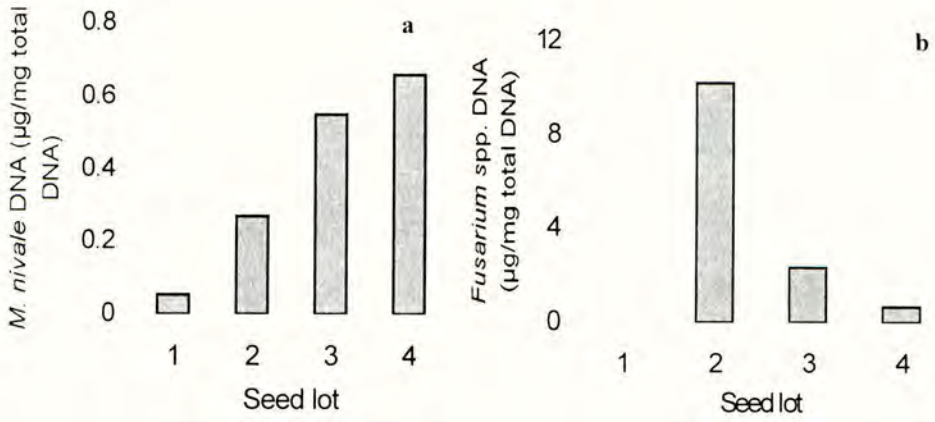

Figure 1. Quantification of (a) *M. nivale* DNA and (b) *Fusarium* spp. DNA in infected seed lots.

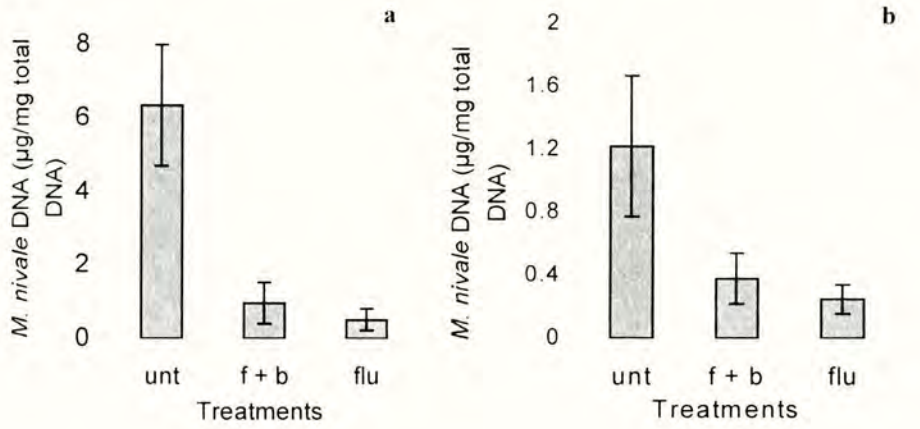

Figure 2. Mean *M. nivale* DNA at (a) Harper Adams and (b) Aberdeen trial sites. Bars indicate SE. Untreated significantly ($P < 0.05$) greater than treated at both sites.

The results for *Fusarium* spp. are not shown as these pathogens were either not detected or present in extremely low amounts in all treatments at both sites with the exception of the untreated for Seed lot 2 which was reduced to very low concentrations when a seed treatment was applied. This suggests either that *Fusarium* spp. was less aggressive as a seedling blight pathogen or that *Fusarium* spp. competed less well with *M. nivale* in mixed infections under these field conditions. The results may therefore suggest that the two treatments used show greater efficacy towards *Fusarium* spp. than *M. nivale*.

Seedlings from the Aberdeen trial site contained more *M. nivale* DNA than those from the Harper Adams trial. This may be due to varying seed bed conditions between the two sites.

This study has demonstrated the benefits from the use of fungicide seed treatments for the control of seedling blight infections during the early stages of crop development. Germination tests give an indication of the likely improvement in crop emergence through the use of seed treatments. The results presented for the quantification of *M. nivale* and *Fusarium* spp. in emerged seedlings was the first time quantitative PCR assays have been used to measure the performance of seed treatments towards seedling blight infections of wheat. Further work will be aimed at quantifying the amount of *M. nivale* sub-species present in these samples.

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The effect of a range of novel and established fungicides on *Fusarium* growth and mycotoxin production

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ABSTRACT

Four fungicides were evaluated for their effect on growth and toxin production of *Fusarium culmorum* and *Fusarium poae*. Productivity of wheat plants was also evaluated. All fungicides showed some activity against *Fusarium* but results were variable. Tebuconazole and Compound A gave promising results for productivity increases probably linked to reduction of disease on the spike. As overall toxin levels were low it was difficult to determine the cause of any reductions, and further work needs to be done in this area.

INTRODUCTION

Fusarium Ear Blight (FEB) infection of cereals was initially described in 1884 as wheat scab. Parry *et al.* (1995) cites over 15 species of *Fusarium* linked to ear blight in cereals, with the most common being *F. culmorum, F. graminearum, F poae, F. avenaceum* and *Microdochium nivale* (previously known as *F. nivale).* FEB causes losses due to the production of smaller and fewer kernels and results in problems for both brewers and bakers by producing enzymes affecting production processes (Schwarz *et al.* 1996; Nightingale *et al.* 1999). *Fusarium* also produces mycotoxins with implications for both human and animal health. The toxins fall into several broad categories: trichothecenes, fumonisins, zearalenone and its analogues, and fusaric acid. In the UK mycotoxins found tend to be the trichothecenes, deoxynivalenol (DON) and T-2 toxin (Turner *et al*, 1998).

The problem for cereal growers in attempting to combat the impact of *Fusarium* is that there are few, if any, fungicides that are totally effective in combating a disease complex that can be caused by so many different agents. Wilcoxson (1996) reviewed a range of work with fungicides and found wide differences in results for work with the same compound. The work detailed in this paper has evaluated two existing fungicides with different modes of action, plus two new compounds under development.

MATERIALS AND METHODS

In-vitro tests

Fungicides used were azoxystrobin, tebuconazole, and new formulations referred to as compounds A and B. The fungicides were assessed using concentrations of 0, 1, 10, 100 and 1000ppm fungicide in PDA. Plugs of *Fusarium* were placed centrally to the plate and the Petri dishes were incubated at 20°C. Growth of the colonies was measured after 6 days. The percentage reduction in growth of the fungi in the presence of the fungicides was calculated.

In-planta trials

Wheat (cv. Cadenza) was pot-grown in John Innes No. 2 compost, watered and fed regularly. Plants were grown at a day - night temperature of 20° - 16°C with a 16h photoperiod. At growth GS 39, GS 59 or both, plants were given field application rate sprays of either fungicide or sterile distilled water. At GS 60 (early anthesis) or GS 65 (mid anthesis) wheat spikes were inoculated with 10^5 conidia/ml of either *F. culmorum*, *F. poae* or a mixture of the two species. Aliquots (10 ml) of each suspension or water were delivered to each spike using a Nalgene chromatography sprayer. The plants were covered with plastic bags for 8 days to increase the humidity levels around the spike. Disease development was assessed at weekly intervals for 9 weeks by scoring the percentage of spike showing infection.

Post harvest assessment

Spikes were harvested, hand threshed and the seed and chaff separated. Productivity was based on grain weights obtained from 30 spikes. Harvested seed was ground and subjected to toxin analysis using Veratox® competitive ELISA kits, provided by ADGEN Ltd, UK, for both DON and T-2 toxin

RESULTS

The effects of fungicides on the growth and development of *Fusarium* differed between the species tested. As an illustration, the data presented here are for *F. culmorum* only.

In-vitro activity of fungicides

Results comparing the *in vitro* toxicity of the fungicides tested are given in Table 1. From these results it can be seen that whilst all compounds had some activity, tebuconazole and Compound B were the most and least active molecules respectively, in this assay.

Table 1. Reduction of the growth of *F. culmorum* on fungicide-amended PDA

	Percentage reduction in fungal growth			
Treatment	Tebuconazole	Azoxystrobin	Compound A	Compound B
1ppm	63	36.5	24.9	0
10ppm	80.2	41.2	41.9	0
100ppm	100	42.6	100	9.3
1000ppm	100	44.2	100	45.8

In planta activity of fungicides

Data illustrating the ability of the fungicides tested to inhibit spike infection of wheat by *F. culmorum* are given in Figure 1. All the fungicides tested were able to reduce the level of spike infection to some extent, with Compound A and tebuconazole being the most

active. It is interesting to note that there is no apparent correlation between *in vitro* and *in planta* activities at the lower *in-vitro* concentrations.

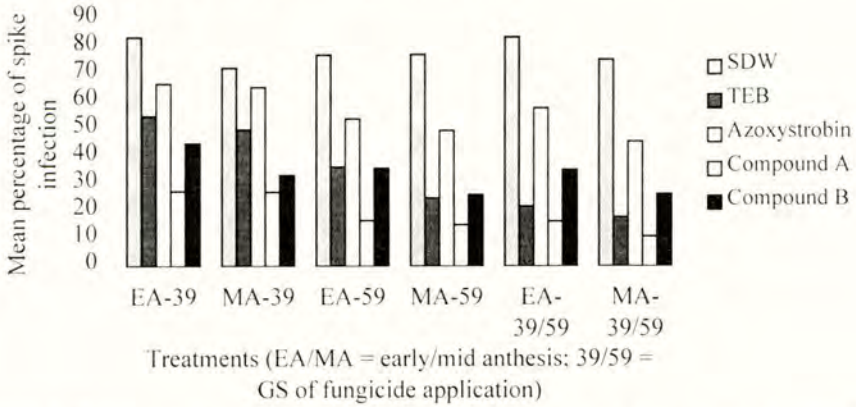

Figure 1: Percentage spike infection by *F. culmorum* on wheat treated with fungicides

Effects of fungicides on wheat productivity

Data given in Figure 2 show the thousand-grain weight (TGW) of wheat treated with fungicides following inoculation with *F. culmorum*. From these results it is clear that tebuconazole and Compound A both increase TGW. This correlates to the results obtained for these fungicides in the assessment for reduction in spike disease (Figure 1).

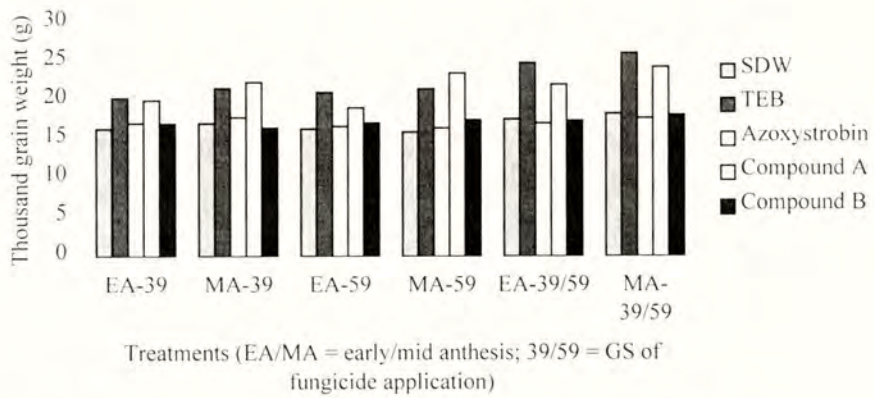

Figure 2: Thousand Grain Weights for spikes infected with *F. culmorum*

Toxin levels in inoculated wheat grain samples

Data given in Figure 3 show the deoxynivalenol content of wheat treated with fungicides following inoculation with *F. culmorum*. Overall toxin levels were very low but it is

possible that tebuconazole and Compound A have a slight effect on DON levels, correlating with the reduction in spike disease (Figure 1).

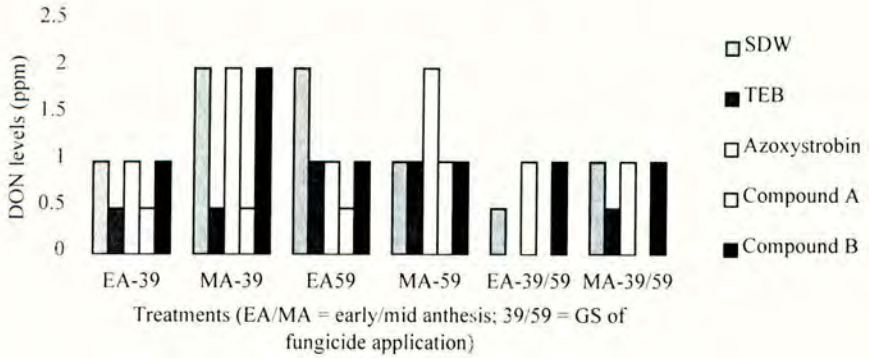

Figure 3: DON levels in wheat inoculated with *F. culmorum*

DISCUSSION

The results found in this work give support to the use of certain fungicides for some control of *Fusarium* infection. However, more work needs to be done to assess fungicides individually and in combination. It is possible that the way forward may be by use of an integrated control strategy incorporating the use of fungicides, specific cultural methods aimed at reducing infection, and the use of biological control.

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The predatory mite *Neoseiulus californicus*: its potential as a biocontrol agent for the fruit tree red spider mite *Panonychus ulmi* in the UK

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ABSTRACT

The non-native predatory mite *Neoseiulus californicus* developed from egg to adult more quickly than the native *Typhlodromus pyri*, and equally quickly with *Panonychus ulmi* or *Tetranychus urticae* as the food source. A strain collected in the UK was able to diapause, whereas one from the USA did not. A strain from Spain exhibited limited diapause propensity. A survey of overwintering refuges established that the strain found in the UK was surviving field conditions.

INTRODUCTION

The predatory phytoseiid mite *Neoseiulus californicus* is not native to the UK; it has, however, been released on several occasions in recent years as a biocontrol agent against the two-spotted mite (*Tetranychus urticae*). It has subsequently been found on strawberry, blackcurrant and hop in several locations in the southeast and west of England, and in a few instances it has occurred on apple (unpublished data). Given the possibility of this species becoming widespread in UK apple orchards, this project is investigating its potential as a biocontrol agent for the fruit tree red spider mite (*Panonychus ulmi*), and its ability to survive winter conditions. In both these respects *N. californicus* is being compared with the native phytoseiid mite *Typhlodromus pyri*, organophosphorous-resistant strains of which are currently exploited for biocontrol of *P. ulmi* in apple orchards in the UK.

Previous studies have concentrated on *N. californicus* as a biocontrol agent for pests of crops in other countries. There has been a considerable amount published on *N. californicus* with *T. urticae* as the prey species; it has been shown to be an effective biocontrol agent against *T. urticae* on strawberry (Greco *et al.*, 1999). However there is little literature concerning predation of *P. ulmi*, although field experiments in Spain and Chile have suggested it does consume this species (Costa-Comelles *et al.*, 1994; Gonzalez, 1971).

MATERIALS AND METHODS

Effect of prey on developmental time of immature phytoseiid stages

A comparison was made of the developmental time of each immature life stage of the phytoseiid mites *T. pyri* and *N. californicus* when fed on either *T. urticae* or *P. ulmi*. Circles of capillary matting, 3.5 cm diameter, were used to line the bottom of plastic pots (4x3cm). The matting was soaked with water, and leaf discs of 2.5cm diameter were placed on top, lower surface upwards. The cultivar used for leaf discs was Greensleeves as it has few leaf hairs, thus facilitating the finding of immature mites and shed exuviae. The wet capillary

matting acted as a water barrier to stop the mites walking off the leaf discs. A phytoseiid egg (either *T. pyri* or *N. californicus*) was placed on each leaf disc, and the hatched larva was provided with prey (either *P. ulmi* or *T. urticae*). Prey was given in numbers such that there was always prey present. The leaf discs were kept in a controlled temperature (CT) room (20°C, 18L:6D) and examined twice a day; the developmental stage of the phytoseiid was recorded and any prey that had been consumed were replaced. Once the phytoseiid reached the adult stage it was mounted in polyvinyl alcohol mountant and examined under a compound microscope to confirm its sex. Mean developmental times were compared by ANOVA.

Overwintering potential

Diapause

During winter conditions temperate phytoseiid mites generally undergo reproductive diapause; females cease laying eggs and become less active (Overmeer, 1985a). An investigation into the ability to diapause was carried out for three strains of *N. californicus*: from Spain, the USA and one collected from the UK. A strain of *T. pyri* that was known to diapause was used as a control. Thirty gravid females of each strain were placed on culture plates (as described by Overmeer (1985b)) to lay eggs, and the culture plate maintained in a CT room at 21°C with a short day length (8L:16D). Day length, rather than temperature, has been shown to be the factor influencing diapause induction in *N. californicus* (Castagnoli *et al.*, 1996). Mites were fed on *T. urticae*, as some species require live prey to be able to diapause due to a requirement for ß-carotene (Overmeer, 1985a). Eggs were taken and placed, one on each square, on a culture plate divided up into 18 with strips of filter paper. The eggs were allowed to develop to adult, females mated with males from the same strain, and each female examined every day for egg laying.

Overwintering refuges

Greater numbers of *N. californicus* were found in hop gardens than in apple orchards, so this investigation of overwintering success was conducted in a hop plantation. During September, 8cm wide bands of hessian sacking were wrapped around dwarf hop bines and secured with insulation tape. Bands were placed in rows in which *N. californicus* had been found on leaf samples taken earlier that month. At fortnightly intervals 14 bands were collected and placed in Tulgren funnels for a minimum of four days to extract the arthropods sheltering in them. Phytoseiid mites collected from the bands were mounted in polyvinyl alcohol mountant and identified under a compound microscope.

RESULTS

Effect of prey on developmental time of immature phytoseiid stages

In the nymphal stages, *N. californicus* developed more quickly than *T. pyri* ($P<0.05$). *Neoseiulus californicus* developed as rapidly on *P. ulmi* as on *T. urticae*. The development time for protonymphs of *N. californicus* feeding on *T. urticae* was shorter than that of *T. pyri* feeding on *P. ulmi* ($P<0.05$) (Figure 1).

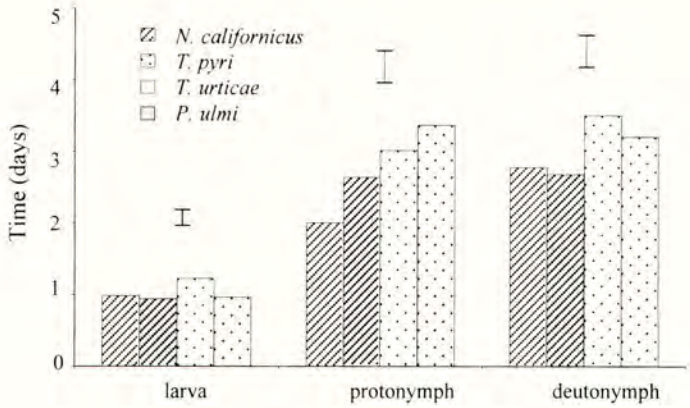

Figure 1. The effect of prey type on developmental time of female phytoseiid immature stages, at 20°C 18L:6D. (bars show 5% LSD for comparison of predators)

Overwintering potential

Diapause

All the *T. pyri* individuals, and 96% of the UK strain of *N. californicus*, underwent diapause (Table 1.). However, only a few individuals from the Spanish strain and none of the US strain were able to diapause. The results were compared by chi-squared test and were significant ($P<0.001$).

Table 1. The percentage of individuals of *T. pyri* and three strains of *N. californicus* that underwent diapause in short day conditions.

	T. pyri	*N. californicus* UK strain	*N. californicus* Spanish strain	*N. californicus* USA strain
% of individuals in diapause	100	95.7	16.1	0
n	19	23	31	34

$\chi^2 = 86.93$ df = 3 $P<0.001$

Overwintering refuges

Phytoseiid mites were found in the hessian bands collected from hop plants from the end of October (1 *T. pyri* per band) and increased in number by the end of November (6 *T. pyri* per band). *Neoseiulus californicus* showed a similar pattern, though numbers did not reach more than 0.5 mites per band. Although *N. californicus* was less abundant than *T. pyri* it appeared to be overwintering as successfully, as live mites were still being found in January and at the beginning of March.

DISCUSSION

If *N. californicus* populations were to increase in UK apple orchards then their ability to control pest mites in relation to *T. pyri* would be important. Given that *N. californicus* develops more rapidly than *T. pyri* and that there were no significant differences between its rate of development on *T. urticae* and *P. ulmi*, it could be suggested that *N. californicus* would be as good a candidate for biocontrol of these two pest species as the native UK phytoseiid *T. pyri*. Further work will be required to determine interactions between these two predatory mite species and consequent control of phytophagous mites in orchards.

The potential for *N. californicus* to overwinter in the UK has implications for policy on future introductions as a biocontrol agent. The ability to diapause is probably a requirement for successful overwintering in the UK. As far as it has been possible to establish the origins of the *N. californicus* introduced into the UK in earlier years, it appears that they were sourced from both Spain and the USA. In the present study, *N. californicus* from the USA did not enter diapause when exposed to short day length. A proportion (16%) of the *N. californicus* originating from Spain did enter diapause, indicating that the gene pool includes a genetic predisposition that enables diapause. On the basis of this initial investigation, it thus appears likely that the *N. californicus* found to be surviving in the UK originated from mites from Spain rather than the USA. Continuing work is examining cold tolerance of these strains and the diapause ability of subsequent generations of the USA and Spanish strains.

ACKNOWLEDGEMENTS

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The importance of field boundaries for whole-farm biodiversity conservation

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ABSTRACT

Field boundaries are widely recognised to be important sources of biodiversity within the agro-ecosystem. The contribution which post and wire and hedgerow field boundary habitats make to farm-scale invertebrate biodiversity was determined using emergence trapping. Emergence of Carabidae and Staphylinidae beetles was recorded and species richness, abundance and diversity measured. The diversity of Carabidae but not Staphylinidae was higher in hedgerow compared to post and wire field boundary habitat. In contrast, the abundance of Staphylinidae was higher in post and wire boundaries but similar for Carabidae between the two boundary habitat types. Conclusions were drawn regarding the importance of field boundaries for the maintenance of farm-scale biodiversity.

INTRODUCTION

The intensification of farming has lead to a widespread reduction in biodiversity within cropped land. Consequently, field boundaries are the principle source of floral diversity in lowland agricultural landscapes (Barr *et al.*, 1993). Floral diversity has been shown to contribute to ecosystem stability (Tilman, 1996), whilst the invertebrate community associated with field boundaries performs many ecosystem functions including biological control of pests and diseases, pollination, contributions to soil dynamics and a food source for higher trophic levels (Altieri, 1999). Floral diversity and structural and landscape heterogeneity enhance invertebrate diversity. Field boundaries are a composite of many features and will vary according to their origin, management, age, proximity to woodland, adjacent land use and the local plant species pool. Their heterogeneity may be a crucial factor in the maintenance of farmland biodiversity.

Specific elements within field boundaries are associated with high densities of natural enemies (e.g. species of Carabidae and Staphylinidae) of crop pests, and methods of manipulating the landscape to increase the abundance of these habitat elements have been developed. For example, beetle banks have been shown to support high densities of the staphylinid *Tachyporus* spp. and the carabid *Demetrias atricapillus* (Thomas *et al.*, 1992). However, emphasis on the creation of a single habitat element, such as grassland strips, is unlikely to maximise biodiversity benefit. In addition, a more diverse community of natural enemies may provide a greater range of pest control through space and time, reducing the likelihood of the pest finding a refuge for population growth (Crawley, 1992).

The study described in this paper investigated the contribution of two field boundary habitat types, namely post and wire fence and established hedgerow, to the maintenance of diversity for two beetle families (Carabidae and Staphylinidae). Conclusions are drawn regarding the farm-scale management of field boundaries for biodiversity maintenance.

METHODS AND MATERIALS

Sixteen field boundaries (11 hedgerows and 5 post and wire fences) were studied on the Seale-Hayne Farm, Devon (a mixed farm of 167 ha.). Emergence tents were constructed from 85% agricultural shade material (Tildenet, Bristol) with a Velcro opening along one seam to enable access by the investigator. They were established to isolate a 1m length and the entire width of the hedgerow field boundaries, or one side of a post and wire fence. The tents enclosed the canopy of the field boundary and were dug 0.2m into the ground. Four pitfall traps containing a water:detergent solution were used to collect ground active invertebrates. Trapping was continuous from the 1st March, when invertebrates are known to start post-winter emergence, until the 9th May 1999. Traps were reset every four days during March and then on a weekly basis. Collected samples were transferred to 70% alcohol preservation fluid.

The tents were sufficiently large to represent all microhabitat components of the field boundary and species caught were therefore considered to represent richness. The number of individuals captured m^{-2} was also calculated to quantify abundance within each boundary. For all analyses, the GLM procedure of SPSS Version 9 was used on $\log_{10}$ transformed data, whilst diversity measurements were calculated using BioDiversity Pro Version 2 (Natural History Museum and Scottish Association for Marine Science).

RESULTS

A generalised linear model, with field boundary type as the factor and tent ground surface area as a covariate (to control for this variable) was used to test for differences in species richness. Significant differences in the number of carabid species due to field boundary type was observed ($P<0.05$, d.f.=1,13), with higher richness in hedgerows, whilst no difference for staphylinids was evident. The covariate of ground surface area was not significant for either carabids or staphylinids. Carabid abundance was not influenced by field boundary type, whilst staphylinids were significantly more abundant in post and wire boundaries ($P<0.01$, d.f.=1,14). Carabid diversity was measured using the Shannon H' ($\log_{10}$) index and the mean was 0.91 for hedges and 0.65 for post and wire boundaries, which were significantly different ($P<0.05$, d.f. =1,14). No significant difference in staphylinid diversity was observed with mean indices of 0.59 for hedgerows and 0.67 for post and wire fences.

Beta-diversity was investigated using richness and abundance measures. The number of new species accumulating as the samples were pooled was plotted (Figure 1) for carabids and staphylinids, expressed as a proportion of the total number of species recorded. For carabids, five hedges represented 60% of carabid species whilst five post and wire boundaries represented only 45%. Five field boundary sites, irrespective of type, represented approximately 60% of staphylinid species. The number of individuals accumulating as the samples were pooled was plotted for both field boundary types and beetle families (Figure 2).

The relationship appeared similar between field boundary types for carabids. Staphylinids were clearly more abundant in post and wire boundaries compared to hedges; 5 post and wire and 5 hedge sites supported approximately 550 and 180 staphylinids m^{-2} respectively.

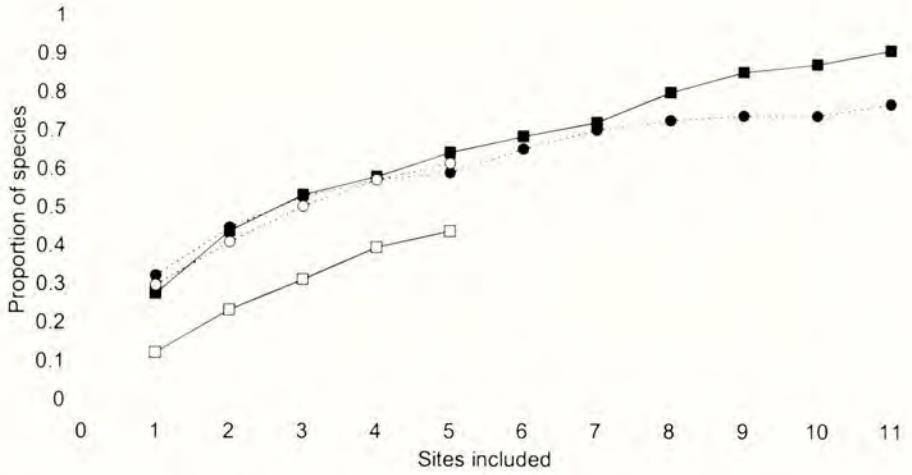

Figure 1. Number of species of carabids (squares) and staphylinids (circles) in hedges (filled markers) and post and wire boundaries (open markers) plotted as proportion of total against cumulative numbers of sites for each field boundary type. In total 38 species of carabid and 32 species of staphylinid were recorded over all 16 sites.

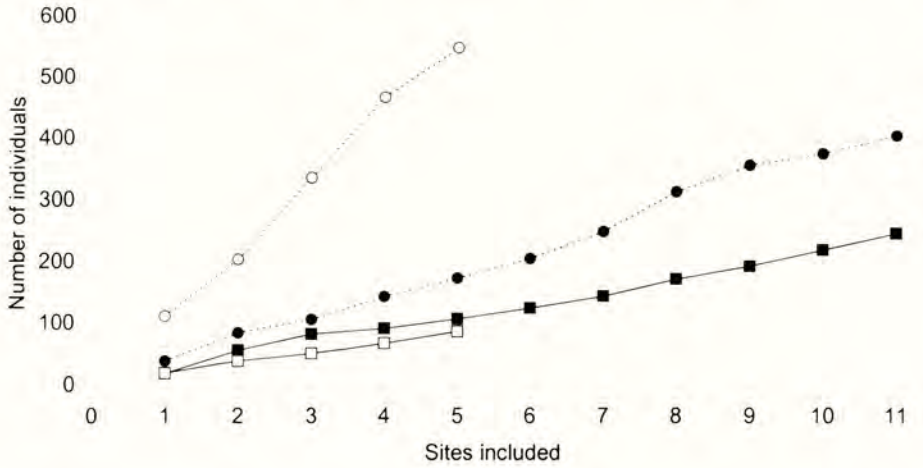

Figure 2. Abundance of carabids (squares) and staphylinids (circles) in hedges (filled markers) and post and wire boundaries (open markers) plotted against cumulative numbers of sites for each field boundary type.

DISCUSSION

Differences in beetle communities were identified between hedgerow and post and wire field boundary types. Hedgerows had a richer and more diverse carabid assemblage when compared to post and wire boundaries, although abundances were similar. Post and wire boundaries supported markedly more staphylinids, whilst both diversity and richness were equivalent. Post and wire boundaries were characterised by flat, grassy strips on either side of the fence line, whilst hedgerows were on a raised bank with a large proportion of bare ground under the woody canopy. Dennis *et al.* (1994) demonstrated that staphylinid overwintering survival was enhanced in a grassy habitat compared to bare ground. The greater structural complexity of the hedgerow field boundary habitat provides a wider range of microhabitats, a factor known to influence insect diversity. Hedgerows may contain a carabid assemblage comprised of overwintering residents as well as 'woodland' species that remain exclusively within the boundary. Hedgerows are a key habitat requirement to support 'woodland' species diversity in farmland and may also increase the diversity of predominately field-dwelling insects (Holland & Fahrig, 2000). A more detailed comparison of the species composition of the two field boundary types is required to identify the principle differences in their assemblages. The high abundance of staphylinid species associated with the grassy strips of post and wire boundaries demonstrates the importance of this habitat type for biological control. However, emphasis on this habitat type at a farm-scale at the expense of hedges would lead to the under-representation of carabids, to the detriment of beneficial ecosystem functions. This study illustrates the importance of the inclusion of a variety of field boundary types for the maintenance of invertebrate biodiversity at the farm-scale.

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New approaches to pest risk analysis for plant quarantine

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ABSTRACT

There is an urgent need for pest risk analysis (PRA) methodologies to provide the scientific basis for plant quarantine decision-making required under the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organisation. The use of 'Mind-mapping' is illustrated to investigate and compare the elements involved in PRA. To combine these elements into an overall risk assessment, mathematical approaches are being developed to account for both the greater impact of extreme scores and the weighting of individual risk elements. Case studies concerning the introduction and/or spread potential of some lepidopteran and dipteran pests are presented.

INTRODUCTION

The Agreement on the Application of Sanitary and Phytosanitary Measures (1995) aimed to prevent the introduction of pests, while ensuring that unnecessary barriers to international trade were not imposed. It is a requirement that decision-making for plant quarantine should be based on Pest Risk Analysis (PRA) with accurate scientific data, rather than the precautionary principle previously used.

The assessment of pest risk in PRA currently lacks methods to (a) handle economic and social criteria and to integrate these with biological criteria; (b) combine the assessments under different criteria into a meaningful overall risk score; and (c) cope with uncertainty throughout the whole assessment. The risk criteria recognised in the International Standards for Sanitary and Phytosanitary Measures (ISPMs) (FAO, 1996) include geographical and regulatory criteria, introduction and/or spread potential and economic factors. The lack of specific guidance discourages detailed examination of risk criteria with the result that some important risk elements may be overlooked.

ANALYSING THE RISK

We subdivided or disaggregated the risk criteria into more specific risk elements until a series of direct, tangible questions could be posed. Mind mapping plots (MindManager, MindJET) were used to structure this process. To distinguish the different impact of each risk element on the overall risk, a weighting (0-1) was given to each of the risk elements. Case studies of some lepidopteran and dipteran pests were used to examine the decision-making relating to an assessment of their introduction and/or spread potential.

Factual information came from various reference materials, including the Crop Protection Compendium (CAB International, 2000), EPPO's Plant Quarantine Retrieval System (OEPP/EPPO, 1999), Quarantine Pest for Europe (Smith *et al.*, 1997), Internet resources, and a review of literature. In the case studies, approximately 40 risk elements were defined for the introduction and/or spread potential criterion; their number and nature depended on the characteristics of the pest and the pathways of introduction. Scores of the potential risk level associated with each element were based on the judgement of the authors, using a 1-9 scale (EPPO, 1998). The weightings allowed the differing importance of each element to be incorporated. Figure 1 shows a part of the Mind-mapping for the introduction and/or spread potential to European countries of the Natal fruit fly (*Ceratitis rosa* Karsch), a polyphagous species attacking a wide range of unrelated fruit crops. The map shows the risk elements, each with a weighting (number in brackets) and a score (number before brackets). Each risk element has an underlying question, an example of which is given in the box at the bottom of Figure 1.

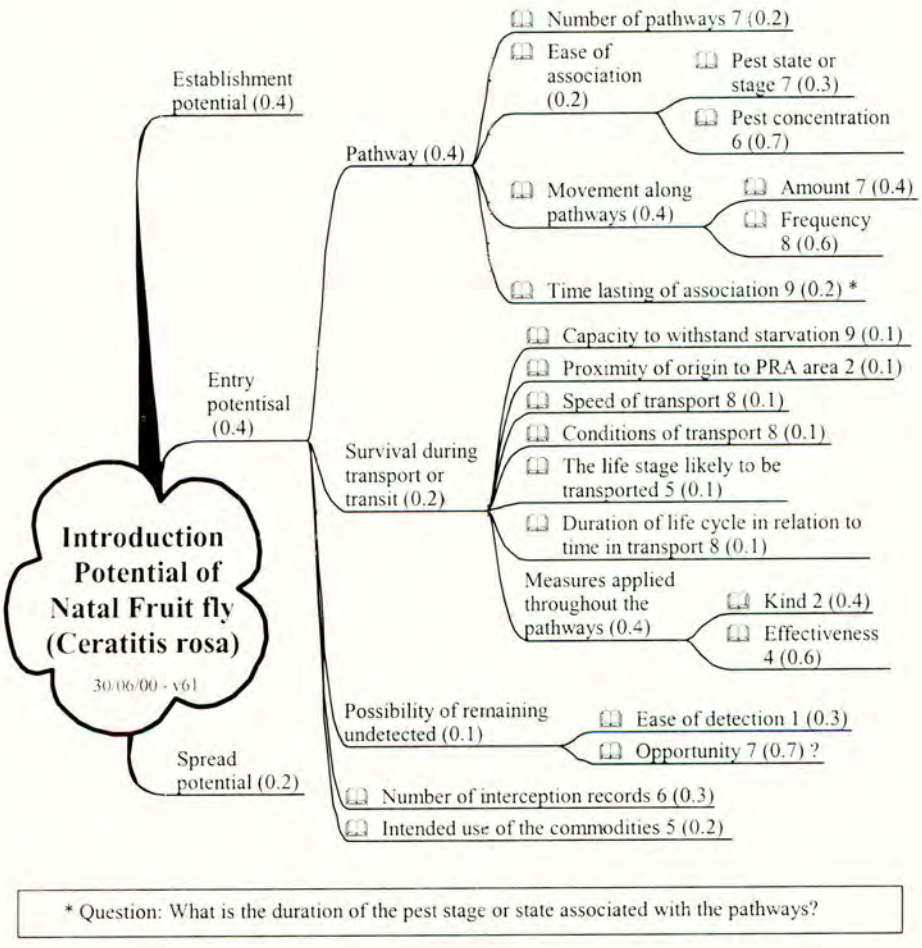

Figure 1. Introduction and/or spread potential for Natal fruit fly

The software package allows items of information (questions, facts from datasheets, etc.) to be associated with each element and these are indicated by a "book" symbol in Figure 1. In all the case studies, it proved possible to specify the introduction and/or spread potential as a series of risk elements, each of which had a simple direct question associated with it, and to which a score and weighting could be attributed.

COMBINING RISK SCORES

The simplest way to combine risk element scores to give an overall score is by simple averaging. However, an extreme score (either very high or very low) for a risk element may in practice have an over-riding impact on the actual decision. We compared simple averaging, weighted averaging (as suggested in the EPPO PRA scheme, 1998), and weighted averaging which incorporated two complementary transformations, one of which gave more influence to high scores and the other to low scores. The equations were (1) average risk $r_a = \left[\sum_{i=1}^{i=n} a_i\right]/n$, where a_i is the score of risk element i, n is the number of risk elements; (2) weighted average $r_w = \sum_{i=1}^{i=n} a_i w_i$, where w_i is the weighting of risk element i, such that $1 = \sum_{i=1}^{i=n} w_i$; (3) transformed weighted averages, with a high-score bias $r_h = 5 + \ln\left[\sum_{i=1}^{i=n} \exp(a_i - 5) w_i\right]$, and a low-score bias $r_l = 5 - \ln\left[\sum_{i=1}^{i=n} \exp(5 - a_i) w_i\right]$. Thus, the biases are achieved by summing the scores as exponents; the scores 1,2…9 transform to e^{-4}, e^{-3}…e^{4} and e^{4},e^{3}…e^{-4}, for the high- and low-score biases, respectively. The three metrics are compared for four pests in Table 1.

Table 1. Comparison of the introduction and/or spread potential risk scores for four pests using different metrics

	fall webworm (*Hyphantria curea*)		codling moth (*Cydia pomonella*)		Fijian fruit fly (*Bactrocera passifiorae*)		Natal fruit fly (*Ceratitis rosa*)	
Average r_a	6.03		5.28		5.95		6.79	
Weighted average r_w	6.34		5.25		5.68		6.75	
High- & low- biased weighted average r_h & r_l	7.54	4.25	6.77	3.34	7.51	3.44	7.68	4.65

Suppose that the final stage of the actions according to whether the risk is high, medium or low. To this end we divided the 1-9 scale into three equal parts: 1-3.66, 3.67-6.33 and 6.34-9. A change from medium to high risk occurred for the fall webworm, if a weighted average rather than a simple average was used. The Mind-map for this species (not shown) revealed that certain higher-scoring risk elements were thought to be more important and were therefore given high weights.

We interpret the high- and low-biased averages in the following way. A high value (≥6.34) of the high-biased score indicates that one or more of the risk elements from which it is derived has a very high score. Such a situation might be regarded as high-risk even if other risk elements have moderate scores. However, a low value (≤3.66) of the low-biased score is

taken to indicate that a constraint exists for one or more of the component risk elements, thereby negating the impact of high scores for other elements.

Comparing the outcomes for the weighted averages and the low/high-biased averages, there was no difference for both fall webworm and Natal fruit fly: high risk for both metrics. With Fijian fruit fly and the codling moth the outcome differed: in both cases being medium for the weighted average and low for the low/high-biased average. The mind-map for the Fijian fruit fly revealed the existence of many importation pathways and a high chance of pest survival during transport. However, the climate and host-range within the PRA area were largely unsuitable – these constraints meant that the overall risk was correctly regarded as low. For the codling moth, marginal climate suitability in the PRA area and a low volume of fruit trade between the source area and the PRA area also led to a low-biased score in the low-risk range (i.e.≤3.66). Again, therefore, given the existence of elements that constrain risk, the prognosis was correctly judged to be low-risk even though some high-risk elements were also present.

CONCLUSIONS

New methodologies to assist in PRA have been described and subjected to some evaluation with case studies. Mind mapping provided a means to collate and manage a diverse data set, and potentially, to reduce ambiguity and increase transparency, thereby making evaluation by third parties easier. To combine risk scores, weighting to reflect the relative importance of different risk elements was judged to improve the accuracy of the assessment in some cases. A pair of mathematical transformations was used to give more emphasis to i) constraints to risk and ii) the existence of high-risk elements. Final risk scores based on these transformations were more in agreement with intuitive judgement than were simple averages or weighted averages. Other work in progress aims to incorporate uncertainty by using intervals or fuzzy variables instead of simple numbers to score risk. In this way it is hoped to include some aspects of the precautionary principle in a rigorous and systematic approach to PRA.

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Different sources of Fusarium inoculum and use of fungicides - how does this affect development of the fungus?

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ABSTRACT

The *Fusarium* spp are known to infect several crops of commercial importance. In this survey it was shown that *Fusarium* spp isolated from Swedish cereals are being able to infect red clover and vice versa. It was also shown that the fungicides commonly used in Sweden had no or very little effect on *Fusarium* spp in field, while these fungicides negatively affect other fungi. This means that the Swedish Fusarium spp not only can survive and possibly increase in amounts in bicrop systems or rotations with ley, but also have an advantage over other fungi when fungicides are applied to the fields.

INTRODUCTION

The *Fusarium* species are important pathogens of several commercial crops all over the world, including small grain cereals and leguminous plants. One of the reasons for the focus on these pathogenic fungi is their capability to produce toxins, affecting both humans and animals. In northern Europe the most common *Fusarium* species on cereals are *F. avenaceum*, *F. culmorum*, *F. sporotrichoides*, *F. poae* and *F. equiseti* (Eriksen & Alexander, 1998).

F. avenaceum is also one of the most frequently isolated fungi in the Swedish red clover root rot *Fusarium* complex (Rufelt, 1986). Other *Fusarium* species common on cereals that have been found in the red clover root rot complex are *F. culmorum* and *F. poae* (Rufelt, 1986). In earlier trials made in Great Britain, Australia and Canada, *F. avenaceum* from leguminous hosts has proven pathogenic on cereals and vice versa (Cormack, 1937; Kollmorgen, 1974; Deadman *et al.*, 1996). According to our knowledge no similar trials have been done so far with *F. culmorum* and *F. poae* or with Swedish isolates of *F. avenaceum*. The use of clover as a source of nitrogen is increasing in bicrop systems as well as in-between cereal crops, especially in organic farming. Therefore it is of great interest whether these *Fusarium* pathogens could infect different species and to which extent.

In trials with fungicides, that have proven efficient towards *Fusarium*, also the mycotoxin content has been lowered (Mesterházy & Bartók, 1996; Homdork *et al.*, 2000). The fungicide Amistar (azoxystrobin) has been commonly used with good effects on most cereal fungi. There have, however, been observations that azoxystrobin has increased the infection of *Fusarium* spp in Sweden. In Norwegian trials with azoxystrobin the amount of *Fusarium* has increased compared to controls, although the increase was not significant (Henriksen, 1999).

The objective of this study was to investigate how Swedish *Fusarium* spp can survive on another host plant and how these fungi are affected by the use of fungicides. Part of the study was concerned with the possibility of some commonly isolated *Fusarium* spp to utilise alternative hosts. The other part was an evaluation of the fungicidal effect on the *Fusarium* infection and the mycotoxin content in small grain cereals in field.

MATERIALS AND METHODS

Cross inoculations

Seeds of red clover, cultivars Sara and Betty, and spring wheat, cultivar Curry, were planted in polyethylene plastic bags, 5 seeds in each bag. The bags were 5 x 30 cm and had four drainage holes in the bottom. There were 4 replicates for each sampling date and pathogen, including controls. The bags were filled with 13cm of leca, 5cm of soil on which the seeds were placed and then an extra 3cm of soil with 0.5cm of sand on top. The isolates used were *F. culmorum* (IBT 1512), *F. poae* (IBT 1514), both isolated from oats in Sweden, and two isolates of *F. avenaceum* (evp83 and evp84, both identified at CBS in Baarn, The Netherlands), isolated from red clover fields in Sweden. After 4 days spore suspensions of each isolate was added to the plastic bags. The bags were kept at 20°C in glasshouses with moisture content of 70%. Samples were taken 2 wks, 4 wks and 6 wks after inoculation when roots were cleaned from soil. The roots and stembases were studied under low power microscope for rots. Infections were rated on a scale from 1-5, after McGee & Kellock (1974), with the difference that the values 4 and 5 on their scale are put together into value 5 in our scale and all other values moved one step up so that the value 0 is lacking in our scale.

Field trial

The kernel infection was examined in samples from five different field trials with winter wheat in the region between Stockholm, Uppsala and Västerås, 1998. Four fungicide treatments and one control were selected from each trial. From a sample of 1 kg harvested and dried wheat kernels from each treatment, 100 kernels were randomly taken out. The plots were treated with the following fungicides at GS 51-55: Tilt Gel 0.2 l/ha (Propikonazol 62% weight), Mentor 0.7 l/ha (Kresoximmetyl 150 g/l, Fenpropimorf 300 g/l), Mentor 0.5 l/ha + Sportak EW 0.5 l/ha (Kresoximmetyl 150 g/l, Fenpropimorf 300 g/l + Prokloraz 450g/l) and Amistar 1.0 l/ha (Azoxystrobin 250 g/l). The kernels were surface disinfected with 4% sodium hypochlorite for 5 min and dried overnight. The kernels were put on Czapek-Dox iprodione dicloran agar (Abildgren *el at.*, 1987), 10 in each dish, and incubated for 9-11 days at 20°C, 12h NUV light/12h dark. After incubation the dishes were examined for *Fusarium spp.* under low power microscope. Conidia were transferred to slides for identification under microscope. From each colony on the dishes agar plugs were cut out for osmos analysis, each plug were transferred to a plastic dish with filter paper covered with a lid to create an osmos chamber. The filter paper was soaked in sugar solution 150g sucrose/l (Svensson, pers.comm.). The chamber was incubated in 12h light/12h dark with 27°C/24°C for one week and was then examined for *F. avenaceum* that gives a yellow to green flourecens under UV light 360 nm. (Svensson, pers. comm.).

RESULTS

Cross inoculations

The differences in infection frequency between the control and the four pathogens were most pronounced 6 wks after inoculation (data not shown). As can be seen in Figure 1 all treatments significantly differ from the control (Kruskal-Wallis one-way analysis of variance).

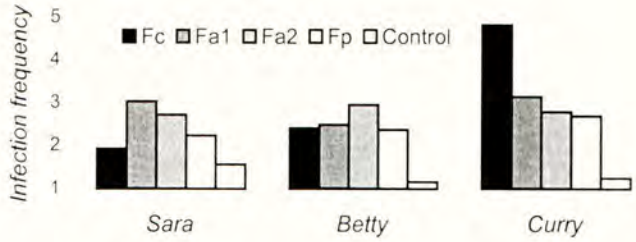

Figure 1. Infection frequencies of *F. culmorum* (Fc), *F. avenaceum* evp83 (Fa1), *F. avenaceum* evp84 (Fa2) and *F. poae* (Fp) on the roots of clover cultivars Sara and Betty, and on stembases of spring wheat cultivar Curry after 6wks exposure to the pathogens.

Field trial

In three of five field trials the percent of *Fusarium* infected kernels were higher in all of the fungicide treated samples than in the untreated control (Figure 2). The azoxystrobin treated sample had the same level of infection , or higher, than the control in all five field trials. The dominant *Fusarium* species isolated from the kernels was *F. avenaceum* but *F. poae, F. sporotrichoides, F. culmorum* and *Microdochium nivale* were also found to some extent (data not shown).

All fungicides in the test reduced the level of infection of other field fungi like *Drechslera tritici-repentis., Septoria tritici* and *Stagonospora nodorum* but the azoxystrobin treatment was the most effective (data not shown). The contents of the mycotoxins deoxynivalenol and nivalenol were analysed but no differences were found (data not shown).

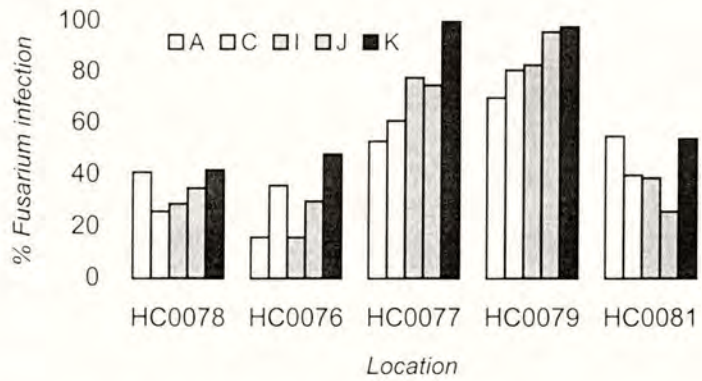

Figure 2. Percent of Fusarium infected kernels in five field trials (HC0076-HC0081) after application of four different fungicides: Propikonazol (C), Kresoximmetyl/Fenpropimorf (I), Kresoximmetyl/Fenpropimorf + Prokloraz (J), Azoxystrobin (K) and one untreated control (A).

DISCUSSION

The *Fusarium* spp investigated can use spring wheat and red clover as alternate hosts, which could prove to be a problem in rotations with ley or in bicrop systems. This could also become a source for increased inoculum. When the climate is promoting growth of fungi in the cereal crop, use of fungicides that have less effect on *Fusarium* spp than on other fungi could give the *Fusarium* spp an advantage. Both these parameters impose the need to carefully plough down crop residues. More research is needed to evaluate the effect of cereal *Fusarium* spp on clover cultivars and vice versa.

Unexpectedly no effect was seen on the mycotoxin content. If the mycotoxins produced by *F. avenaceum* also had been analysed, the results might have been different. To elucidate the effects more research is needed.

ACKNOWLEDGEMENTS

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The interaction between potato cyst nematodes and *Rhizoctonia solani* diseases in potatoes

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ABSTRACT

Glasshouse studies were undertaken to determine the interaction between the fungal pathogen *Rhizoctonia solani* and the potato cyst nematode *Globodera rostochiensis*. Results showed that when potato plants were inoculated with *R. solani*, a significant increase in the number of juvenile nematodes invading roots was observed. Preliminary results from subsequent field studies indicate that the severity of stem canker caused by *R. solani* increased as nematode population increased.

INTRODUCTION

The pathogen *Rhizoctonia solani* Kühn occurs worldwide (Banville, 1989) and is responsible for causing the diseases stem canker and black scurf in potatoes. Previous studies have shown that stem canker can result in yield losses in some early crops (Hide *et al.*, 1989). The potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida* are the most important pests of potatoes wherever they are grown (Hockland *et al.*, 2000). According to Haydock & Evans (1998) the yield losses caused by PCN has an approximate value of £43 M in the UK.

Nematode-fungus complexes are common on many crops and can cause synergistic, antagonistic or additive effects with respect to disease development and yield suppression (Abdel-Momen & Starr, 1998). Prior to this investigation, yield reduction (Mazurkiewicz-Zapalowicz & Waker-Wójciuk, 1994) and severe necroses (Grainger & Clark, 1963) have been recorded in the co-occurrence of *R. solani* and *G. rostochiensis*. In contrast to these synergistic effects, Janowicz *et al.* (1994) produced evidence for an antagonistic relationship. Their results show a significant reduction in the final density of cysts, eggs and juveniles of *G. rostochiensis* when *R. solani* was present. There has been to date no clear account of the interaction between PCN and *R. solani*. Since both synergistic and antagonistic effects have been described, it is, therefore, important to find a method that will fully define the relationship. This paper reports preliminary findings from the first year of a PhD investigation.

MATERIALS AND METHODS

Glasshouse experiment

Tubers of the cv. Maris Peer (sprout length c.10 mm, seed size: 45-55) were planted to a depth of 10 cm in John Innes sterilised peat based loam in pots c.15 cm in diameter. All tubers were assessed for any visible sclerotia (black scurf) and several were examined microscopically for *R. solani* mycelium using the guidelines of Parmeter & Whitney (1970).

Pots were arranged in a randomised block design with 20 replicates of each treatment. Plants were grown in the presence of PCN only, *R. solani* only or PCN and *R. solani*. Plants grown in the absence of PCN and *R. solani* provided controls. *Rhizoctonia solani* inoculum (for both field and glasshouse experiments) was prepared by taking 6 mycelial plugs from the growing margin of a 7-day-old culture of AG3. These were used to inoculate 1 kg of sterilised maize/sand medium (20 g maize meal: 980 g sand: 250 ml distilled water) (Dhingra & Sinclair, 1995). This was incubated for 40 days at 25°C. 20 ml of the inoculum was applied to the tuber at planting. Sterilised sand was applied to non-*R. solani* treatments.

Cysts of *G. rostochiensis* were extracted from heavily infested field soil. Several egg counts were undertaken to facilitate a representative calculation of cysts required (Shepherd, 1986). All PCN treatments contained approximately 12 eggs g/ soil.

Plants were harvested at 4 and 6 weeks after planting at which time assessments of stem canker severity, analysis of PCN root invasion (Hooper, 1986) and growth analyses (stem numbers, fresh weight, dry weight, % dry matter) were undertaken.

Field experiment

A field trial site at Harper Adams University College was selected on the basis of highly variable final populations (Pf) of *G. rostochiensis* in 1999. Prior to planting, plots (4 rows, 5 metres in length) were marked out and sampled for initial PCN populations (Pi). These data were used to create 2 population ranges that *R. solani* inoculated and uninoculated treatments could be randomly assigned to. The cv. Desirée (sprout length: c. 10mm, seed size: 35-45, grade: VTSC 1) was selected for its low resistance to *R. solani* (Little *et al.*, 1988) and susceptibility to PCN (BPC Seed Potato Variety Handbook, 2000). Tubers were planted to a depth of 15 cm on 16 May 2000. Inoculation with *R. solani* was achieved using the method described above for the glasshouse experiment.

RESULTS

Glasshouse

Significant increases ($P<0.05$) were found in nematode invasion at 4 and 6 weeks in plants grown in presence of *R. solani* and PCN compared to plants grown in presence of PCN alone. Figure 1 demonstrates a trend at 4 weeks which correlates to that found at 6 weeks.

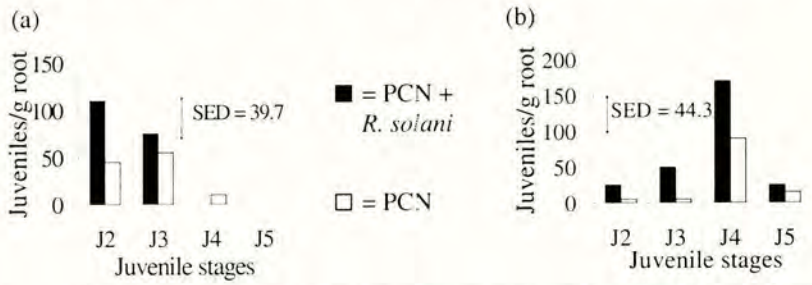

Figure 1. The effect *R. solani* infection of potato plants (cv. Desirée) on invasion of roots by potato cyst nematode juveniles at 4 (a) and 6 (b) weeks after planting.

At 4 weeks a heavier influx of J2s appeared to occur in plants grown in presence of both *R. solani* and PCN. No significant differences were found to occur in the growth analysis data. Analysis of stem canker severity was shown not to differ significantly between plants inoculated with *R. solani* and plants inoculated with *R. solani* and PCN.

Field experiment

Assessment of disease severity 4 weeks after planting revealed that, increasing Pi's resulted in an increase in the severity of *R. solani* infection of potato stems and stolons (figure 2).

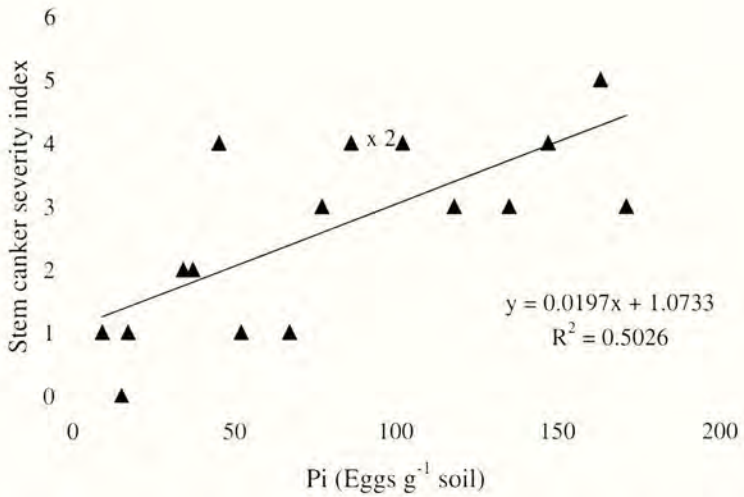

Figure 2. Relationship between Pi and stem canker severity on potato plants (cv. Desirée) harvested 4 weeks after planting.

DISCUSSION

These preliminary results suggest a synergistic interaction between *G. rostochiensis* and *R. solani*. Results from the glasshouse studies indicate that *G. rostochiensis* may gain increased access to potato roots when *R. solani* is present. Several authors have made similar observations. For example Gokulapalan & Nair (1983) recorded increased numbers of the nematode *Hirschmanniella oryzae* on the roots of rice infected by *R. solani* in comparison to control plants where *R. solani* was absent. In addition Nordmeyer & Sikora (1983) found that clover roots colonised with the pathogen *Fusarium avenaceum* received elevated penetration by the nematode *Heterodera daverti*. This effect could be repeated by artificially treating the roots with *F. avenaceum* culture filtrates.

The relationship between stem canker severity and Pi (figure 2) provides further evidence for a synergistic interaction between pest and pathogen and is in agreement with the results found by Mazurkiewicz-Zapalowicz & Waker-Wjciuk (1994). However, conflicting data found in

some of the glasshouse work emphasises the need for further work in this area. Future work will explore the interaction in greater detail by comparing both *G. rostochiensis* and *G. pallida* with *R. solani* anastomosis groups. Ultimately, a better understanding of this interaction could provide more advanced strategies of control.

ACKNOWLEDGEMENTS

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Biological control of *Leptosphaeria maculans* (anamorph *Phoma lingam*) causal agent of Blackleg/Canker on oil seed rape by *Cyathus striatus*, a Bird's Nest Fungus

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ABSTRACT

Blackleg/canker of oil seed rape cannot at present be effectively controlled by chemical or other means. After light leaf spot, it is the most economically significant disease which affects rape, so research into the disease is crucial. *Cyathus striatus* shows potential as a biocontrol agent for *Leptosphaeria maculans*. Experiments have shown that *C. striatus* possesses a greater ability for primary resource capture, by producing more cellulase and lignase than *L. maculans*. Fungi from the genus *Cyathus* are also know to produce an antibiotic complex called cyathin, which may be detrimental to *L. maculans*, although this has yet to be proved.

INTRODUCTION

The ascomycete fungus *Leptosphaeria maculans* causes disease on Cruciferae, particularly *Brassica* species including oil seed rape. Symptoms on rape include; leaf spots, canker, black leg, dry rot, root, collar and stem rot. Infection results in poor plant growth, premature ripening, lodging and death. Yield losses of up to 58% have been recorded in susceptible cultivars (Smith *et al.*, 1988). Chemically there is no consistent, effective method of control, (Gladders *et al.*, 1998), so research into a biocontrol agent is all the more viable. Some crop protection is given by fungicides notably prochloraz, (Sansford *et al.*, 1996), although seed treatment is far more effective than spraying, (Parry, 1990). However, with any agrochemical there is a possible environmental risk, so if any alternative to chemical control is as or more effective, it should be investigated. *Cyathus striatus* has potential as a biocontrol agent as members of this genus are known to produce an antibiotic complex called cyathin (Allbutt *et al.*, 1971). It has been observed that in rape fields, where *Cyathus sp.* is present, the disease caused by *L. maculans* is less severe, (Pers. Com. A Hall, R Williams). Tewari & Briggs (1995) also used *Cyathus sp.* for straw degradation. The overall aim of the project therefore, is to investigate the relationship between *L. maculans* and *C. striatus*, with a view to the development of biological control.

MATERIALS AND METHODS

Competitive ability

Initially, the competitive ability of both fungi was assessed *in vitro* by comparing their growth rates on a variety of media; potato dextrose agar, V8 juice agar, malt extract agar, salts agar (Robinson *et al.*, 1993) and sterile rape straw on tap water agar (TWA). Mean colony diameter of isolates was recorded daily by taking an average of two measurements of each

colony diameter. Seven isolates of each fungus were assessed, using five replicates of each isolate. Statistical analysis of variance (ANOVA) was calculated using the StatView for Macintosh package. The fungi were also observed in dual culture, by inoculating 5mm diameter plugs onto agar plates 25mm apart.

Cellulase and lignase production

Cellulase production by both fungi was investigated by measuring the reduction in tensile strength of sterile cotton strips. Shirley Soil Burial Test Fabric (BS 2576) was cut into strips measuring 20cm by 25mm. These were then dampened with sterile tap water, placed into boxes and inoculated with agar blocks 25mm by 10mm. Five strips in a box were used for each isolate. The controls were one box containing strips 'inoculated' with sterile agar only and one containing damp strips alone. The boxes were then sealed and incubated at 30°C for one week. The tensile strength of the strips was then recorded using a tensometer (Hounslow tensometer 600N beam), which measures the force required to break them. Two isolates of both fungi were assessed.

Lignase production by both fungi was assessed by gauging the colour change of lignin agar. This dark brown media was created by adding 150ml of lignin suspension (a by-product of the wood pulping industry) to 250ml of distilled water and 15g of bacteriological agar No.1. As the lignin in the media was broken down, a lightening in colour could be observed, first to orange then yellow. A mycological colour chart was used to record changes. The growth rates of colonies were also recorded as an indicator of ability to utilise lignin.

Cyathin complex production by *C. striatus*

To assay for production of cyathin complex, isolates of both fungi alone and also in dual culture, were grown in 250ml, foam bunged flasks. The medium was adapted from Allbutt *et al.* (1971) and contained mainly glucose and small quantities of asparagine, potassium dihydrogen orthophosphate, calcium nitrate, magnesium sulphate, zinc and thiamine. The flasks were inoculated with plugs of fungus and incubated still, at 25°C, for four weeks. Samples of the growth medium were removed at one week intervals. To assay for antibiotic production (Allbutt, 1971), 5mm diameter, sterilised filter paper discs were dipped into the samples and placed in the centre of a plate freshly inoculated with a suspension of penicillin-resistant *Staphylococcus aureus*. These were then incubated for one to two days at 30°C or until some bacterial growth was visible. HPLC analysis was also carried out on the fungal medium samples.

RESULTS AND DISCUSSION

Competitive ability

Cyathus striatus was shown to have a significantly faster growth rate ($P = 0.0001$) than *Leptosphaeria maculans* on all media (Table 1). On straw on TWA, *C. striatus* growth was visibly greater also, but difficult to quantify. This demonstrates the superior ability of *C. striatus* for primary resource capture. This may have an implication in the field, as *L. maculans* overwinters as pycnidia on straw debris. If *C. striatus* outcompetes *L. maculans* on

straw in the field, fewer pseudothecia will be produced by the pathogen and therefore fewer ascospores, which are the initial disease inoculum at the start of the growing season.

Table 1. Summary of mean growth rates of *L. maculans* and *C. striatus* on various media ± standard error.

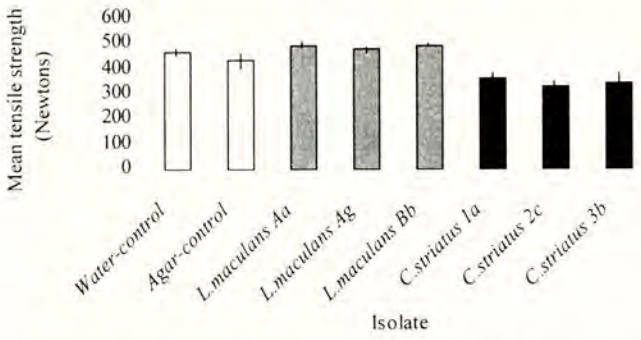
Mean growth rate of colonies of all isolates (mm/day) (n=6)				
	PDA	V8 agar	MEA	Salts agar
L. maculans	3.67 ± 0.31	3.71 ± 0.15	2.28 ± 0.12	2.61 ± 0.11
C. striatus	6.77 ± 0.05	7.55 ± 0.07	6.89 ± 0.08	4.90 ± 0.13

In dual culture, the following observations were noted. The shape of the *C. striatus* colonies was not round as in single culture. Initially, *C. striatus* appeared to be slightly inhibited by the presence of *L. maculans* and tended to grow away from it. After approximately one week, when the fungi met, the *C. striatus* colonies produced a line of brown pigment, which appeared as a barrier between the fungi, it then proceeded to produce aerial hyphae, which overgrew the *L. maculans*. Despite the fact that not all the fungal isolates which were paired met, the observed overgrowth demonstrates an ability for secondary resource capture by *C. striatus*.

Cellulase and lignase production

C. striatus isolates were shown to produce significantly more cellulase than *L. maculans*. The cotton strips which had been inoculated with *C. striatus* isolates had a lower mean tensile strength than those inoculated with *L. maculans*, (Figure 1), demonstrating that they were in a more advanced stage of degradation. This supports the thesis that *C. striatus* has a better competitive ability than *L. maculans* and so can degrade rape straw more efficiently.

Figure 1. Mean tensile strength of cotton strips after one week of treatment (bars indicate standard error).



Lignase production by *C. striatus* has also been demonstrated, with a clear colour change of lignin agar from brown to yellow after two weeks. *L. maculans* isolates which had been incubated for the same length of time in the same conditions, did not result in the agar

changing colour. As on other media, *C. striatus* grew at a faster rate than *L. maculans* on the lignin agar.

Cyathin complex production by *C. striatus*

At this stage this work has not given useful results, because of problems both with the HPLC analysis and particularly the bioassay for cyathin. There was some indication of the presence of compounds not found in the media-only control flasks, but more detailed analysis is required to classify them.

FUTURE WORK

Future work will concentrate on the production and evaluation of cyathin. In addition, once a protocol has been devised for ascospore production, this will be used to test the hypothesis that straw invasion by *C. striatus* inhibits ascospore production by *L. maculans*.

ACKNOWLEDGEMENTS

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Fungicides for control of ergot in cereal crops

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ABSTRACT

Twenty-three out of 34 fungicides tested showed activity against *Claviceps purpurea* in the laboratory but none gave commercially acceptable control of the disease in the field. Applications of strobilurin fungicides usually increased the mean weight of ergots and, less frequently, the number. Poor field performance is attributed to the failure of fungicides to reach the target (i.e. the ovary), and improvements in control may need compounds with novel properties or novel ways of applying them.

INTRODUCTION

Ergot, caused by the ascomycete fungus *Claviceps purpurea*, affects the ears of temperate cereals and grasses throughout the world. It infects the ovaries and produces sclerotia (ergots) in place of the grain. The disease is sporadic in its occurrence in the UK and is seldom, if ever, severe enough to have significant effects on yield. However, the ergots contain harmful toxins, and grain sold for animal feed must contain no more than 0.001% ergot by weight. Grain sold for human consumption must contain no detectable ergots when standard sampling procedures are followed. Despite the small effects on yield, contamination by ergot can, therefore, decrease the market value of grain, and have severe financial implications for individual growers.

Appropriate husbandry (e.g. ploughing to bury the sclerotia, sowing uncontaminated seed and controlling grass weeds) can decrease the risks of infection but does not protect against inoculum originating in hedgerows and nearby fields. No fungicides are currently approved in the UK for application as sprays to control ergot. Benomyl, which forms carbendazim in the plant, has been shown to be capable of reducing infection (Puranik & Mathre, 1971) but is not reliably effective under commercial conditions (e.g. Wood & Coley-Smith, 1980). Since the introduction of benomyl (and carbendazim itself), many new fungicides, from a number of unrelated chemical groups, have been developed. However, because ergot is not considered by the agrochemical industry to be a major target, it is unlikely that many of these have been tested for their activity against *C. purpurea*. The object of the work reported in this paper is to identify more effective compounds and to determine how best to use them to obtain effective control.

MATERIALS AND METHODS

The number of candidate compounds that was obtained for testing was relatively large. They were, therefore, subjected to *in vitro* tests with a view to screening-out compounds with little innate activity against *C. purpurea* so that field tests could concentrate on the more promising compounds. However, a large proportion of the compounds showed significant activity in the laboratory and the decision was, therefore, taken to test all of them in the field.

Laboratory screen

A total of 34 compounds was screened in the laboratory. Potato dextrose agar (PDA) plates (at 10% of normal strength) were uniformly inoculated with a fine spray of conidia suspended in water (*c.*1 x 10^6 spores/ml) and left to dry. A 6mm diameter filter paper disc was then saturated in a chemical suspension of the required concentration and placed in the centre of each plate. Each chemical was tested at 4, 20, 100, 500 and 2500 ppm with 4 replicate plates per concentration. Plates were incubated for 14 days at 25°C and the radii of the fungal inhibition zones measured after 8 and 13 days.

Field experiments

In 1998 and 1999, different field experiments were sown with winter wheat, spring wheat or winter rye. Different cereals were used because they flower at different times and this helps to spread the workload. However, over the two years all of the available compounds were tested at the manufacturers recommended rates on wheat. Plots (3m x 3m) were sprayed at anthesis, 2-3 days before or after inoculation and there were four replicates per treatment. Approximately 10-15 ears in each corner of every plot were inoculated using an injection method to place an aqueous suspension of conidia containing *c.*1 x 10^6 spores/ml within the floral cavities. Other plots, sown with winter wheat or winter rye and inoculated in the same way, were used to test the effects of applying selected compounds of particular interest at double the recommended rate, using a conventional hydraulic and/or electrostatic sprayer, or with or without the addition of a wetter. Carbendazim was used as a standard in all experiments. Just before harvest, inoculated ears and those that had become infected as a result of secondary spread were sampled and average numbers and weights of ergots in each ear determined.

RESULTS

Twenty-three of the 34 compounds tested showed evidence of at least some activity *in vitro*. Carbendazim inhibited fungal growth at concentrations of 20 ppm and above (Table 1). A few compounds were active at a lower concentration but most were active only at 100 ppm and above. Fenpropidin and spiroxamine were active only at the largest concentration tested. At 500 ppm, fungitoxicity of most of the twenty-three compounds was comparable to or better than that of carbendazim (Table 1).

Although many compounds were active against *C. purpurea* grown *in vitro*, none was very effective when applied to crops at anthesis. A summary of the maximum decreases, compared to unsprayed controls in each experiment in each year, and the fungicides involved is presented in Table 2. Most of the decreases were small and not significant. Effects on secondary spread, when it occurred, tended to be larger than effects on ergots in the inoculated ears, and were caused by different compounds. The compounds that had

Table 1. Inhibition by fungicides of *C.purpurea* grown *in vitro,* measured 8 days after inoculation.

Fungicide	Min. inhibitory concentration (ppm)	Inhibition at 500 ppm (% of carbendazim)	Fungicide	Min. inhibitory concentration (ppm)	Inhibition at 500 ppm (% of carbendazim)
Carbendazim	20	100	HGCA 1	100	154
Tebuconazole	100	197	Fenpropidin	2500	-
Cyproconazole	100	224	Fenpropimorph	500	93
Difenconazole	100	130	Tridemorph	4	370
Epoxiconazole	100	160	Cyprodinil	100	46
Propiconazole	100	215	Mancozeb	500	68
Flusilazole	100	202	Iprodione	500	7
Bromuconazole	100	230	Chlorothalonil	4	103
Fluquinconazole	100	48	HGCA 2	4	119
Prochloraz	4	220	Fludioxinil	20	134
Flutriafol	500	118	Spiroxamine	2500	-
Triadimenol	500	165			

Table 2. Maximum decreases in ergot, in each field experiment (% of unsprayed).

Crop	Year	Inoculated ears			
		No. of ergots/ear (Fungicide)		Wt. of ergots/ear (Fungicide)	
Spring wheat	1998	10	(Silthiofam)	17***	(Silthiofam)
	1999	7	(Fludioxinil)	13	(Carbendazim)
Winter wheat	1998	7	(Tridemorph)	8	(Tridemorph)
	1999	19	(Kresoxim-methyl)	4	(Silthiofam)
Winter rye	1998	5	(Kresoxim-methyl)	-	(No decreases)
	1999	12	(Flusilazole)	10	(Chlorothalonil)
		Ears infected by secondary spread			
		No. of infected ears/plot[1] (Fungicide)		Wt. of ergots/ear (Fungicide)	
Spring wheat	1998	10	(Silthiofam)	3	(Experimental 2)
	1999	-	-	22	(Carbendazim)
Winter wheat	1998	-	-	-	-
	1999	-	-	19***	(Cyproconazole)
Winter rye	1998	32***	(Epoxiconazole)	14	(Carbendazim)
	1999	51***	(Fluquinconazole)	1	(Carbendazim)

[1] Not counted in every experiment (no spread on winter wheat in 1998), *** indicates significant at P<0.001

Table 3. Increases in weight of ergots per ear after applying strobilurins at ear emergence (% of unsprayed).

Strobilurin	Spring wheat	Winter wheat	Winter rye
Azoxystrobin	Not tested	81***	28*
Kresoxim-methyl	21***	96***	15*
Trifloxystrobin	4	Not tested	82***

* Indicates significant at P<0.05, *** indicates significant at P<0.001

significant effects on secondary spread were all azoles. Applying sprays before or after inoculation, adding a wetter or using an electrostatic sprayer (which deposits *c.* 20 times more chemical on the ear than a conventional sprayer; Cayley *et al.*, 1984) had little effect on the level of control that was achieved. Significant increases in the number and, especially, the mean weights of ergots were seen in some experiments and were consistently associated with the application of strobilurin and related compounds (Table 3).

DISCUSSION

Two thirds of the compounds showed activity against *C.purpurea* when tested *in vitro*, and some of those that did not (e.g. the strobilurins) are known not to perform well in agar and could not, therefore, be excluded from the field experiments. Our results showed, however, that strobilurins consistently increased the weights of ergots, and sometimes the number, confirming results obtained in Germany (Werner *et al.*, 1999). The explanation is uncertain but it may be an indirect consequence of the effect that strobilurins have on grain yield rather than a direct effect on the fungus itself. None of the fungicides that showed activity *in vitro* gave commercially acceptable levels of control when applied to field plots at normal rates and volumes at anthesis. This may seem surprising because the ears are at the top of the canopy and are, therefore, an accessible target. However, the real target is the ovary, which is protected by the glumes. Their importance is illustrated by the improvement in fungicidal control that can be achieved when glumes are clipped to allow fungicides to enter the floral cavity (Puranik & Mathre, 1971). Many of the compounds tested are systemic (in the xylem) and might, therefore, be expected to move readily to the ear. However, fungicide that is deposited on the leaves would first have to move basipetally before it was in a position to move to the ear. Using an electrostatic sprayer to increase deposition on the ear did not significantly improve control of ergot. Current experiments are testing the effects of applying fungicides in high volumes of water which may help to wash them between the glumes and into the floral cavities. Radio-labelled fungicides are also being used to study their movement when applied using different methods.

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Pathogenicity of *Verticillium dahliae* isolates to spring linseed cultivars

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ABSTRACT

Thirty *Verticillium dahliae* isolates from different hosts were tested for their pathogenicity to linseed (*Linum usitatissimum*) cv. Antares at 22°C. Four of the isolates were tested for their pathogenicity to eight spring linseed cultivars. All isolates tested, irrespective of their original host, were pathogenic to linseed and all cultivars were susceptible to *V. dahliae*.

INTRODUCTION

In the UK, *Verticillium dahliae* was observed for the first time on *Linum usitatissimum* (linseed cultivars) in 1990 at Rothamsted (Fitt *et al.*, 1992). This paper reports results of experiments to examine the pathogenicity of *V. dahliae* isolates to spring linseed cultivars.

MATERIALS AND METHODS

Thirty isolates of *V. dahliae*, from tomato, potato, hop, strawberry, maple, quince, sunflower, cotton, olive, chrysanthemum, linseed or soil, were tested for their pathogenicity to the spring linseed cultivar Antares and four of these isolates were tested for their pathogenicity to eight spring linseed cultivars (Jupiter, Agristar, Windermer, Master, Mikael, Omega, Barbara and Coniston). Pathogenicity experiments were done in controlled environment cabinets at a night/day temperature of 22°C, with a 16 h photoperiod and a light intensity of 600 $\mu E\ m^{-2}\ sec^{-1}$. Linseed plants were inoculated by dipping roots of seedlings at growth stage 22-23 (Freer, 1991) into a *V. dahliae* spore suspension with 1.4×10^7 spores/ml. Inoculated plants in pots were arranged in a randomised block design, with nine replicates per isolate for five pathogenicity experiments with cv. Antares and three replicates per isolate for the pathogenicity experiment with the eight linseed cultivars. Plants were assessed 1, 2 and 3 wk after inoculation for main stem height, number of tillers and % leaf area with chlorosis or necrosis on each main stem or tiller. The visual symptoms (% leaf area with chlorosis or necrosis) caused by *V. dahliae* were assessed using a disease index: 0, no visual symptoms; 1, 0-20%; 2, 20-40%; 3, 40-60%; 4, 60-80%; 5, 80-100% area affected. A plant disease index was calculated as the mean of the disease indices of the stem and tillers of each plant.

RESULTS

All thirty *V. dahliae* isolates tested were pathogenic to the spring linseed cultivar Antares. Infection by *V. dahliae* generally decreased the number of tillers and the height of the linseed main stems to cause severe stunting, with the greatest plant disease indices caused by VD4, VD8 and VD9 (Table 1). Isolates from linseed did not produce more severe disease symptoms than isolates from other hosts. The plant disease index ranged from 4.6 for highly pathogenic

Table 1. Effects of *Verticillium dahliae* on the number of tillers, stem height and plant disease index on spring linseed cv. Antares.

	Isolate code	Host of origin	No. of tillers	Height (cm)	Plant disease index*
Expt 1	VD1	Hop	4.7	17.8	2.9
	VD3	Hop	6.3	28.1	1.1
	VD4	Hop	4.0	17.8	3.5
	VD5	Hop	5.6	20.1	3.0
	VD8	Tomato	2.8	15.9	4.0
	VD10	Strawberry	3.9	18.8	3.1
	Control		10.0	52.9	0.0
	SED (12df)		0.79	2.35	0.29
Expt 2	VD2	Hop	4.2	23.8	2.8
	VD9	Strawberry	2.3	23.7	4.0
	VD11	Strawberry	5.3	30.5	2.5
	VD12	Strawberry	4.3	22.0	2.9
	VD14	Strawberry	6.3	29.8	1.0
	VD17	Potato[1]	3.2	22.8	3.8
	Control		4.5	36.5	0.0
	SED (12df)		1.3	1.89	0.38
Expt 3	VD5	Hop	4.8	24.9	3.0
	VD9	Strawberry	2.5	20.5	4.4
	VD20	Mint (linseed once)	6.0	31.7	0.5
	VD33	Sunflower	6.3	28.1	2.9
	VD36	Maple	4.4	25.1	2.3
	VD37	Quince	5.1	22.6	2.6
	VD40	Soil	3.4	22.7	3.2
	VD41	Soil	4.9	32.8	1.0
	Control		5.3	44.6	0.0
	SED (20df)		0.91	1.51	0.30
Expt 4	VD42	Soil	1.3	21	4.6
	VD43	Soil	4.2	24.3	2.6
	VD44	Soil	4.8	27.9	1.9
	VD45	Cotton	4.8	30.2	1.5
	VD46	Olive[2]	4.1	31.4	0.2
	VD51	Chrysanthemum [2]	4.8	30.0	1.0
	VD53	Artichoke[2]	4.7	25.4	2.4
	Control		5.2	32.7	0.0
	SED (14df)		0.48	1.14	0.24
Expt 5	VD5	Hop	3.3	17.1	3.3
	VD9	Strawberry	2.1	14.2	4.3
	VD42	Soil	4.0	17.6	2.8
	VD56	Linseed	5.1	21.7	2.5
	VD58	Linseed	5.9	28.3	2.2
	VD59	Linseed	6.3	34.0	1.5
	VD60	Linseed	4.9	26.2	2.0
	VD61	Linseed	4.6	21.0	3.0
	Control		6.4	40.4	0.0
	SED (16df)		0.61	1.53	0.33

* Plant disease index: 0-5 scale based on % area with necrosis or chlorosis, mean of disease indices of stem and tillers of each plant. [1]Canadian isolate. [2]Greek isolates

Table 2. Effects of *Verticillium dahliae* on the number of tillers, stem height and plant disease index on eight cultivars of linseed.

Cultivar	Control	VD5	VD8	VD9	VD33
	Number of tillers				
Agristar	1.3	1.7	3.7	1.0	4.7
Barbara	2.7	2.7	2.3	0.7	7.0
Coniston	2.0	1.3	0.7	0.7	7.7
Jupiter	2.7	2.3	1.3	0.0	3.3
Master	1.7	0.3	0.0	0.0	2.0
Mikael	7.0	1.7	3.7	1.3	4.3
Omega	7.3	4.7	2.0	2.7	4.3
Windermer	6.0	1.3	2.0	1.7	8.0
	Stem height (cm)				
Agristar	30.7	28.7	33.7	25.7	40.0
Barbara	33.7	29.0	30.7	32.0	31.3
Coniston	41.3	28.7	24.7	21.3	29.3
Jupiter	36.7	27.3	25.3	23.0	30.0
Master	31.0	21.0	19.7	20.3	22.0
Mikael	38.0	26.0	26.0	23.7	29.7
Omega	35.7	21.4	21.3	23.3	32.0
Windermer	43.3	29.7	26.3	28.7	30.0
	Plant disease index*		(0-5 scale)		
Agristar	0.8	3.7	2.8	4.8	3.7
Barbara	0.0	4.3	3.8	4.3	3.6
Coniston	0.0	4.8	5.0	5.0	4.0
Jupiter	1.3	4.8	4.5	5.0	3.5
Master	0.3	5.0	5.0	5.0	4.8
Mikael	1.0	4.0	4.1	4.3	3.6
Omega	0.0	4.6	4.1	4.5	3.1
Windermer	0.0	5.0	4.3	5.0	3.5

SED (df 77)	No. of tillers	Height	Plant disease index
Cultivar	0.90	1.31	0.28
Isolate	0.72	1.04	0.22
Cultivar x isolate	2.05	6.09	0.63

*Plant disease index: 0-5 scale based on % area with necrosis or chlorosis; mean of disease indices of stem and tillers of each planisolates

(VD42, Table 1) to 0.2 for the least pathogenic isolate (VD46, Table 1). In the other experiment, the four *V. dahliae* isolates tested were pathogenic to all eight linseed cultivars.

They developed similar symptoms (necrosis, chlorosis, decreased number of tillers and stunting) to those on Antares and the differences in pathogenicity between isolates were similar to those on cv. Antares, with VD33 producing the smallest disease index and VD9 the greatest (Table 2). The plant disease index ranged from 2.8 to 5.0 (Table 2). The cultivars showed differences in their susceptibility to *V. dahliae*; Agristar had the lowest disease index for all isolates except VD9 (Table 2).

DISCUSSION

These pathogenicity tests suggested that all *V. dahliae* isolates were pathogenic to linseed, irrespective of their original host, and the differences in pathogenicity of isolates could not be related to their original host or geographical origin. Such lack of pathogen specialisation in *V. dahliae* isolates and the ability of isolates from a given host to cause symptoms on other hosts has been reported for a many crops (Bhat & Subbarao, 1999) and suggests a lack of host specificity in the fungus *V. dahliae*. They also suggested that all spring linseed cultivars were susceptible to *V. dahliae*.The differences in pathogenicity between isolates could not be explained by host adaptation as the most pathogenic isolates were from hosts other than linseed and the linseed isolates were not the most pathogenic. A possible explanation is that differences in pathogenicity between isolates may reflect differences in the cropping history of the fields from which they came (Tjamos, 1981). Plant maturity might may have played a role in expression of the symptoms (Schnathorst, 1981) since different cultivars of linseed tested were all susceptible to *V. dahliae*, but the late and medium maturity cultivars Agristar and Barbara had lower plant disease index to the early maturity cultivar Master.

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Prediction and manipulation of blackdot (*Colletotrichum coccodes*) in potato crops

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ABSTRACT

The effect of blackdot on potatoes was approached holistically. The prediction of disease using stem bases or stolons early on crop growth was effective on Estima, but less so for Maris Piper and King Edward. The ability of bio-control and varying humidity to control the spread of disease in the stored crop has been investigated with variable results.

INTRODUCTION

In the last twenty years, significant advances have been made in the control of blemish diseases of potato, primarily silver scurf. Black dot is now identified as a major blemishing disease, affecting presentation at point of sale and ultimately reducing profitability for potato producers (Read and Hide, 1995). Work at SAC, Aberdeen, has focused firstly on prediction in the growing crop, with the objective to provide a tool which would enable farmers to be aware of their potential disease levels allowing time for remedial agronomic decision making. Secondly, to manipulate the disease in field and store, biological control measures have been implemented (to determine the ability of micro-nutrient supplements and to reduce disease) and controlled atmosphere chambers have been used to monitor disease expansion post harvest.

MATERIALS AND METHOD

Predictive technique

Potato plants from 80 commercial crops throughout the UK were examined microscopically and macroscopically for blackdot disease development on the epidermis of stems, stolons and tubers in July and August and again on tubers at harvest. Disease was measured as incidence and % coverage. Correlation between timing and level at harvest was determined by regression analysis.

Storage

Experimental chambers were set up containing appropriate salt solutions to give humidity levels of 95%(h) and 80%(l) relative humidity (see Table 1). Diseased Estima potatoes were placed in the chambers, situated in a dark constant temperature room at 15°C for 2 weeks before being transferred to 4°C for the remaining 10 weeks.

Table 1. Storage conditions (temperature and relative humidity) of four treatment regimes used in experiment 2.

Treatment	Storage conditions		Notation used in results
	Weeks 0-2	Weeks 3-12	
1	15C 95% rh	4C 95% rh	(h)
2	15C 95% rh	4C 80% rh	(h/l)
3	15C 80% rh	4C 95% rh	(l/h)
4	15C 80%rh	4C 80% rh	(l)

Observations were made regarding lesion increase, using Elipse formula (long diameter x short diameter x 0.7854, Anon 1998) at 2,4,7, 10 and 12 weeks after treatment started.

Bio-control method

Diseased Estima seed potatoes were planted in 14 litre pots containing peat. The peat was amended with micro-nutrient solutions to give 15 different nutrient treatments, which included different concentrations of iron, zinc and sulphur. This was replicated 8 times. Plants were grown under greenhouse conditions following commercial practices for disease and pest control. Once senescence took place the potatoes from each bucket were cleaned, weighed and analysed for black dot incidence and % surface area infected. At time of writing results are not available.

RESULTS

Predictive technique

Disease levels on stem bases and stolons in July and August correlated highly with disease levels on tubers at harvest for Estima crops in 1998 and 1999 (Table 2). However correlation was poor for Maris Piper and King Edward.

Table 2. Regression analysis of mean area of diseased tubers compared to mean area (mm^2) of diseased stolons and stems 1998/9.

Variety	Variable Compared (% area)	Co-efficient of correlation	P-Value
Estima	Tuber (July) vs. tuber @ harvest 98	0.74	0.16
Estima	Stembase (July) vs. tuber @ harvest 98	0.71	0.18
Estima	Stolon (July) vs. tuber @ harvest 98	0.80	0.10
Estima	Tuber (August) vs. tuber @ harvest 98	0.50	0.39
Estima	Stembase (August) vs. tuber @ harvest 98	0.53	0.36
Estima	Stolon (August) vs. tuber @ harvest 98	0.57	0.31
Estima	Stembase (Sept.) vs. tuber @ harvest 99	0.52	0.001
Estima	Stolon (Sept.) vs. tuber @ harvest 99	0.80	0.001
Maris Piper	Stembase (Sept.) vs. tuber @ harvest 99	0.17	0.11
Maris Piper	Stolon (Sept.) vs. tuber @ harvest 99	0.05	0.4
King Edward	Stembase (Sept.) vs. tuber @ harvest 99	0.002	0.95
King Edward	Stolon (Sept.) vs. tuber @ harvest 99	0.002	0.95

Storage results

There were no significant effects of humidity on disease expansion during storage (Figure 1).

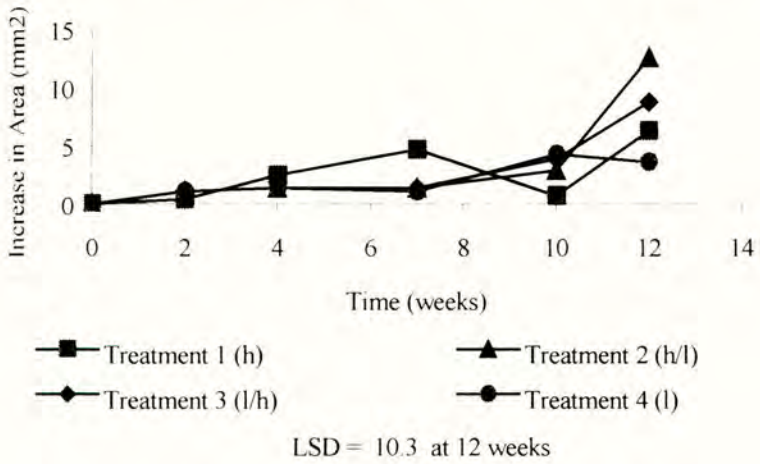

Figure 1: Increase in area of black dot lesions in response to four different humidity regimes calculated using Elipse formula.

DISCUSSION

Predictive

The examination of below ground parts from July onwards provides a good indication of likely blackdot development later in the growing crop. This is particularly so for Estima, a variety with low resistance to blackdot. However, prediction is less easy for Maris Piper and King Edward. This can be attributed to the susceptibility of the variety, as Maris Piper and King Edward show higher levels of resistance to the disease (Hilton *et al*, 1999). If this technique becomes highly predictable, as is hoped, then it can be utilised in decisions regarding burnoff, storage and crop marketing. However, further research is required to ensure the predictive nature, not just for Estima, but a wide range of cultivars on the market. Evaluation of plant parts as well as conidia and sclerotia in soil will continue for a further two seasons.

Storage

At present there is no solid evidence (Figure 1) to determine accelerated or decelerated lesion development in response to humidity control in store. However results do suggest a possibility of control. Therefore, further work into this area will be undertaken. In particular the effects of precise humidity levels in lesion will be examined over a longer duration.

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Effect of the chitin synthesis inhibitor lufenuron on the American bollworm

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ABSTRACT

The activities of the chitin synthesis inhibitor, lufenuron against embryonic and post embryonic stages of the American bollworm, *Helicoverpa armigera* were evaluated by exposing eggs of different embryonic stages and the first instars to various concentrations of lufenuron under laboratory conditions. Larval hatch was very high (> 95%) but mortality of the first instars shortly after hatch or during moult to the second instar stage was also high (> 90%). The few larval instars that were able to develop to the pupal and adult stages had various degrees of morphological deformities. Although lufenuron had no effect on the development of the embryos, its larvicidal activities could help in reducing the damage caused by the American bollworm.

INTRODUCTION

The America bollworm (*Helicoverpa armigera*) is one of the most destructive agricultural pests in the world (Han *et al.*, 1999). Control of the American bollworm has become increasingly difficult because of the development of resistance to most of the conventional insecticides (Han *et al.*, 1999). Chemicals that inhibit the formation or deposition of chitin (a major component of the insect's cuticular exoskeleton) should affect all developmental stages in which there is a moult (Mosson *et al.*, 1995). Lufenuron, like other benzoylphenyl ureas, blocks the synthesis and deposition of chitin during development, thereby inhibiting the moult between the life stages (Anonymous, 1997). The mechanism by which this inhibition occurs is still poorly understood (Soltani *et al.*, 1984). This paper reports the effects of lufenuron on embryonic and postlarval stages of the American bollworm.

MATERIALS AND METHODS

Insects and chemical

Newly emerged *H. armigera* adults were collected from a colony maintained at the Plant Protection Research Institute in Pretoria. The moths were put in oviposition chambers (plastic containers, 20 cm in height by 11 cm in diameter, with screened tops for ventilation) and fed 5% sucrose solution (King *et al.*, 1985). Eggs were oviposited on nylon netting in the chambers. All experiments were conducted at a constant temperature of 28 ± 1 °C and 12L: 12D photoperiods. Match® 050 EC (Novartis South Africa (Pty)

Ltd) as an emulsifiable concentrate containing 50 g a.i./l lufenuron was used for the bioassays. The formulated insecticide was diluted in distilled water for all the assays.

Bioassays

Tomato fruits were dipped in different concentrations (0.02, 0.04 and 0.12 g a.i./l) of lufenuron for 5 minutes, air-dried and transferred to rearing chambers (plastic containers 7 cm in height and 12 cm in diameter with screened tops). *H. armigera* eggs (n = 400/egg stage) at different embryonic stages (white; ring and blackhead) were transferred from the oviposition substrates to the treated tomato fruits with a fine camel's hairbrush. Each concentration plus the control (in distilled water) was replicated four times and the plastic containers were covered with nylon netting until larval hatch. Newly hatched larvae were transferred to individual test tubes containing treated larval diet/medium to avoid cannibalism. Controls were fed untreated diet and the test tubes were stoppered with cotton wool plugs. The experiment was monitored daily and the number of dead larvae were recorded and observed under a stereomicroscope (X16) for morphological abnormalities. Data were corrected for natural mortality using Abbott's formula (Abbott, 1925).

RESULTS

Embryo-larvicidal effects

Very low embryocidal effect was recorded in the four egg stages and there was no significant difference in the percentages of larval hatch (> 95) in the treated and untreated experiments (Figure 1). Embryonic development in all the eggs reached the final black head stage. Mortality of first instars from the lufenuron treated substrates was high (> 90%) compared with the untreated substrates (Figure 2).

Larval mortality was most common during the moult to the second instar stage and nearly all the dead larvae exhibited morphogenetic disorders such as black shrivelled body, reduced body size and ruptured exoskeleton. Few larval instars (< 2%) exposed to the lower concentrations, were able to develop to the pupal and adult stages. Larvae that fed on treated medium and survived to become pupae had higher morphological deformities compared to the larvae that fed on untreated medium. Various degrees of morphological deformities such as ruptured and deformed cocoons, leaking hemolymph, and pupae with larval features (larviform) were recorded in the treated substrates. There was a significant decrease in adult emergence in the treated experiments (Figure 3). Findings from this investigation show that the larvicidal activities of lufenuron could help in reducing the damage caused by the American bollworm.

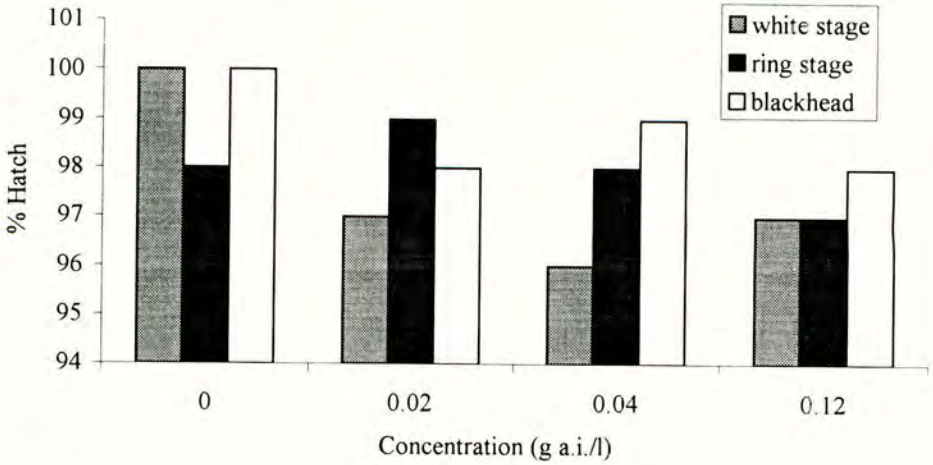

Figure 1. Effect of lufenuron on hatch of American bollworm eggs

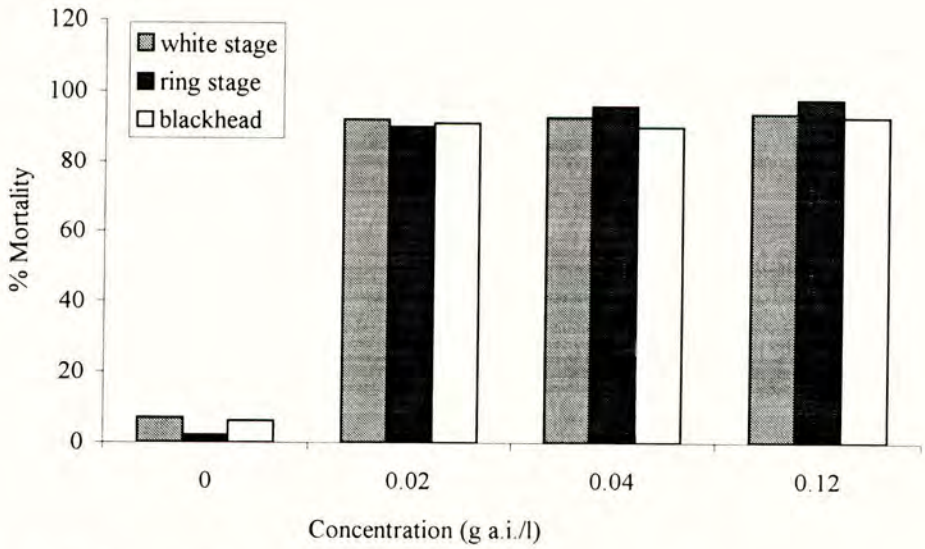

Figure 2. Effect of lufenuron on larval stages of the American bollworm

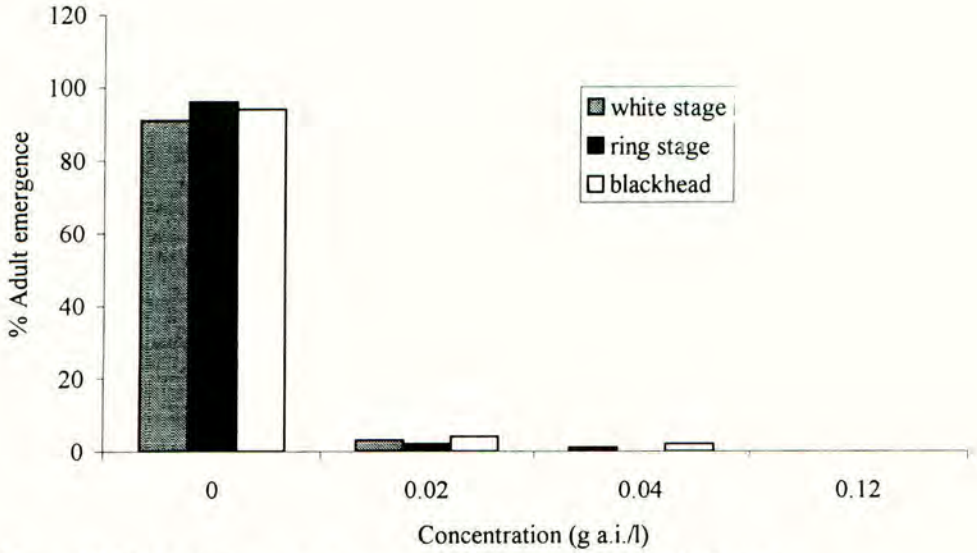

Figure 3. Effect of lufenuron on the emergence of adult American bollworm

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Pathogenicity of bacterial symbionts from entomopathogenic nematodes to larvae of *Galleria melonella*

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ABSTRACT

Introduction of larvae of *Galleria mellonella* to sand enriched with two types of bacterial suspensions of the symbionts *Xenorhabdus nematophilus* isolated from *Steinernema carpocapsae* nematode and *Photorhabdus luminescens* isolated from *Heterorhabditis bacteriophora* nematode, has resulted in high mortality to the larvae. More investigations are needed if these bacteria are to be used in biocontrol of soil pest insects.

INTRODUCTION

The bacterial symbionts *Xenorhabdus nematophilus* and *Photorhabdus luminescens*, associated with the entomopathogenic nematodes *Steinernema caropocapsae* and *Heterorhabditis bacteriophora* respectively. Both genera *Xenorhabdus* and *Photorhabdus* bacteria are belonging to the family Entrobacteriaceae. Both bacteria are very similar and they were considered to be one genus till recent studies on DNA-DNA hybridisation, carried by (Boemare *et al.*, 1993), (Rainey *et al.*, 1995) and (Burnel *et al.*, 1997) showed that these bacteria belong to different genera. The genus *Xenorhabdus* contains five described species *X. nematophilus*, *X. poinarii*, *X. beddingii*, *X. javonicus* and *X. bovienii*. The genus *Photorhabdus* contains only one species, however recent studies carried out by Fischer-Le Saux *et al.*, (1999) has proposed the subdivision of this species into four subspecies.

Dauer juveniles (DJs) of *Steinernema* and *Heterorhabditis* nematodes carry both *Xenorhabdus* and *Photorhabdus* in their guts. The Juveniles release the bacteria into the insect haemocoele after penetrating an insect host. The bacteria proliferate and cause septicaemic death after series of physiological events (Boemare, *et al.*, 1997). Inside the carcass of the host the bacteria provide a suitable nutrient environment for nematode reproduction. The purpose of this paper is to test the pathogenicity of the two types of bacterial suspensions of *X. nematophilus* and *P. luminescens* against larvae of the greater wax moth *G. mellonella*.

METHODS AND MATERIALS

Late instar larvae of *Galleria mellonella* larvae were obtained from The Meal Worm Company. *G. mellonella*. Bacteria obtained from infected larvae with either *S. carpocapsae* or *H. bacteriophora* as described by Elawad (1998).

Mass production of the bacterial cells was done by inoculating a single colony of the bacterium into 500 ml of distilled water nutrient broth solution containing 15g nutrient broth (BDH) in flask stoppered by sterile cotton wool and placed in a shaking incubator at 150 rpm for one day at 28°C. Two types of bacterial suspension by prepared, suspension of

bacteria in sterile tap water and suspension of bacterial cell in broth. The bacterial free suspension in tap water was prepared were centrifugation at 4100 rpm for 20 min. The supernatant broth solution was drawn off and placed by sterile tap water. The concentrations of both bacterial suspensions were determined by measuring the optical density using a spectrophotometer adjusted to 600 nm wavelength.

In the first experiment ten larvae of *G. mellonella* were placed in 100 g of sterilised sand in plastic containers (9cm diam. And 5 cm deep) and treated with 25 ml of bacterial suspensions or broth alone or sterile distilled water alone. The moisture content of the sand was adjusted 20% and the concentration of bacterial cells was adjusted to $4X10^7$ ml and the mortality of the larvae and pupae was recorded after 8 days. Six treatments were used: (1) two bacterial suspensions in sterile distilled water, (2) two bacterial suspensions in broth, (2) broth solution alone, (4) distilled water alone. Replication was 4 fold i.e. total number of 40 larva was tested per each treatment. In the second experiment for estimation of LC_{50} for the two types of bacterial suspensions, ten larvae were placed in sand as explained in experiment one. Six concentrations 400, 4000, 40000, 400000, 4000000 40000000 for each bacterial suspension and the mortality of the larvae was recorded after 8 days and the replication was 4 fold i.e. 40 larva per concentration. In the third experiment to estimate the LT_{50} values LC_{50} for the two types of bacterial suspensions, ten larvae were placed in sand as in experiment one, the mortality was recorded daily over a period of 10 days and the replication was 4 fold. All experiments were carried out at 28°C. SAS computer programme was used to calculate X^2, LC_{50} and LT_{50} values.

RESULTS

Both bacterial suspensions of *Xenorhabdus nematophilus* and *Photorhabdus luminescens* in broth and in water caused high mortality to *G. mellonella* larvae $P > 0.001$, see Table 1. It seems that bacterial suspensions in broth were causing high mortality than bacterial suspension in water. The cause of mortality was confirmed by isolation of primary forms of both bacteria from the carcass of all dead larvae. The LC_{50} values for the two bacterial suspensions in broth were highly significant than bacterial suspensions in water $P > 0.001$, however LC_{50} value for *Photorhabdus* in broth is highly significant than *Xenorhabdus* suspension in broth see Table 2. LT_{50} values for broth and water suspensions are highly significant $P > 0.001$, however there was no significant difference in the LT_{50} values for *Photorhabdus* in broth and *Xenorhabdus* suspension in broth see Table 3.

Table 1 The percent mortality of *G. mellonella* larvae introduced to sand enriched with two types of bacterial suspensions of *X. nematophilus* and *P. luminscens*

Treatment	%Mortality
Sand + *X. nematophilus* suspension in water	87.5
Sand + *X. nematophilus* suspension in broth	92.5
Sand + *P. luminscens* suspension in water	90.0
Sand + *P. luminscens* suspension in broth	95.0
Sand + broth only	35
Sand + Water only	20.0

Table 2. LC_{50} bacterial suspensions of *X. nematophilus* and *P. luminscens* to larvae of *G. mellonella*

Treatment	LC_{50}
Sand + *X. nematophilus* suspension in water	77102.6
Sand + *X. nematophilus* suspension in broth	1480.7
Sand + *P. luminscens* suspension in water	81210.1
Sand + *P. luminscens* suspension in broth	401.5

Table 3. LT_{50} for *X. nematophilus* and *P. luminscens* to larvae of *G. mellonella*

Treatment	LT_{50}
Sand + *X. nematophilus* suspension in water	4.4
Sand + *X. nematophilus* suspension in broth	2.9
Sand + *P. luminscens* suspension in water	4.6
Sand + *P. luminscens* suspension in broth	3.0

DISCUSSION

Larvae of *G. mellonella* introduced to sand enriched with two different bacterial suspensions of the symbionts, *X. nematophilus* and *P. luminescens* were killed. The results reported here are similar to previous reports by (Dudney, 1997), (Elawad *et al.*, 1999). It seems that the bacterial cells have gained access to the larvae via the spiracles similar to the way the nuclear polyhedrosis virus *Autographa californica* have accessed to the haemocoele via spiracles and trachea (Kirkpatrick *et al.*, 1994). The bacterial suspension of both *Xenorhabdus* and *Photorhabdus* bacteria in broth are more pathogenic than water suspensions. Possible reason for that is the presence of toxic bacterial metabolic products in the broth. More studies are needed to quantify and identify these toxic substances. Application of bacterial suspensions against some of the soil pest insects could be adopted as a mean of biocontrol of some insect pests. However, application of bacterial suspension in the natural soils should be preceded by through investigations on the persistence as well as studies on the effect of these bacteria on other organisms present in the soil.

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