POSTER SESSION 8C

POST-GRADUATE STUDENT POSTERS

Session Organiser: Professor P E Russell
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Poster Papers: 8C-1 to 8C-15

Behaviour-modifying chemicals of the damson-hop aphid, *Phorodon humuli* (Schrank)

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ABSTRACT

The role of two widespread plant volatiles, benzaldehyde and methyl salicylate, in the autumn migration of *Phorodon humuli* was investigated. Results from a field trapping experiment showed that the release of these two plant volatiles together reduced the number of male aphids trapped in response to the sex pheromone, (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol. Males did not respond to either plant volatile released singly, nor to the combination of plant volatiles in the absence of the sex-pheromone. Electroantennograms of gynoparae indicated the presence of peripheral odour perception to both plant volatiles.

INTRODUCTION

The damson-hop aphid, *Phorodon humuli* (Schrank), has a host alternating (heteroecious) life-cycle. In spring, winged parthenogenic female aphids fly from primary hosts, various species of *Prunus* including plum (*P. domestica* L.) to hops (*Humulus lupulus* L.) the only secondary host. In autumn, winged females (gynoparae) and males re-colonise *Prunus* (Born, 1968; Eppler, 1986). Perhaps uniquely among host-alternating aphids, *Phorodon humuli* is a serious pest of high-value crop plants among both alternate hosts.

Olfactory cues have been shown to be important in both the spring (Campbell *et al.*, 1993) and autumn (Campbell *et al.*, 1990) migrations of *P. humuli*. On primary host plants, males respond to a sex pheromone, the two diastereoisomers (1*S* and 1*R*,4a*R*,7*S*,7a*S*)-nepetalactol (Campbell *et al.*, 1990) released by sexual females (oviparae), the offspring of gynoparae. Water traps releasing a synthetic mixture of these isomers have been shown to trap males, however, the addition of primary host plant volatiles has an additive or synergistic effect (Lösel *et al.*, 1996).

The work presented here looks at the behavioural responses of *P. humuli* to the sex pheromone and to two general plant volatiles; benzaldehyde and methyl salicylate. Benzaldehyde is known to be a major component of *Prunus* spp. volatiles and has been shown to synergistically increase trap catches of *Rhopalosiphum padi* (L.) in the presence of its sex pheromone (Hardie *et al.*, 1994). Methyl salicylate is produced by both *Prunus spinosa* and hop (Cook, 1996). In behavioural studies with spring migrants of *Phorodon humuli*, methyl salicylate was shown to negate the aphid's positive responses to two other host-plant volatiles (Campbell *et al.*, 1993).

METHODS

Field trials

Water traps were made from 15 cm diameter clear plastic Petri dishes filled with water containing 0.2% Lipsol detergent. Vials releasing the test volatiles were suspended above the surface of the water by wires. Traps were set on canes 0.6 m above ground level within a hop garden in Kent in the autumn of 2000. Benzaldehyde (50 mg) and (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol (10 mg) were each released from 1 ml chromacol vials (Type:08-CPV) with one 1 mm hole drilled in the top. Methyl salicylate (35 µl) was released from polythene vials (Fisons WP/5) with four 1 mm holes drilled in the top. The field experiment employed a complete factorial design replicated in four blocks with the three volatiles as factors each at two levels (present and absent). Blank control vials were added as necessary so that all traps presented an identical visual image. Traps were emptied daily and the numbers of *P. humuli* males trapped were recorded. Data were examined by analysis of variance using GENSTAT. In order to stabilize variances, the daily counts were transformed to square roots ($n^{0.5}$) and summed for the whole sampling period.

Electroantennography

The overall response of the odour receptors on an aphid's antenna, known as primary and secondary rhinaria, was recorded using the improved electroantennogram (EAG) technique developed by Park and Hardie (1998). Benzaldehyde, methyl salicylate and (*E*)-2-hexenal (the positive control) were dissolved in paraffin oil to make 1% (v/v) solutions. Twenty-five µl of each solution, or paraffin oil as the blank control, was applied onto a piece of filter paper, and the filter paper inserted into a glass Pasteur pipette. During odour stimulation 60 ml/min of air was passed through the pipette, for 2 seconds, and into a constant airflow of 1.8 l/min of humidified air passing over the insect. The EAG responses of 10 gynoparae to benzaldehyde, methyl salicylate and the blank control presented in random order at 2 min intervals were recorded. EAG responses were normalised using the mean response to (*E*)-2-hexenal presented before and after the test volatiles. Data were analysed in GENSTAT using t-tests.

RESULTS

Field trials

Significantly more male *P. humuli* were caught in traps releasing (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol than in traps not releasing this volatile ($t = 8.16$, $P < 0.001$) (Figure 1). Benzaldehyde and methyl salicylate released singly or in single combination with (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol or in combination with each other did not affect trap catches. However, the addition of both plant volatiles significantly reduced the number of males caught in response to (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol ($t = 2.96$, $P < 0.01$).

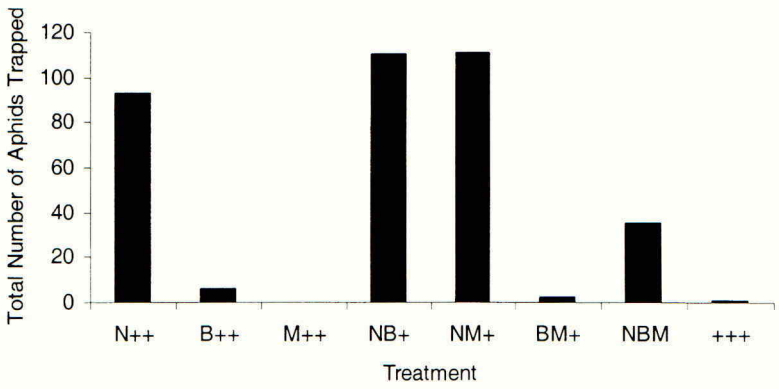

Figure 1. Cumulative numbers of *Phorodon humuli* males trapped in each treatment. N = (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol; B = benzaldehyde; M = methyl salicylate; + = visual blank.

Electroantennography

The EAG responses of *P. humuli* gynoparae (Figure 2) were significantly larger to both benzaldehyde and methyl salicylate than to the paraffin oil control ($t = 7.02$, $P < 0.001$ and $t = 4.21$, $P < 0.001$, respectively). The EAG response to benzaldehyde was also found to be significantly larger than to methyl salicylate ($t = 3.37$, $P = 0.003$). However, EAG responses to both benzaldehyde and methyl salicylate were smaller than to the positive control, (*E*)-2-hexenal.

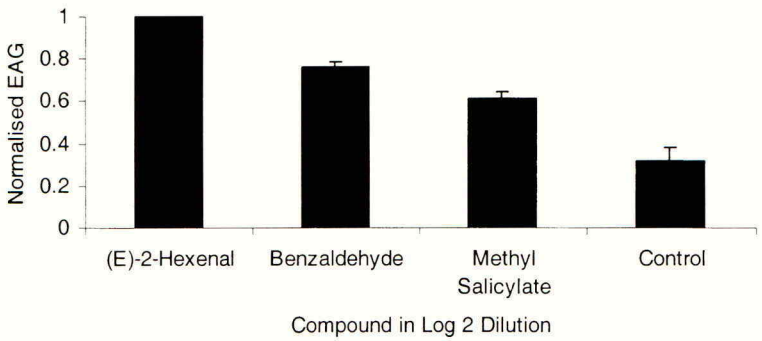

Figure 2. EAG responses of *Phorodon humuli* gynoparae to benzaldehyde and methyl salicylate. Responses normalised to that of (*E*)-2-hexenal. (n = 10).

DISCUSSION

Field data presented here confirm the efficacy of (1*RS*,4a*R*,7*S*,7a*S*)-nepetalactol in increasing trap catches of male *P. humuli* (Campbell *et al.*, 1990). The addition of benzaldehyde and methyl salicylate, however, did not explain the synergistic effects of adding host plant extracts to this pheromone (Lösel *et al.*, 1996). In contrast, the addition of both plant volatiles reduced the numbers of males caught in traps releasing the pheromone. EAG results with gynoparae suggest that at least this morph among autumn migrants of *P. humuli* can detect both of these plant volatiles.

ACKNOWLEDGEMENTS

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Maturation of ascospores of A-group and B-group *Leptosphaeria maculans* (stem canker) on winter oilseed rape debris

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ABSTRACT

Ascospores of both A-group and B-group *L. maculans* matured faster at 15-20°C than at 5-10°C. At 5-10°C, maturation of B-group ascospores was slower than that of A-group ascospores but there was no difference at 15-20°C. A-group pseudothecia were mainly produced on the surface of stem bases and B-group pseudothecia were mainly produced under the epidermis of upper stems. At Rothamsted in 2000/2001 and 2001/2002, ascospores matured earlier on stem bases than on upper stems. Ascospores matured and were released earlier in 2001 than 2000 and were then released from autumn to spring. Seasonal differences in first maturation of ascospores were related to differences in summer rainfall. The first phoma leaf spotting appeared in untreated plots of winter oilseed rape 8-15 days after the first release of ascospores.

INTRODUCTION

Stem canker (blackleg), caused by *Leptosphaeria maculans*, is a serious disease of oilseed rape worldwide (West *et al.*, 2001). The population of *L. maculans* can be divided into two main sub-groups, which are often termed A-group and B-group (Williams & Fitt, 1999). Previous epidemiology work has mainly been on the A-group, with little information about B-group *L. maculans*. In the UK, both A-group and B-group are present. Stem canker epidemics are initiated by ascospores released from infected oilseed rape debris and can be controlled only by fungicides applied in autumn during the phoma leaf spot stage of epidemics. To control the disease effectively, it is necessary to understand the maturation of ascospores of both A-group and B-group *L. maculans* on oilseed rape debris.

MATERIALS AND METHODS

Naturally infected UK oilseed rape stem bases and Polish oilseed rape upper stems were collected after harvest. UK stem bases (colonised by A-group) and Polish upper stems (colonised by B-group) were used to study the maturation of ascospores of A-group and B-group *L. maculans*, respectively. These stem bases and upper stems were incubated in growth cabinets (5, 10, 15 or 20°C) under continuous wetness. Maturation of ascospores on the debris was assessed weekly under a light microscope.

In 2000/2001 and 2001/2002, infected UK oilseed rape stem base (A-group) and upper stem (predominantly B-group) (> 10 cm above the ground) debris were collected after harvest and incubated under natural conditions at Rothamsted. Maturation of ascospores on stem bases and upper stems was assessed weekly. A Burkard spore sampler was used to monitor the release of air-borne ascospores of *L. maculans*. To study relationships between timing of ascospore release and timing of phoma leaf spot development, field experiments were done at Rothamsted in 2000/2001 and 2001/2002. No fungicides were applied. In both seasons, after the first release of ascospores was observed, 30 plants (cv. Apex) were sampled weekly from plots to assess the presence of phoma leaf spots.

RESULTS

Effects of temperature on maturation of ascospores in controlled environments

In controlled environments, ascospores of both A-group and B-group *L. maculans* were produced at 5-20°C, but matured faster at 15-20°C than at 5-10°C. At 5-10°C, maturation of B-group ascospores was slower than that of A-group ascospores but at 15-20°C there was no difference between them. Pseudothecia of A-group *L. maculans* were produced on the surface of stem bases, with the whole pseudothecium exposed, and there were ostioles on the top of pseudothecia after release of ascospores (Figure 1a). Pseudothecia of B-group *L. maculans* were produced under the epidermis of the upper stems, with only the neck of the pseudothecium exposed (Figure 1b). If the epidermis was peeled off, the shapes of B-group pseudothecia were similar to those of A-group pseudothecia. The density of A-group pseudothecia produced on stem bases was higher than that of B-group pseudothecia produced on upper stems, at all four temperatures tested.

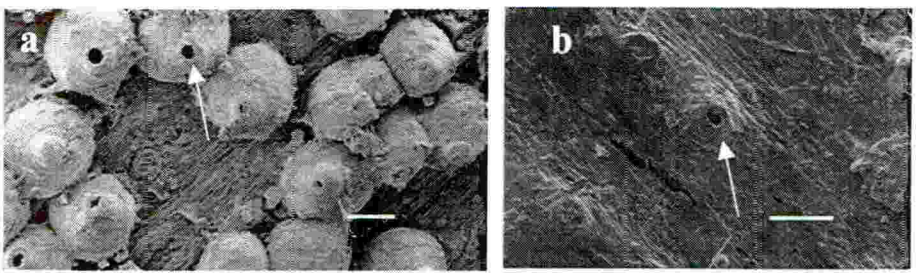

Figure 1. Mature pseudothecia, with ostioles after ascospore release (marked with arrows) of A-group (a) and B-group (b) *L. maculans* produced at 15°C on oilseed rape stem base and upper stem debris (cv. Lipton), respectively (bars = 100 μm).

Maturation of ascospores on UK oilseed rape debris under natural conditions

In 2000/2001 and 2001/2002, under natural conditions mature ascospores in pseudothecia were observed from September to February. The first mature ascospores were observed on stem bases 2 weeks earlier than on upper stems in both seasons; there were no differences in proportions of mature pseudothecia between stem bases and upper stems (Figure 2a). Ascospores on both stem bases and upper stems matured earlier in 2001 than 2000.

Comparing the two seasons for rainfall and average temperature during the period between harvest and the first mature pseudothecia suggested that differences in rainfall had a greater influence on the date of first maturation of ascospores.

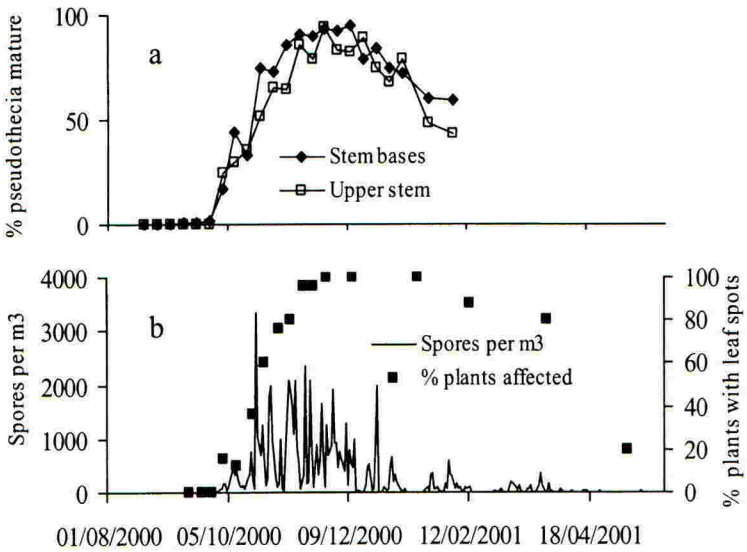

Figure 2. Changes with time in percentage of mature pseudothecia on UK stem base (A-group) and upper stem (mainly B-group) debris (a) under natural conditions in relation to numbers of ascospores of *L. maculans* in the air and incidence (% plants affected) of phoma leaf spots (■) in untreated winter oilseed rape (cv. Apex) at Rothamsted (b) in 2000/2001.

Seasonal periodicity in release of ascospores and timing of phoma leaf spots

There were differences in the numbers and patterns of ascospores released between 2000/2001 and 2001/2002. The first releases of ascospores were detected on 24 September 2000 and 18 September 2001, and ascospores continued to be released until late spring in both seasons. The majority of ascospores were released between October and late December (Figure 2b). After the first ascospores were detected, subsequent ascospore releases were associated with occurrence of rain or heavy dew.

Phoma leaf spotting was first observed on 9 October 2000 and 26 September 2001. The time from the first observed release of ascospores to the first observed leaf spots on plants in unsprayed plots was 15 days in 2000 and 8 days in 2001. In 2000/2001, the phoma leaf spotting epidemic was severe; the incidence of affected plants reached 36% in late October and increased to 100% in late November (Figure 2b); the severity (% leaf area affected) was 5.5% in late October and increased to a maximum of 34% in mid-November. In contrast, in 2001/2002, the epidemic was early but less severe, with an incidence of 4% plants affected in late September and a maximum incidence of 70% in late October; the % leaf area affected was only 0.6% in late September and increased to a maximum of 16.3% in late October.

DISCUSSION

The controlled environment experiments indicate that ascospores of both A-group and B-group *L. maculans* mature on oilseed rape stem debris faster at 20°C than at 5°C. Interactions between temperature and maturation of A-group and B-group ascospores may explain why the upper stems are mainly colonised by B-group, while stem bases are mainly colonised by A-group in the UK (West *et al.*, 1999). Results of this study suggest that in the UK low temperatures in winter can delay maturation of B-group ascospores more than that of A-group ascospores. In spring as temperatures increase, maturation of B-group ascospores may start again and the number of air-borne B-group ascospores may increase. Subsequently, infection of upper stems is mainly associated with B-group *L. maculans*.

Results of ascospore maturation experiments in natural conditions suggest that weather factors (temperature and rainfall) affected maturation of ascospores and the timing of the first major ascospore release and subsequent phoma leaf spot development. In 2001/2002, there was more rain during the period from harvest until the first mature ascospores were observed than in 2000/2001, and ascospores matured earlier than in 2000/2001. Subsequently ascospores were released 1 week earlier and the phoma leaf spots were observed two weeks earlier than in 2000/2001. Results of this study suggest that the first release of ascospores can be predicted by monitoring weather conditions and maturation of ascospores. Accurate forecasting of severe epidemics can improve disease control and decrease fungicide use when the risk of crop damage is low.

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Alternatives to methyl bromide method for the management of root-knot nematodes (*Meloidogyne* spp.) in greenhouse-grown tomato

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ABSTRACT

The effectiveness of solarization in combination with soil amendments, in the control of root-knot nematodes (RKN) on greenhouse-grown cherry tomato was investigated during the growing season 2000-01. Solarization for 4 or 8 wks, in a hermetically-sealed greenhouse, significantly decreased the root galling index. In contrast, no significant differences were observed between soil amendments and the control treatment. Both solarization treatments also reduced significantly *Fusarium oxysporum* populations, eliminated all important soilborne diseases and increased total fruit yield by ~ 25%, compared to that obtained from uncovered soil. In a preliminary study in the same greenhouse site, biocontrol products based on the micro-organisms *Burkholderia* sp., *Bacillus* sp., *Myrothecium* sp. and *Penicillium* sp. provided insufficient control of RKN. According to the results of this study, solarization is an important plant protection tool for the control of RKN and other soilborne diseases of greenhouse grown tomato, under the climatic conditions of Cyprus.

INTRODUCTION

Soilborne diseases and root-knot nematodes (RKN) are very destructive pathogens in tomato and other vegetable crops. Their management in Cyprus is presently based on pre-plant soil fumigation with methyl bromide (MB), a compound whose phase-out procedure was initiated in Montreal Protocol (1992) due to its ozone depleting action (Maragos, 1999). Solarization, as an alternative to MB in countries with hot climates, has shown promising results for the control of many soilborne fungi and some plant-parasitic nematodes (Barbercheck & Broembsen, 1986; Kumar, *et al.*, 1993). For some other major plant-parasitic nematodes such as RKN, however, results of various studies with solarization are ambiguous and contradictory (Chellemi, *et al.*, 1997). The insufficiency of solarization to provide optimum RKN control comprises one of the most important barriers for its commercialization on a large scale. The aim of this study was to evaluate and improve the effectiveness of solarization against root-knot nematodes by combining it with various soil amendments.

MATERIALS AND METHODS

This study was undertaken in 2000-01 in a greenhouse with natural RKN infestation at the experimental station of the Agricultural Research Institute at Zygi, Cyprus. The experiment was a split-plot design with three main plots arranged in strips and accommodating two solarization treatments (4 and 8 wks) and the uncovered (non-solarized) control. Each plot was subdivided into 7 subplots: (a) the untreated control, (b) Deny (spores of the bacterium *Burkholderia cepacia*), (c) Nemaclean (plant extracts), (d) calcium cyanamide, (e) Rugby (cadusafos), (f) Antinem (chitin) + Acidam (sulfur with *Thiobacillus* sp.), and (g) Nemacur (fenamiphos). In each subplot, sixteen tomato seedlings (cherry type – cv. Bar 138-8) were planted in a double row system whereby observations in each row were kept separately. Therefore, each combination (solarization x soil amendment) was replicated six times.

Solarization was carried out in July – August 2000 by mulching the wet soil with transparent polyethylene sheet, 70 μm thick. During solarization, the greenhouse was covered with clear plastic sheet, 200 μm thick, and kept hermetically closed in order to increase still further the soil temperatures. Soil temperature was recorded at depths of ~10 and ~20 cm using mercury-in-steel distance type thermographs. The RKN disease was assessed at different stages of the crop taking into account the following parameters: (a) number of 2nd stage juveniles in soil, (b) root-gall index (RGI) on a 1-10 scale and (c) number of females and other stages in the root tissue using the Fuchsin Acid stain. The population of the fungus *Fusarium oxysporum* was determined in soil samples taken before and after solarization. Other soilborne diseases, such as corky root rot (*Pyrenochaeta lycopersici*), Fusarium crown and root rot (*F. oxysporum* f.sp. *radicis-lycopersici*, *Phytophthora* sp.), and tomato wilts (*F.oxysporum f.sp. lycopersici*, *Verticillium dahliae*) were assessed at the end of the experiment, when plants were uprooted, by using a 1-10 scale index. The total fruit yield was also recorded for each plot.

In the same greenhouse, a preliminary, small-scale study was conducted in order to evaluate the effectiveness in the management of RKN of four formulations based on the biocontrol agents *Burkholderia* sp., *Bacillus* sp., *Myrothecium* sp. and *Penicillium* sp.. These biocontrol agents were applied in soil alone or in combination with 4 wk solarization, using similar techniques as described in the main experiments.

RESULTS AND DISCUSSION

The mean maximum temperature in solarized soil reached 55 and 53°C at depths of 10 and 20 cm, respectively, while in the uncovered soil it was 45 and 43°C, respectively.

The population density of *F. oxysporum* was reduced by 65 and 94% in the 4 and 8 wk solarization treatments, respectively. Root galling severity was lower in solarized treatments compared to the non solarized plots throughout the various growth stages of the crop. Three months after transplanting, the RGI was 1.5 in the non-solarized treatments as opposed to the solarized ones, where it was kept at nearly zero level. At the end of the crop season, average RGI values in the 4 and 8 wks solarization were 3.3 and 1.5,

respectively, compared to 8.1 in the uncovered treatment. Differences among soil amendments (averaged over solarization treatments) were not significant (Figure 1).

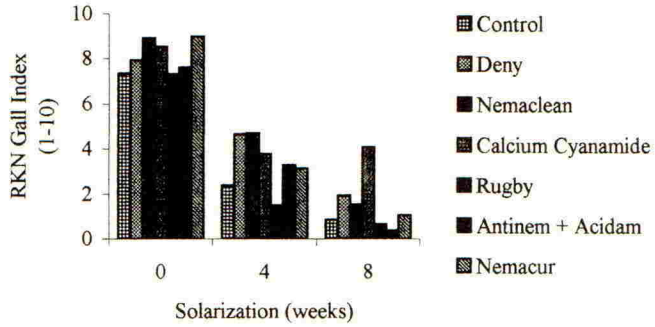

Figure 1. Effects of solarization and soil amendments on root galling caused by RKN at the end of the crop season

Similar results showing a drastic effect of solarization against RKN were reported by Ijani *et al.* (2000). In contrast, Barbercheck & Broembsen (1986) and Ioannou (2000) reported a weakness of solarization in the management of RKN. The drastic effect of solarization against RKN in the present study may be attributed to its improved application technique, since solarization was applied in a hermetically closed greenhouse which may have helped in reaching higher soil temperatures (Ioannou, *et al.*, 2000). In agreement with other reports (Katan, 1981), solarization also managed effectively many other soilborne pathogens, including *Fusarium* sp., *Phytophthora* sp, and *Verticillium* sp. The total yield in both 4 and 8 wk solarization treatments (averaged over soil amendment treatments) was increased by approximately 25% compared to the non-solarized treatment (Figure 2).

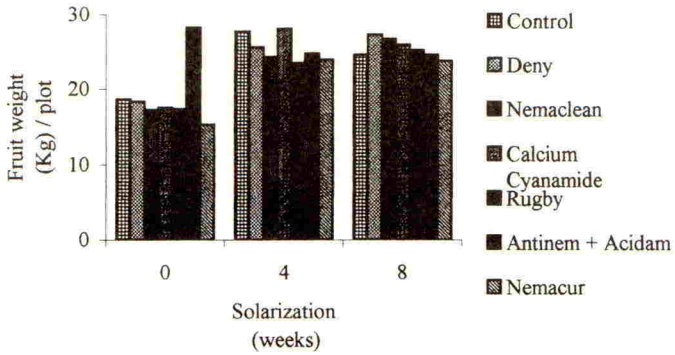

Figure 2. Effects of solarization and soil amendments on plant yield

A similar yield increase in solarized soil was also observed by Barbercheck & Von-Broembsen (1986), Ioannou (2000) and Ioannou *et al.*, (2000). The increase of yield in solarized soils may be attributed to the effective control of soilborne pathogens, the changes of nutrient status in soil and the stimulation of beneficial organisms (Gruenzweig,

et al., 1993). An equal yield increase was also observed in the Antinem + Acidam treatment in non-solarized soil (Figure 2), on which further studies should be conducted.

The results of the preliminary experiment with biocontrol agents showed that some of them, such as *Bacillus* sp. could limit the RKN disease of tomato plants, and further studies on a large scale should be conducted.

This study has shown that disinfecting the soil with 4 or 8 wks solarization in a hermetically closed greenhouse during the summer months decreases the severity of RKN and other soilborne diseases and significantly increases yield. A 4 wk solarization is nearly as effective as an 8 wk, especially with regard to fruit yield, suggesting that the shorter treatment may be sufficient for soilborne management.

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Variety mixtures and the blighted organic potato

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ABSTRACT

There is a need to better understand the usefulness of variety mixtures for the prevention of late blight in organic potatoes. It is only in certain variety combinations that mixture responses, in terms of disease and/or yield, are important. This, coupled to evidence from different plot sizes, indicates that the usual mixture mechanisms of barrier effects, the spatial separation of susceptibles and induced resistance, are not always important in potato variety mixtures. It is proposed that plant-plant interactions may also have an important role.

INTRODUCTION

Late blight, caused by *Phytophthora infestans* is a devastating disease of potatoes. The potential for devastation is heightened in organic production where chemical inputs are heavily restricted. There is therefore an urgent need to provide organic growers with a means to manage late blight.

Variety mixtures, the growing of different varieties as intimate neighbours in the same space and time, can impede the progress of crop disease epidemics. But, in theory, potato variety mixtures are not well suited to restricting late blight epidemics. This is due, in part, to the large plant size, the importance of lesion expansion to disease progress and a fast rate of disease progress. However, there is evidence for the usefulness of potato variety mixtures in preventing late blight (Garrett & Mundt 2000). There is therefore a continuing need to better understand the ecology and epidemiology of this mixture pathosystem before its usefulness can be properly evaluated.

The understanding of this mixture pathosystem is aided by the incorporation of different spatial scales into experiments. Firstly, there is the indication that genetic heterogeneity provides greater disease suppression when used over large areas (Zhu, *et al.,* 2000). And secondly, the epidemiological influences of one plot on another are greater at smaller plot sizes. This means, amongst other effects, that the ratio of autoinfection (secondary infection from spores originating from inside the plot) to alloinfection (secondary infection from spores originating from outside the plot) is greater in smaller plots.

METHODS

In 2001 late blight progress was monitored on a trial with treatments of pure stands, two component, three component and four component variety mixtures. The varieties were chosen for characteristic tubers for separation at harvest and to diversify for late blight resistance: Nicola and Sante are susceptible, Robinta is intermediate and Cara and Verity are resistant. None of these varieties show clear or important race-specific resistance when grown in the

UK. The trial consisted of four blocks, two with large plots (9m x 7.5m) and two with small plots (4.5m x 4.5m). All treatments were randomised within blocks. Therefore the trial examined the effect of increasing the number of varieties in a mixture, this also has the effect of providing different concentrations of resistant plants. The trial also allowed for an appreciation of specific variety interactions, both in terms of disease progression and yield effects.

Phytophthora infestans was allowed to naturally infect the trial, the infection pattern being general. The progression of disease severity was visually assessed (using the key of Fry, 2000) on plants of each variety, in the centre of the middle bed of each plot. Yield was measured from the last week in September to the first week in October.

Disease severity was summarised as AUDPC and Rate of Disease Progress. Rate of Disease Progress was calculated as the regression slope coefficient of a logit transformation of disease severity over time. Both AUDPC and Rate of Disease Progress were analysed using anova in Genstat. Firstly using linear contrasts to look for specific varietal interactions; and secondly, partioning the sums of squares into the amount that can be explained by a linear relationship of the treatment factor with disease, and this effect interacted with block size, thereby examining a quantitative treatment factor. The quantitative treatment factor was the proportion of resistant genotypes, where the resistant genotypes were Cara and Verity.

RESULTS

The influence of resistant tissue in a mixture was proportional to its concentration (Figure 1).

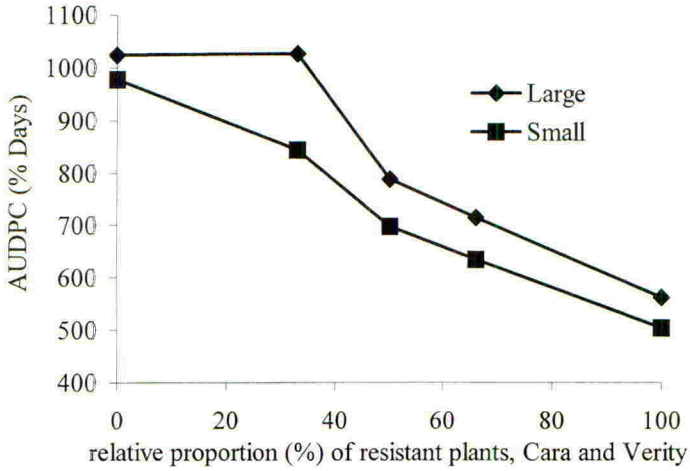

Figure 1. Mean Area Under the Disease Progress Curve (AUDPC, % days) for treatments with different proportions of resistant genotypes, the relationship was linear ($P < 0.001$), with differences between concentrations ($P < 0.001$). There was a significant difference between block size ($P < 0.001$) and the relationship was best described by parallel lines. The coefficient of variation was 31.4% (179 d.f.).

In addition, there was a mean reduction of 10.6% in AUDPC for small blocks (Figure 1), this reduction was not apparent for the rate of disease progress statistic (Figure 2). It was only in specific variety combinations where the influence of one variety on another, either in terms of disease severity or yield, proved significant (Data not shown).

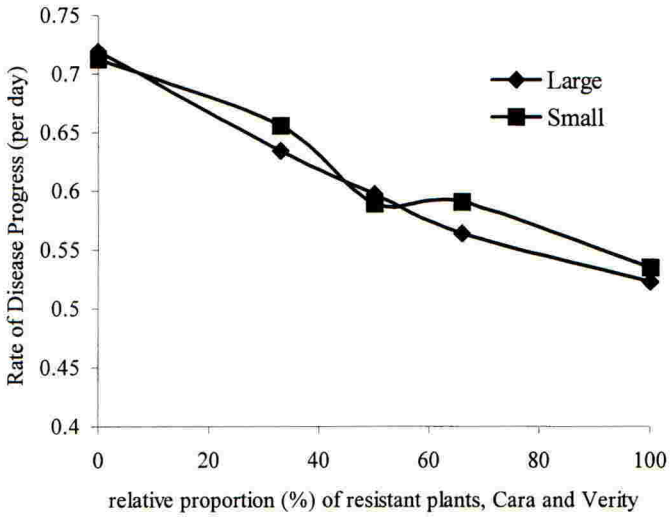

Figure 2. Mean disease rate for treatments with different proportions of resistant genotypes, the relationship was linear ($P < 0.001$) with differences between concentrations ($P < 0.05$). There was no difference between block sizes and the relationship was best described by a single straight line. The coefficient of variation was 30.4% (178 d.f.).

DISCUSSION

There were certain combinations of varieties, in random mixtures, where the performance of one variety (either in terms of disease and/or in terms of yield) was significantly affected by the presence of another variety: for example, the presence of Verity significantly decreased disease severity (AUDPC) and increased yield on Cara, and the presence of Cara significantly reduced disease severity (AUDPC) on Sante but concomitantly decreased yield. Therefore, host-diversity effects in potatoes are highly dependent on particular variety combinations.

Host-diversity effects in potatoes against late blight have been attributed to a limited amount of autoinfection coupled to the physical barriers provided by resistant plants to inoculum spread, and a decrease in the proportion of susceptible tissue in mixed plots. An additional mechanism of disease restriction often attributed to variety mixtures is that of induced resistance (Wolfe, 1985). If these mechanisms are important to all potato variety mixtures then we expect nonlinear responses in Figures 1 and 2. This is because we expect the resistant plants to always protect the more susceptible plants; this would manifest itself as a non-proportional response to a reduction in the concentration of resistant genotypes. Hence, if barrier effects or dilution of susceptibles were proving important for all mixtures then we would expect a non-proportional response to low and medium concentrations of susceptible

plants. Also, if induced resistance was always important, we would expect curvilinear responses to dilution of resistant genotypes, since induced resistance should be more marked in the more resistant genotypes (Stromberg, 1995).

That said, in Figure 1 we see an average 10.6% difference in AUDPC, between block sizes. Figure 2 shows no difference in rate of disease progress between block sizes. Therefore the epidemic was not slowed but delayed, most probably resulting from the greater target area of susceptible tissue, increasing the chances of initiating an epidemic, in the blocks with large plots. Also, if the above mixture mechanisms were universal, then interactions with block size would also be expected, since the autoinfection:alloinfection ratio is different.

Given a lack of evidence for the mixture mechanisms of induced resistance, a dilution of susceptibles or barrier effects; how can significant variety interactions be explained? The competitive ability of potatoes is highly dependent on cultivar characteristics. In addition, Finckh & Mundt (1992) found that plant-plant competitive interactions had epidemiological effects in genetically diverse wheat populations. So, we can speculate that inter-plant competition is important in potato variety mixtures, both in terms of yield but also in its influence on disease.

The ecological and epidemiological repercussions of mixing potatoes are complex. We hope that by using simulation models and continuing field trials we will achieve a better understanding potato variety mixtures and consequently assess the usefulness of this approach against late blight.

ACKNOWLEDGEMENTS

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Insect growth regulators inhibit acetylcholinesterase activity in B-biotype *Bemisia tabaci*

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ABSTRACT

The insect growth regulators buprofezin and novaluron were found to be inhibitors of acetylcholinesterase (AChE) in B-biotype *Bemisia tabaci*. This is a new mode of action for these compounds. AChE inhibition did not occur in the buprofezin resistant strain of B-biotype *B. tabaci* and our results indicate a buprofezin resistant B-biotype *B. tabaci* has evolved an insensitive form of acetylcholinesterase as a resistance mechanism.

INTRODUCTION

B-biotype *Bemisia tabaci* (Homoptera: Aleyrodidae) was first detected in Australia in October 1994 (Gunning *et al.*, 1995). B-biotype *B. tabaci* is a severe pest worldwide and is a pest in many ornamental, agricultural and horticultural industries in Australia. Control of B-biotype *B. tabaci* is hampered by resistance to many conventional insecticides. Consequently, insect growth regulators such as buprofezin and novaluron, are being investigated for use against B-biotype *B. tabaci* in Australia.

MATERIALS AND METHODS

B-biotype *B. tabaci* were collected from the field. A culture was segregated into two populations. One of these populations was laboratory selected for 8 generations to produce a strain resistant to buprofezin (LD_{50} of 595ppm). The other was not selected with buprofezin, and designated the buprofezin unselected B-biotype *B. tabaci* strain (LD_{50} of 3ppm).

Acetylcholinesterase assay

Fifty buprofezin resistant and non selected B-biotype *B. tabaci* were separately mass homogenised in 1ml of 0.1M phosphate buffer (pH 7.5 and containing 0.1% Triton X-100). Homogenates of buprofezin resistant and unselected B-biotype *B. tabaci* were incubated with increasing concentrations of buprofezin (0.02, 0.05, 0.2, 0.5, 2, 5, 20, 50, 200 and 500ppm) for one hour at 25°C. Aliquots (10µl) were pipetted onto a clean microplate and assayed for AChE activity. AChE activity was measured by the Ellman method (Ellman *et al.*, 1961). AChE activity was plotted as a percentage of the uninhibited control against insecticide concentration.

Homogenates of buprofezin resistant and unselected B-biotype *B. tabaci* were incubated with increasing concentrations of novaluron (0.1ppm, 1ppm, 10ppm, 100ppm and 1000ppm) for 30 minutes. AChE activity was measured by the Ellman method (Ellman *et al.*, 1961).

Polyacrylamide gel electrophoresis

Buprofezin unselected and resistant B-biotype *B. tabaci* were homogenised individually in 20µl of 1.6% triton X-100 containing 10% sucrose. Aliquots (15µl) were pippetted into wells of 7.5% polyacrylamide gels (Byrne & Devonshire, 1991). Gels were run for 90 minutes at 250V at maximum current, and stained for AChE activity as described by Karnovsky & Roots (1964).

RESULTS AND DISCUSSION

AChE of unselected B-biotype *B. tabaci* was markedly inhibited by buprofezin. At 0.5µM concentration some 90% of AChE activity was inhibited (Figure 1). AChE from the resistant B-biotype *B. tabaci* strain showed only a slight response to buprofezin, with inhibition level increasing up to 20% at the highest buprofezin concentration (Figure 1).

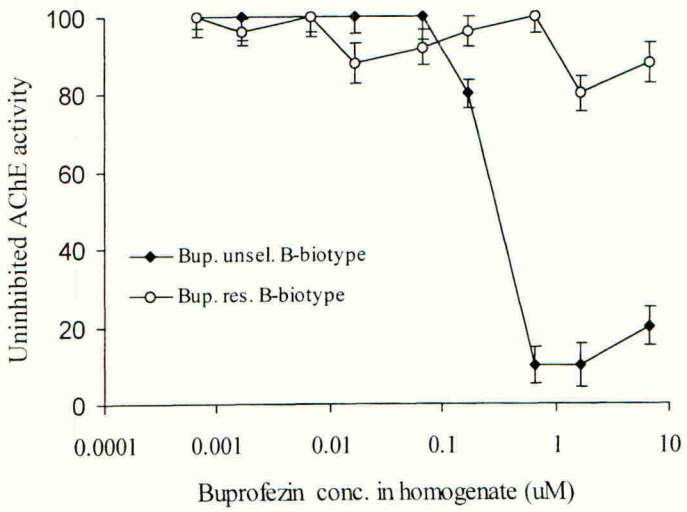

Figure 1. Effects of buprofezin on *in vitro* AChE activity of adult B-biotype *B. tabaci* (1hr incubation).

The electrophoretic mobility of AChE bands differed between buprofezin unselected and resistant strains, with relative mobility (Rm) values of 0.15 and 0.16 respectively (Figure 2). The different Rm values suggest that these are two different forms of AChE, and may indicate

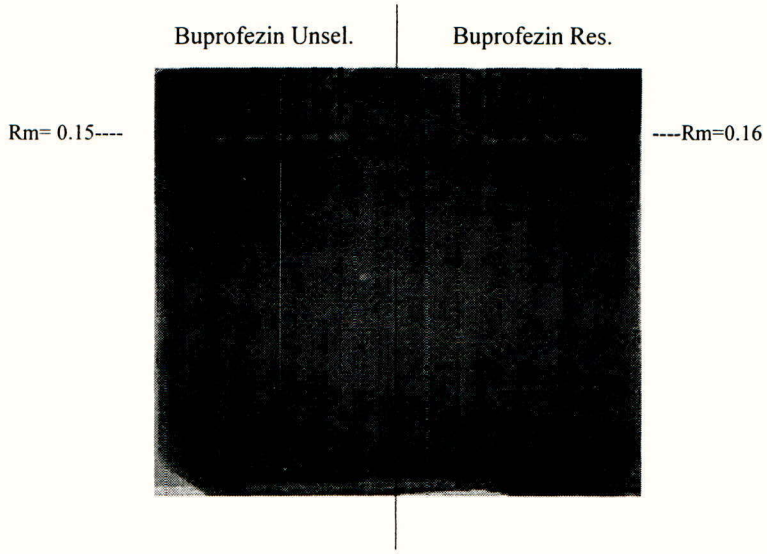

Figure 2. Polyacrylamide gel showing acetylcholinesterase bands of buprofezin unselected and resistant B-biotype *B. tabaci*.

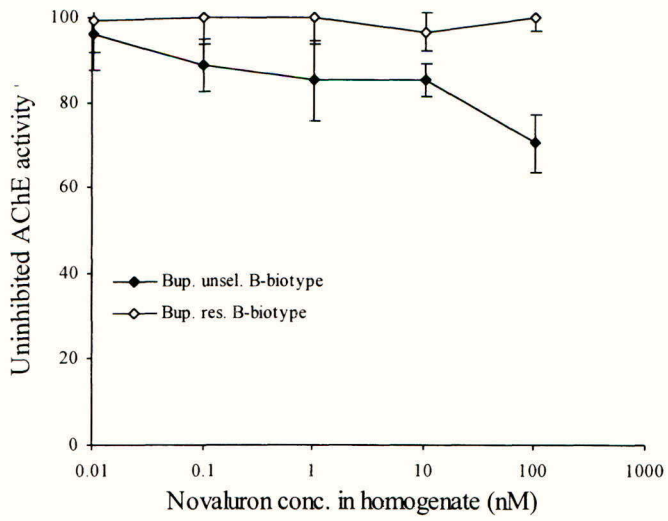

Figure 3. Effects of novaluron on *in vitro* AChE activity of adult B-biotype *B. tabaci* (30 minute incubation).

some sort of structural modification. Resistant *B. tabaci* apparently have a modified AChE which is insensitive to inhibition to buprofezin. Results also showed that B-biotype *B. tabaci* AChE was inhibited by novaluron in the unselected strain, but the buprofezin resistant AChE remained uninhibited (Figure 3). Novaluron, like buprofezin, is a chitin synthesis inhibitor. These data may indicate that there is cross-resistance between buprofezin and novaluron in B-biotype *B. tabaci* through an altered acetylcholinesterase resistance mechanism.

These data show a new mode of action for buprofezin, in addition to its more conventional action of moult disruption. This is the first report of a commercial insect growth regulator acting as an AChE inhibitor. From an evolutionary viewpoint, *B. tabaci* would not have evolved this resistance mechanism unless the target site was important. The finding that resistant B-biotype *B. tabaci* have a mutant form of AChE further emphasises the importance of AChE attack as a mode of action of buprofezin.

ACKNOWLEDGEMENTS

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Behavioural consequences of pyrethroid resistance in the peach-potato aphid, *Myzus persicae* (Sulzer)

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ABSTRACT

Myzus persicae (Hemiptera: Aphididae) clones possessing the knockdown resistance (*kdr*) mechanism are less responsive to alarm pheromone than those lacking the mutation. In order to investigate further the effect of *kdr* and the newly discovered *super-kdr* (or *skdr*) mutations on aphid alarm response, controlled crossing experiments were set up in the laboratory using *M. persicae* clones generated from field populations. Aphid progeny carrying previously untested combinations of *kdr* and *skdr* mutations were subsequently tested in discrimination-dose alarm pheromone bioassays. The results showed that aphids possessing both the *kdr* and *skdr* mechanisms exhibited lower levels of disturbance after exposure to measured amounts of synthetic alarm pheromone than aphids with the *kdr* mutation only. Our findings have potential important implications for the survival of *M. persicae* expressing both resistance mutations, as the alteration in behaviour of these forms may result in greater vulnerability to predation and parasitism.

INTRODUCTION

Pyrethroid-resistant *M. persicae* clones have been found to carry a leucine-to-phenylalanine mutation (designated L1014F) within the IIS6 transmembrane segment of the *para*-type sodium channel protein, and there is overwhelming evidence that this amino acid substitution is responsible for the *kdr*-type nerve insensitivity phenotype (Martinez-Torres *et al.*, 1999). An additional mutation, the methionine-to-threonine replacement (M918T) in the short cytoplasmic domain between segments IIS4 and IIS5 of the channel protein, has also been recently detected together with the L1014F mutation in some *M. persicae* clones, and is correlated with an enhanced form of pyrethroid resistance termed *super-kdr* or *skdr* (unpublished data).

It has long been suggested that the accelerated microevolutionary process of insecticide resistance development by insects may confer detrimental effects on the biological 'fitness' of resistant individuals rendering them less competitive than their susceptible counterparts in the absence of insecticides (Roush & McKenzie, 1987). Furthermore, there is increasing evidence that some of these costs are conditional, only becoming apparent under environmental or physiological stress when any differences in survival, reproduction or competitive ability are likely to be at a premium. Previous studies have revealed that *M. persicae* with *kdr* show much lower levels of response to synthetic aphid alarm pheromone, *(E)*-β-farnesene, than aphids lacking the *kdr* mechanism (Foster *et al.*, 1999). The current work investigates this phenomenon further by testing the alarm response of *M. persicae* clones produced from crossing experiments and carrying various combinations of *kdr* and *skdr* mutations.

MATERIALS AND METHODS

Clone origins and rearing

A total of sixty *M. persicae* clones generated in the laboratory by crossing aphid individuals with different combinations of *kdr* and *skdr* mutations were used in the experiments. Information on the aphid clones used in the crosses is given in Table 1. Sexual crosses were conducted following the method of Blackman *et al.* (1996). Parental aphids and the progeny of crosses were reared as virginoparous, predominantly apterous colonies on excised Chinese cabbage leaves in small plastic box-cages and kept under 20 ± 1°C and a 16 h light / 8 h dark regime.

Table 1. *Myzus persicae* clones used in the crossing experiments.

Clone	Origin	*Kdr* genotype[1]	*Skdr* genotype[1]
794J	UK	RR	SS
800F	Italy	SS	SS
2169G	UK	SR	SR
108T	cross[2]	SR	SS

[1] Determined by direct DNA sequencing.
[2] Product of a sexual cross between clones homozygous for the presence or absence of the *kdr* mutation.

Kdr and *skdr* genotyping of aphid progeny

The IIS4-IIS6 region of the aphid *para*-type sodium channel gene containing the *kdr* (CTC, leucine to TTC, phenylalanine; Martinez-Torres *et al.*, 1999) and *skdr* (ATG, methionine to ACG, threonine; unpublished data) mutations, was amplified from genomic DNA by two rounds of PCR following a slightly modified version (in the primers and the times of the amplification programmes) of the protocol described by Martinez-Torres *et al.* (1999). *Kdr* and *skdr* genotypes were established by direct DNA sequencing of 550 bp fragments using dye terminators and internal sodium channel primers on an Applied Biosystems 373A automated sequencer.

Aphid alarm pheromone bioassays

Aphid response to synthetically-produced alarm pheromone was assessed in the absence of insecticides in seven separate experiments. For each clone tested, apterous adult individuals were transferred to inverted Chinese cabbage leaf discs held on 1% agar inside small plastic tubs and left overnight to produce developmentally-synchronized cohorts of first instar nymphs. Each replicate batch of aphids was then assayed by applying a 1µl (0.1 mg/ml in hexane) droplet of *(E)*-β-farnesene to the central part of each leaf surface with a fine-needle syringe. All bioassays were carried out at a constant temperature of 22°C and aphid behaviour was monitored for 2 min (Foster *et al.*, 1999). Aphids that withdrew their stylets and walked away were scored as responding. Mean percentage responses between aphid clones were compared by analysis of variance (ANOVA).

RESULTS

Bioassay results on aphid alarm pheromone response are presented in Figure 1. *M. persicae* progeny-clones homozygous susceptible for the *kdr* and *skdr* mutations, and the 800F standard susceptible clone, responded similarly ($P > 0.05$) exhibiting the highest response to the synthetic alarm pheromone ($P < 0.05$). Clones heterozygous for the *kdr* mutation only, showed a moderate response to the chemical stimulus and they clearly affiliated with *kdr* heterozygous 108T and the *kdr* homozygous resistant 794J clones ($P > 0.05$). Aphids heterozygous for both mutations showed low levels of disturbance by the alarm pheromone and they responded similarly to the 2169G field clone ($P > 0.05$) rather than to the *kdr* homozygous resistant (794J) or the *kdr* heterozygous (108T) ones ($P < 0.05$). The aphid progeny homozygous resistant for the *kdr* mutation and heterozygous for the *skdr* mutation, proved the least responsive of all ($P < 0.05$). Thus, the responses of clones with each particular combination of mutations were consistent, and differed significantly from clones with other combinations.

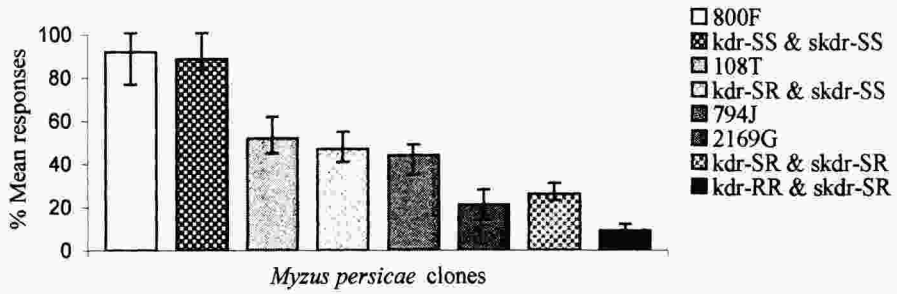

Figure 1. Mean percentage responses of *Myzus persicae* standard and progeny clones to synthetic alarm pheromone.

DISCUSSION

Evidence for the likelihood of the *kdr* mechanism having potential pleiotrophic effects on aphid behaviour is becoming increasingly well documented. It is now believed that the L1014F mutation associated with *kdr* resistance in *M. persicae* (Martinez-Torres *et al.*, 1999) contributes directly to the observed reduction in sensitivity of the aphid nervous system to stimuli that promote survival, such as overwintering success (Foster *et al.*, 1996), movement from senescent leaves (Foster *et al.*, 1997) and response to alarm pheromone (Foster *et al.*, 1999). In these experiments, the alarm pheromone response of *M. persicae* clones generated from laboratory-based sexual crosses, verified and extended previous results. *Kdr-SS* aphids were significantly more responsive to synthetic alarm pheromone than *kdr*-SR or *kdr*-RR clones, which were in turn significantly more responsive than *kdr*-SR, *skdr*-SR clones. However, the least responsive aphids possessed combinations of sodium channel mutations (*kdr*-RR, *skdr*-SR), not yet encountered in the field. The current results also suggest that the *skdr* mutation recently identified in UK *M. persicae* clones (unpublished data) may confer cumulative pleiotrophic effects on nerve function and subsequent alteration of aphid behaviour, which in turn could influence the dynamics of resistance in the field. Moreover, the altered behaviour of clones carrying the *skdr* mutation could place these genotypes at a stronger

selective disadvantage relative to aphids lacking the mutation or even to those possessing the *kdr* mutation only, because of an increased risk of predation and parasitism. This is being investigated further.

ACKNOWLEDGEMENTS

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Symbiotic bacteria from entomopathogenic nematodes acting as biological agents against fungal pathogens of tomato seedlings

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ABSTRACT

Biological control of tomato seedling diseases by the bacterial *Pseudomonas oryzihabitans* and *Xenorhabdus nematophilus* were evaluated *in vitro*, and *the* glasshouse. In laboratory Petri dishes assays the bacteria significally inhibited the growth of *Pythium aphanidermatum* and *Rhizoctonia solani* after 48 h. In the glasshouse the application of the bacteria resulted in significant improvement of plant growth when applied to plant treated with the root pathogens.

INTRODUCTION

Seedling diseases cause an estimated average annual yield loss of 5% and are usually the major disease problems for a great number of crops like cotton and tomato all over the world. Several soil-borne fungi are responsible; *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani*, and *Fusarium* spp. cause seed rot, pre-emergence, and post-emergence damping-off and can lead to significant stand losses during plant establishment. Biological control using antagonistic organisms to minimize the use of chemical pesticides has become more important in recent years. Beneficial organisms including bacteria and fungi, applied as seed treatments provide great opportunities and benefits for crop protection against soil-borne pathogens. The objective of this research was to determine the potential of using strains of *Pseudomonas* (*Flavimonas*) *oryzihabitans* and *Xenorhabdus nematophilus*, both bacterial symbionts of the entomopathogenic nematodes *Steinernema abbasi* and *S. carpocapsae*, respectively to suppress soilborne pathogens causing tomato damping-off. The fungistatic effect was tested both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Preparation of bacteria and fungal inoculum

All fungal isolates and bacterial strains tested were derived from the University of Reading collection. *P. oryzihabitans* and *X.nematophilus* strain were cultured in media as described by Vagelas (2000). Two isolates of *R. solani* and *P. aphanidermatum*, respectively, were cultured on PDA plates at for 7 days. Then *R. solani* inoculum was taken and grown in

Potato Dextrose Broth (PDB) (Difco, 100 ml in 250 ml flasks) for 10-12 days in shake culture at room temperature. The mycelium that was grown in broth was filtered through cheesecloth, rinsed in sterile distilled water (SDW) and fragmented in SDW in a Waring Blender for approximately 10 seconds. Concentration of the mycelial suspension was adjusted to give 50% light transmission (590 nm) on a Bausch and Lomb Spectronic-20 spectrophotometer. Inoculum was pipetted (5 ml/pot) onto the soil surface in pots where 10 day old tomato seedlings were grown. *P. aphanidermatum* oospore suspensions were produced by taking 9 mm plugs from 3 days old cultures on PDA and placing them in 20ml cornmeal medium (CMM) and incubated at in the dark for 3–6 weeks, as described by Martin (1992). The oospores were separated from hyphae by mixing the culture with an equal volume of sterile double distilled water (SDDW) and blending in a Waring Blender at room temperature for 1–2 min. Hyphal mass was removed from the suspension by filtration through a 150 mm sieve and washed twice in SDDW, by centrifugation. Oospores were resuspended in SDDW and stored.

P. oryzihabitans and *X. nematophilus* fungistatic effect *in vitro*

Bacterial cells of *P. oryzihabitans* and *X. nematophilus* were spread uniformly on the surface of Nutrient Agar (NA) in 9 cm Petri dishes then 4 mm plugs of *R. solani* or *P. aphanidermatum* were placed in the middle at the dish. Mycelial growth was recorded and compared with the controls (untreated). Plates were randomly placed and incubated, in the dark. All possible combinations between bacteria and fungi tested, with 10 replicates for each treatment, whereas control plates were kept without bacterial inoculation. Bacteria were tested in four concentrations (10^6, 10^5, 10^4, cells/ml).

In vivo greenhouse experiment

Assays for the ability of the bacteria to suppress *R. solani* and *P. aphanidermatum* were conducted in 1 L trays. Inoculum of the pathogens was mixed with sandy loam (1:3, w/w) soil mixture. Four concentrations of the bacteria strains (10^6, 10^5, 10^4, 10^3, cells/ml) were prepared and tested to control the pathogen.Bacteria were added by applying 100 ml of a washed suspension directly as a soil drench.

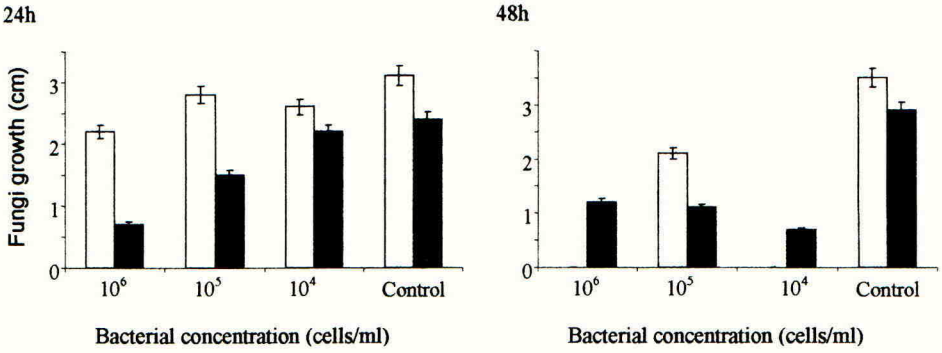
RESULTS AND DISCUSSION

P. oryzihabitans and *X. nematophilus* fungistatic effect *in vitro*

The results showed that bacteria cells produced freely diffusible compounds that were able to suppress fungal growth. Similar results were reported by Andreoglou *et al.* (2001). There were recorded significant interactions (P=0.05) between bacteria and fungi that tested. *P. oryzihabitans* (Figure 1.(a)) was found the most inhibited bacterial strain tested compared to *X.nematophilus* (b) in the first 48 h of incubation. After the period of 72h there is not mycelial growth in all bacterial treatments.

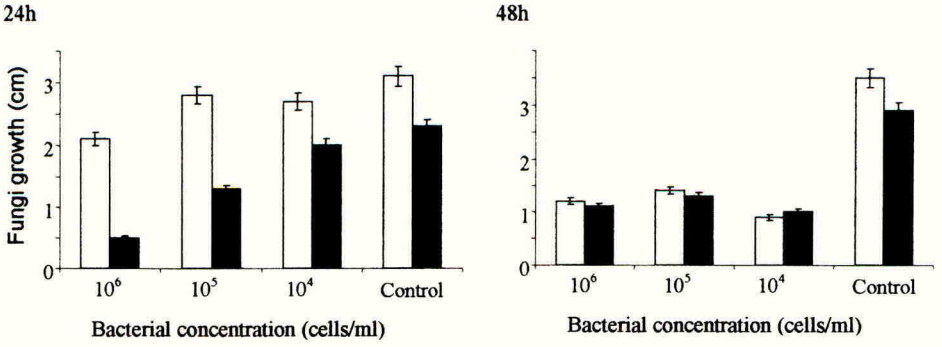
Figure 1.

a. Effect of *P. oryzihabitans* on the development of *P.aphanidermatum* and *R.solani*



* Bars representing *P.aphanidermatum* (□) and *R.solani* (■) mycelia growth (cm.) in 24 and 48 h. Bars show means; Error bars show mean +/- 1.0 SE.

b. Effect of *X.nematophilus* on the development of *P.aphanidermatum* and *R.solani*



* Bars representing *P.aphanidermatum* (□) and *R.solani* (■) mycelia growth (cm.) in 24 and 48 h. Bars show means; Error bars show mean +/- 1.0 SE.

***In vivo* tests**

Treatments with both bacteria significantly (P=0.05) increased tomato plant height and fresh weight, and decreased root weight compared with control in pathogen infested soil. *P. oryzihabitans* proved more biologically effective compared to *X. nematophilus* since it was resulted greater (P=0.05) plant height, and fresh weight (Table 1) and lower (P=0.05) root weight.

Table1. Biological control of *R. solani* and *P aphanidermatum* with the bacteria symbiotically associated with *S. abbasi* in soil *P. oryzihabitans*.

Treatments	Stem length (cm)	Stem fresh weight (g)	Root fresh weight (g)
Untreated	23.1 c*	119.7d	41.1f
R.solani	11.2 a	16.6a	12.2ab
R.solani / *P. oryzihabitans* 10^6cells/ml	18.2 bc	98.2cd	18.2bc
R.solani / *P. oryzihabitans* 10^5cells/ml	21.5 bc	56.4abc	22.6bcd
R.solanii / *P. oryzihabitans* 10^4cells/ml	21.6bc	121.8d	40.7f
R.solani / *P. oryzihabitans* 10^3cells/ml	18.7bc	102.7cd	27.3cde
P.aphanidermatum	9.5a	17.7a	13.6ab
P.aphanidermatum / *P. oryzihabitans* 10^6cells/ml	16.1bc	94.5cd	16.1bc
P.aphanidermatum / *P. oryzihabitans* 10^5cells/ml	20.7bc	53.7abc	21.2bcd
P.aphanidermatum / *P. oryzihabitans* 10^4cells/ml	22.2bc	119.6d	38.3f
P.aphanidermatum / *P. oryzihabitans* 10^3cells/ml	17.9bc	101.4cd	26.7cde

*Values in columns followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

P. oryzihabitans both *in vitro* and *in vivo* showed significant and rapid fungistatic effect against *R. solani* and *P. aphanidermatum*. *X. nematophilus* also showed similar fungistatic effect but after of 72 h of incubation with the fungi involved. This is probably due to its less motility in comparison with *P. oryzihabitans* and hence having smaller production of antibiotics and toxins.

ACKNOWLEDGEMENTS

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Studies on population dynamics of *Bacillus subtilis* and *Fusarium oxysporum* f.sp. *lentis*, the causal organism of lentil vascular wilt

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ABSTRACT

Vascular wilt, caused by *Fusarium oxysporum* f.sp. *lentis*, is one of the most important disease of lentil (*Lens culinaris* Medikus) worldwide. Understanding the dynamics between a pathogen and a biological control agent (BCA) in soil is a determining factor in predicting the success of biological control. By studying the population density fluxes over a period of time, it can be observed how the BCA and pathogen influence each others population and how certain environmental conditions influence this relationship. In a growth room study, a population of *Bacillus subtilis* rapidly increased up to 10^7 (cfu/g soil) in the 10 days following application of 5 ml/plant ($8x10^6$ cfu/ml^{-1}) of bacterium to 15-days-old lentil (ILL 4605) plants and slightly decreased to 10^6 (cfu/g soil) in 5 weeks after application. In a glasshouse experiment, *B. subtilis* reached a population density greater than 10^9 (cfu/g) in soil 2 weeks after application either alone or in the presence of the pathogen. *B. subtilis* was effective at decreasing the wilt incidence by 70%.

INTRODUCTION

Vascular wilt is the most important disease of lentil (*Lens culinaris* Medikus) worldwide (Saxena, 1993). The causal organism, *Fusarium oxysporum* f.sp. *lentis*, is a soilborne fungus which can infect plants throughout the growing season. Microbial antagonism is an important factor for biological control of soilborne pathogens (Baker & Cook, 1974). *Bacillus subtilis* was selected for its biocontrol potential against lentil vascular wilt (El-Hassan, *et al.*, 1997).

Initial inoculum density of the introduced biocontrol agent can be significant in determining the level and persistence of rhizosphere colonization by the biocontrol agent and the level of biocontrol achieved (Parke, 1990). The ability of the biocontrol agent to control disease may also be affected by the inoculum density of the pathogen. In this study, the effects of inoculum density of both pathogenic and suppressive *B. subtilis* on populations of these antagonists on rhizosphere were examined. The relationship between inoculum density or rhizosphere populations and wilt incidence were also investigated.

MATERIALS AND METHODS

Microorganism cultures and inoculum preparation

The antagonistic bacterium *Bacillus subtilis* (IMI 388877) was originally isolated from Syrian lentil soils (El-Hassan, *et al.*, 1997). *Fusarium oxysporum* f.sp. *lentis* (isolate no. 31) was obtained from stems of wilted plants collected from a sick plot at ICARDA during the

1999/2000 crop season. Fungal inoculum for inoculation purposes was prepared as described by Bayaa and Erskine (1990).

To prepare bacterium inoculum, *B. subtilis* was grown on nutrient broth (NB) by using 100-ml of NB, in 250-ml Erlenmeyer flasks inoculated with 1-ml bacterial suspension, incubated at 25±2 °C on a rotary shaker at 150 rpm for 3 days. The culture was then purified by centrifugation (4100 g) at 25 °C for 30 min and washing twice with sterile distilled water (SDW) before adjusting to give a concentration of $8x10^6$ cfu/ml^{-1}.

Population dynamics of *B. subtilis* and disease severity in a growth room

Loamy soil and silver sand were passed through a 2-mm sieve and autoclaved separately for 30 min at 121 °C on 3 consecutive days. Soil and sand were dried for 3 days at room temperature and mixed at ratio of 2:1 (v/v) loamy-sand soil. Lentil seeds (ILL 4605) were surface-sterilized with 5% bleach for 5 min, rinsed three times in SDW and then dried in a laminar flow cabinet for 2 h. One seed was sown in each pot containing 250 g soil. When the seedlings were 2 weeks old, each pot was inoculated with 8 ml suspension of $2.5x10^6$ microconidia/ml^{-1} (Erskine & Bayaa, 1996) of a single-spore isolate of *F. oxysporum* f.sp. *lentis*. At the same time, a 5-ml sample of *B. subtilis* suspension, prepared as described above, was dripped into 3-cm holes beside the seedlings. Disease severity on individual plants was assessed using a 1-9 scale: 1= no symptoms and 9= whole plant or a unilateral shoot is wilted and/or dry (Bayaa & Erskine, 1990). The experiment was set-up in a randomized block design with 3 replications (10 pots each) under controlled conditions at 25 °C.

Population dynamics of *B. subtilis* and disease severity in the glasshouse

The experiment was conducted in trays each containing 2 kg loamy-sand soil under glasshouse conditions at 25±5 °C. Ten surface-sterilized lentil seeds were sown in each tray. Two weeks after planting, each tray was inoculated with a 60 ml suspension of *F. oxysporum* ($2.5x10^6$ microconidia/ml^{-1}). A 6-ml sample of *B. subtilis* suspension was dripped around the seedlings. Wilt incidence was recorded at 5-day intervals starting when the symptoms began to appear until most of the plants had reached maturity (Bayaa & Erskine, 1990). The experiment was set-up in a randomized block design with 3 replications.

Inoculum densities of the suppressive bacterium (cfu/g soil) whether from the growth room or the glasshouse were quantified at 5 and 7-day intervals after inoculation as follows. Five grams of soil from rhizosphere around roots were weighed and dried. Three 1g samples were placed individually in 100 ml of SDW in screw-cap glass jars. Samples were well mixed and stirred for 30 min. Serial dilutions were made from soil solution, vortexed for 30s and 0.2 ml aliquots plated into NA plates. After 2-days of incubation, colony-forming units of *B. subtilis* per gram of soil were visually counted.

Statistical analysis

The percentage values of wilt incidence in both experiments were transformed to their square root values and population sizes of *B. subtilis* transformed to $\log_{10}$ of cfu values before conducting analysis. Data were analyzed according to standard analysis of variance (ANOVA) procedures by GenStat Fifth Edition.

RESULTS

Population dynamics of *B. subtilis* and disease severity in growth room and glasshouse

Results obtained have confirmed that *B. subtilis* survived well in soil with the population close to the initial level after 30 and 49 days in the growth room and the glasshouse, respectively. The population of *B. subtilis* had rapidly increased up to 10^7 cfu/g in growth room and to 10^9 cfu/g soil in glasshouse during 10-14 days and slightly decreased to between 10^6 to 10^7 cfu/g soil after 30 to 49 days from application (Figure 1, A & C).

Disease severity of lentil 30% (in the first score on the control), caused by *F. oxysporum* f.sp. *lentis*, increased over time with symptoms first visible 41 and 48 days after planting in growth room and glasshouse, respectively. In the soil drenched with *B. subtilis,* there was a significant decrease in wilt incidence and only 30% of plants died compared to 100% plants killed (in the final score) in the corresponding controls during 61 and 73 days growth. The results clearly show that the reduction in disease severity over time compared with control is probably related to an increase in population of *B. subtilis* in the rhizosphere around the roots in the 15 days after inoculation (Figure 1, B & D).

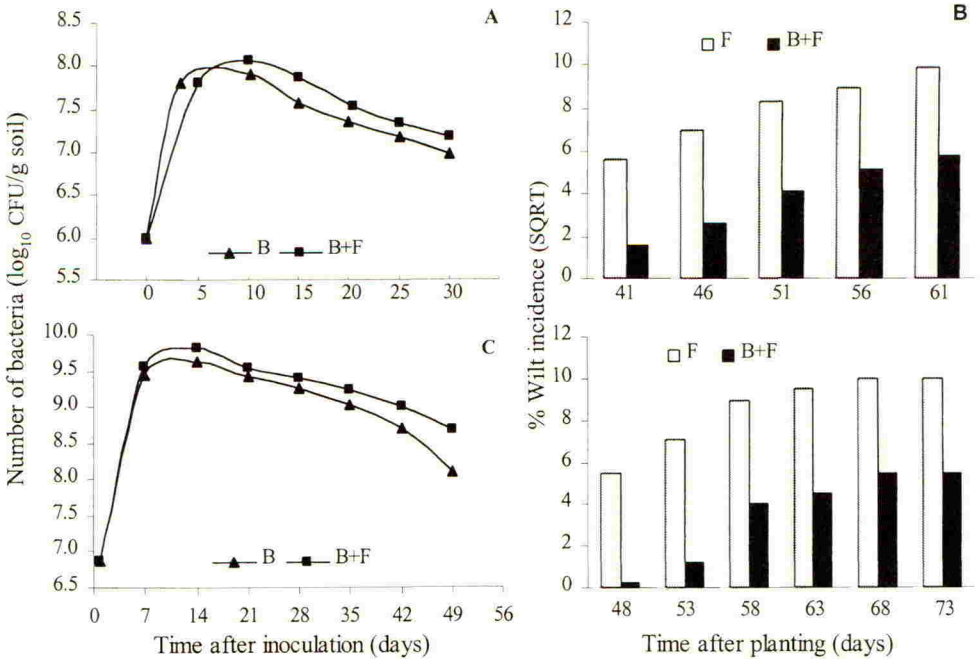

Figure 1. Population sizes of *Bacillus subtilis* and disease severity of lentil planted in sandy loam soil (F: *F. oxysporum* f.sp. *lentis* alone, B: *B. subtilis* alone, B+F: *B. subtilis oxysporum* f.sp. *lentis*). **A** & **B**: Population dynamics and disease severity i growth room; **C** & **D**: Population dynamics and disease severity in glasshouse Populations were determined by dilution plate technique on NA medium. Diseas severity was expressed as percentage of wilted plants. The populations an disease severities for each sampling date are the means of three replications.

DISCUSSION

Timing of application, application strategy and establishment of biocontrol agents at the target area are the critical elements for successful biocontrol (Baker & Cook, 1974; Kim, *et al.*, 1997). Nutrient competition is a potential mechanism for suppression of plant pathogens by endophytic bacteria (Hallmann, *et al.*, 1997). The rhizosphere competence of *B. subtilis* along with its ability to colonize the rhizosphere and then produce broad-spectrum antibiotic(s) (El-Hassan, *et al.*, 2001) can explain the biocontrol activity of this bacterium against *Fusarium* wilt of lentil under certain conditions and densities. These findings, in general, confirm previous studies made by using strains of *Bacillus* sp. and *Pseudomonas fluorescens* as a rhizosphere colonizer with biocontrol potential on various crops (Kim, *et al.*, 1997). The results indicate that *B. subtilis* has established well in soil which have strong activity against the pathogen and reduced wilt incidence of lentil. However, further studies of bacterial populations are needed to identify preferable types of soil and its relation with moisture and temperature to enhance the potentiality of *B. subtilis* as a biocontrol agent.

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Differences between *Rhizoctonia solani* isolates from potato crops

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ABSTRACT

Hyphal fusion compatibility, rDNA IGS1 size and EC_{50} to pencycuron were determined for isolates of *Rhizoctonia solani* associated with potato. Isolates of anastomosis group (AG) 2-1 and AG3 were sensitive to pencycuron, unlike isolates of AG5 and AG8, which were insensitive. Hyphal fusion compatibility and IGS1 lengths revealed that isolates of AG2-1 from potato in the UK are genetically diverse, as opposed to those of AG3 which are closely related to one another.

INTRODUCTION

Rhizoctonia solani (Teleomorph: *Thanatephorus cucumeris*) is an important soil-borne pathogen of potatoes. Infection of stems, stolons and roots decreases tuber yield and quality. Development of sclerotia on progeny tubers results in the tuber blemish disease, black scurf, which significantly reduces marketability of harvested tubers. Sclerotia infested seed tubers can also initiate disease in subsequent crops. Isolates of *R. solani* are placed into anastomosis groups (AGs) on the basis of hyphal fusion compatibility between isolates. Hyphal fusion amongst *R. solani* isolates can be classed into four categories, ranging from C0 (no interaction) to C3, where perfect hyphal fusion occurs as isolates belong to the same vegetatively compatible population (VCP). Presently there are 13 known AGs designated AG 1 to 13 (Carling *et al.*, 2002), with sub-groups existing in AG1 through to 9. In addition to hyphal fusion compatibility, host range, nutritional requirement, morphology and DNA sequences are characteristics used to define such sub-groups.

Despite a lack of survey data on the relative incidences of individual AGs in UK potato crops, isolates belonging to AG3 are generally accepted as the predominant cause of potato disease, with AG2-1 considered the next most important group in many temperate areas. However, isolates of AGs 4,5,7, 8 and 9 have been implicated in potato disease elsewhere. This study presents an analysis of *R. solani* isolates associated with potato, particularly the two groups considered important to potato production, AG3 and AG2-1.

MATERIALS AND METHODS

Isolates were taken from the Harper Adams University College culture collection or obtained from A.K.Lees, Scottish Crop Research Institute, Dundee, Scotland. Nuclear condition and hyphal fusion were observed using the bare slide technique of Kronland & Stanghellini (1988). Each individual isolate in the study was paired with each other in all combinations. Each hyphal interaction was categorised according to the criteria in Table 1. EC_{50} values for pencycuron were calculated from radial growth measurements of isolates grown on potato dextrose agar amended with a range of concentrations of pencycuron, each treatment was replicated three times. Differences in ribosomal DNA (rDNA) between isolates were analysed

using intergenic spacer 1 (IGS1) length. DNA was extracted using a Puregene® Genomic DNA isolation kit. Primers adjacent to the IGS1 region were used in a PCR with the same conditions as described for diagnostic PCR by Edwards *et al.*, (2001) except anneal temperature was 60°C. PCR products were observed after electrophoresis through a 2% (w/v) agarose gel (containing 0.5µg/ml ethidium bromide) in TAE buffer (40mM tris-acetate, 1mM EDTA, pH8).

Table 1. Categories of hyphal fusion in *R. solani* (adapted from Carling, 1996; Cubeta & Vilgalys, 1997)

Category	Description of hyphal interaction	Relationship	
C0	No recognition	Different AG	
C1	Hyphal wall contact only, no evidence of membrane contact	Different AG or diverse members of the same AG	*Increasing genetic relatedness*
C2	Hyphal wall and membrane fusion evident, death occurring in fused and adjacent cells	Same AG but different VCP	
C3	Hyphal wall and membrane fusion with no evidence of cell death (perfect fusion)	Same AG and same VCP, possibly clonal	

RESULTS

Observation of nuclear state revealed all isolates were multi-nucleate. Classification of each hyphal interaction is shown in Figure 1. Perfect fusion (C3) was observed where isolates were paired with themselves. C3 interactions were also observed in three different pairings between AG3 isolates, the remainder of the pairings amongst AG3 isolates were classed as C2. C1 or C2 interactions were observed between AG2-1 isolates. Isolate X81 was one of the pair in all three C1 interactions between the AG2-1 isolates. C1 interactions were observed between some AG2-1 and AG3 isolates. C1 interactions were observed in some pairings involving the AG8 isolate with both AG2-1 and AG3 isolates. The AG5 isolate showed no reaction (C0) when paired with other isolates in the study.

AG/isolate		2-1						3						5	8
		X1	X46	X52	X81	R22	R42	X22	X34	X40	X72	UN	I3	R48	R28
2-1	X1	C3	C2	C2	C2	C2	C2	C0	C0	C1	C0	C0	C0	C0	C0
	X46		C3	C2	C1	C2	C2	C0	C1	C0	C0	C0	C1	C0	C0
	X52			C3	C2	C2	C2	C1	C0	C0	C0	C1	C0	C0	C0
	X81				C3	C1	C1	C0	C0	C0	C0	C0	C0	C0	C1
	R22					C3	C2	C0	C0	C0	C0	C0	C0	C0	C1
	R42						C3	C0	C1	C0	C1	C0	C0	C0	C1
3	X22							C3	C2	C3	C2	C2	C2	C0	C0
	X34								C3	C2	C2	C3	C3	C0	C1
	X40									C3	C2	C2	C2	C0	C0
	X72										C3	C2	C2	C0	C1
	UN											C3	C2	C0	C1
	I3												C3	C0	C1
5	R48													C3	C0
8	R28														C3

Figure 1. Classification of hyphal interaction between isolates in all combinations.

Anastomosis grouping, pencycuron EC_{50} and IGS1 size are shown in Table 2. Isolates belonging to AG2-1 and 3 showed sensitivity to pencycuron, whilst the AG5 and AG8 isolates were insensitive to pencycuron. IGS1 length is given to the nearest 10bp. IGS1 length was approximately 680bp for isolates belonging to AG3 and AG8. Three different lengths of IGS1 were observed amongst the AG2-1 isolates, the IGS1 of isolate X81, was considerably shorter than the other 2-1 isolates. The IGS1 length of the AG5 isolate was not shared by any other isolate in the study.

Table 2. Anastomosis group, pencycuron EC_{50} and IGS1 size of isolates

Code	Origin	Host	AG	Pencycuron EC_{50} (ppm)	IGS1 size (bp)
X1	England[1]	Potato tuber	2-1	0.0010	570
X46	England[1]	Potato stolon	2-1	0.0008	550
X52	Scotland[1]	Potato stem	2-1	0.0006	550
X81	Scotland[1]	Potato tuber	2-1	0.0005	510
R22	USA[2]	Unknown	2-1	0.0010	570
R42	The Netherlands[2]	Cauliflower	2-1	0.0010	570
X22	England[1]	Potato stolon	3	0.0007	680
X34	Scotland[1]	Potato stem	3	0.0013	680
X40	England[1]	Potato stem	3	0.0005	680
X72	Scotland[1]	Potato tuber	3	0.0007	680
UN	England[1]	Potato tuber	3	0.0008	680
I3	England[1]	Potato stem	3	0.0008	680
R48	France[2]	Potato tuber	5	>250	620
R28	England[2]	Potato root	8	>250	680

[1] From the Harper Adams University College culture collection
[2]From the Scottish Crop Research Institute, Dundee

DISCUSSION

Potato isolates of *R. solani* can belong to one of several AGs, knowledge of which anastomosis group is present may be important in disease control. Here, isolates of AG5 and AG8 were insensitive to pencycuron, which is typical of those AGs (Kataria & Gisi, 1999). Other fungicides may also have action limited to certain AGs. In addition, separate anastomosis groups could be affected differently by environmental conditions and have different host ranges.

IGS1 length has been shown to be a useful diagnostic aid in determining AG and perhaps sub-groups within an AG. Since a C1 hyphal fusion reaction can indicate membership of either the same or different AG, IGS1 lengths may resolve ambiguity in some cases. IGS1 length should not be used as a diagnostic test alone, as indicated here, members of the same sub-group can have significantly different lengths. However, IGS1 length could be used in conjunction with other PCR diagnostic tests for AG. For example, primers designed for the determination of AG2 sub-groups (Carling *et al.*, 2002) and AG3 (Lees *et al.*, 2002) only produce a product when DNA from those specific AGs are present. Low yield or poor quality of DNA may give a false negative result. PCR amplification of the IGS1 region, in addition to acting as a control checking for suitable DNA quality, can also provide some indication of AG identity.

In this study isolate X81 produced only a C1 reaction with pairings involving other members of AG2-1. Its considerably different IGS1 length to the other AG2-1 isolates and different

culture morphology suggests X81 could represent a distinct group from the other AG2-1 isolates used in this study. Such differences in IGS1 length and hyphal fusion compatibility suggest that potato isolates of AG2-1 belong to a diverse group. AG3 isolates, however, represent a closely related, homogenous group. The greater genetic diversity within AG2-1 may increase the risk of resistance to fungicides. In potato production, the greater genetic diversity within AG2-1 isolates may reduce the effectiveness of control through crop rotation practices, as AG2-1 isolates can proliferate on a variety of hosts including brassicas, lettuce, cereals, tulips, spinach and sugar beet (Tu *et al.*, 1996). Therefore, greater AG2-1 soil borne inoculum may be present at potato planting. However, isolates of AG3 appear to be specific to Solanaceous hosts, none of which are grown in rotation with potato in Britain. This could suggest that an important cause of AG3 disease is infested seed tubers.

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Effect of mycelial inoculum level and cultivar susceptibility on *Rhizoctonia solani* development on potato stems and seed tubers

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ABSTRACT

The effects of soil-borne mycelial inoculum levels on the development of *Rhizoctonia solani* on potatoes was examined under natural and controlled environmental conditions. Results showed that black scurf increased steadily as inoculum level increased and it was higher under controlled conditions. Sclerotia developed on mother tubers even at low inoculum levels. At the inoculum levels tested under natural conditions there was no increase in stem canker incidence. Potato cultivars differed in their susceptibility to *R. solani* but none of the cultivars tested showed complete resistance to the disease.

INTRODUCTION

Rhizoctonia solani Kühn is the causal agent of stem canker and black scurf diseases of potato. *R. solani* is known to occur wherever potatoes are grown and can severely affect young sprouts of seed tubers resulting in delayed emergence and reduced yield. Both tuber-borne and soil-borne inocula are considered important for the initiation of disease and are significant factors in the epidemiology of *R. solani* (Harrison, 1978).

Potato cultivars have been reported to differ in their susceptibility to stem canker (Hide *et al.* 1989). Other studies have suggested that the higher the frequency of potatoes in a rotation the more severe the infection by *R. solani* will be on potato plants (Scholte, 1987). Although much work has been done on the control of disease with different chemicals and biological control agents (Harrison, 1978), cultivar resistance to *R. solani* may be the most economical and efficient way to control the disease.

The aim of this study was to determine the relationship between soil-borne mycelial inoculum levels and disease development under controlled and natural environmental conditions. Additionally, the susceptibility of seven potato cultivars to *R. solani* was examined at early stages of plant growth.

MATERIALS AND METHODS

Soil moisture and soil inoculation

A sandy clay loam soil (Countesswells series; pH 6.1, 13.1 mg/l P, 122 mg/l K, 197 mg/l Mg), collected from McRobert farm, Craibstone Estate, Aberdeen, was used throughout the experimental procedure. Air-dried soil was sieved through a 6.7mm sieve and stored in plastic

bags. The water holding capacity (WHC) of the soil was calculated and maintained at 40 % for both experiments as described by Kyritsis & Wale (2002).

A culture of *Rhizoctonia solani* AG3, originally isolated from black scurf sclerotia on a potato tuber (isolate PK00RS01), was used as inoculum source. The mycelial inoculum was prepared as described by Kyritsis & Wale (2002) and mixed in appropriate amounts into each soil bag to give the desired inoculum levels for each treatment.

Experiment 1: Disease development trials

The effects of soil-borne mycelial inoculum on development of stem canker and black scurf on the seed tuber, was studied in a pot experiment under controlled and natural conditions. Nine levels of soil-borne mycelial inoculum (0, 1/80, 1/40, 1/20, 1/10, 1/8, 1/4, 1/2 and 1/1 with 1/1 equal to 1 Petri dish of *R. solani* per 1 kg soil) were used. Disease free micro-propagated potato seed tubers (15-20mm) cv. Desiree were planted 9-10cm deep. Ten tubers were planted in each pot (180mm in diameter). Plants were grown under controlled environmental conditions (no lights, 10°C) and under natural conditions, on a sand bed overlaid with a black plastic polyethene sheet to prevent weed growth. Each treatment for every environmental condition was replicated four times. Planting of the pots which were kept outdoors, took place on the second week of May, to correspond with typical plating dates in Scotland. In controlled environment cabinets, pots were watered to their initial weight twice a week to maintain soil moisture of 40 % WHC. Outdoor plants received water only by rainfall.

Experiment 2: Potato variety resistance trial

A randomised block design pot experiment with four replicate pots per treatment was used to investigate the susceptibility of seven commercial potato cultivars to *R. solani*. Three inoculum levels (1/8, 1/10 and uncontaminated control) were used on the basis of the results from experiment 1. Ten disease free micro-propagated potato seed tubers (15-20mm) were planted at 9-10cm depth in each pot. The experiment was carried out at 10°C in controlled environment cabinets.

The pots were examined regularly and plants were carefully separated from soil when stems from the control plants had emerged. Plants were examined for incidence and severity of stem canker and black scurf development on the seed tuber. Incidence was expressed as the percentage of diseased stems and tubers per pot respectively. Stem canker severity was measured as the percentage area of stem affected by *R. solani* lesions. Pruned stems were also included in the disease score. Black scurf severity was measured as the percentage of tuber area covered with sclerotia using the ADAS disease assessment key (Key No. 2.4.1).

RESULTS

In controlled environmental conditions, low levels of inoculum in the soil resulted in limited development of stem canker incidence (Figure 1a). As the inoculum level increased in the soil (1/10, 1/8, 1/4) there was a significant increase in stem canker incidence ($P<0.05$). Above the 1/4 inoculum level incidence of stem canker did not increase further. By contrast, under natural conditions no significant difference in incidence of stem canker at any inoculum level was observed, although stem canker incidence was higher at low inoculum levels in comparison

with controlled conditions. As the greatest increase in stem canker occurred at 1/10 and 1/8 inoculum levels, they were selected for use in experiment 2.

Black scurf incidence on the seed tuber at 10°C increased steadily as the inoculum level increased (Figure 1b), significant increases being when inoculum increased from 0 to 1/80 and 1/20 to 1/10 ($P<0.05$). At inoculum levels greater than 1/4 the amount of disease remained constant. A similar pattern was observed under natural conditions. Black scurf incidence under controlled conditions was always higher than under natural conditions especially at inoculum level 1/10 or above ($P<0.05$).

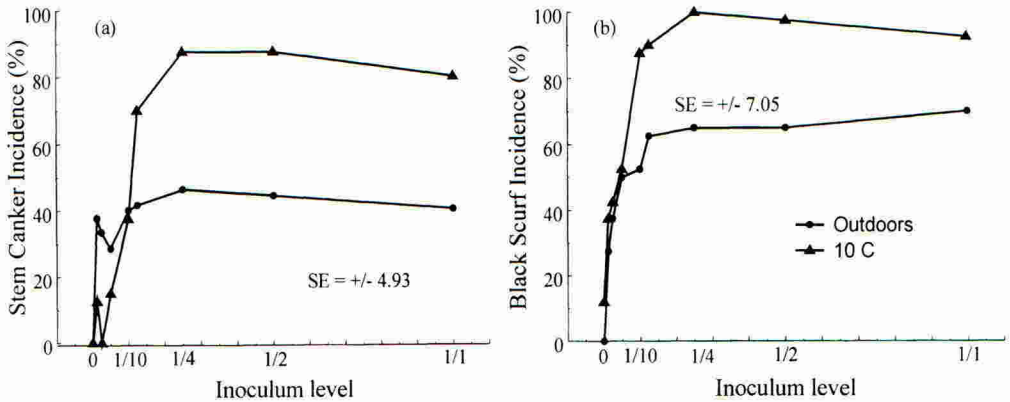

Figure 1: Effect of soil-borne mycelial inoculum level on incidence of stem canker (a) and black scurf on seed tubers (b) under controlled (10°C) and natural conditions (outdoors).

Potato cultivars differed in their susceptibility to *R. solani* (Table 1). Significant differences ($P<0.01$) were observed in the development of black scurf on seed tubers. Similarly, cultivars showed significant differences ($P<0.01$) in the percentage area of stem affected by *R. solani* lesions but no differences were observed in stem canker incidence. None of the cultivars showed complete resistance to the pathogen. The overall reaction of the cultivars to the disease, ranked cultivars such as Osprey, Nadine and Estima among the most susceptible and others, such as Sante and Maris Piper, among the least affected cultivars.

DISCUSSION

The fact that soil-borne inoculum can initiate severe stem canker and black scurf has been demonstrated in these experiments. In experiment 1, a standard curve between soil-borne mycelial inoculum level and disease development was established under uniform conditions which can be used in future experimentation. In Britain, most stem canker develops between planting and stem emergence (Hide & Firmager, 1989) when soil temperatures average about 10°C. Consequently, the tests in controlled environment cabinets took place at this temperature. Results showed that the incidence of disease was much greater at 10°C than under fluctuating natural conditions especially at moderate and high inoculum levels. The weather in May 2002 was particularly warm, favoured rapid emergence and thus disease escape in the natural conditions. This was also supported by the fact than at 10°C no stems emerged at inoculum levels 1/8 or higher, whereas emergence was consistently above 60% when plants were grown

under natural conditions. The results also suggest that the fungus is attracted more to seed tubers than to stems, thus the incidence of black scurf was higher than stem canker at all inoculum levels. Sclerotia developed on tubers even at low inoculum levels.

Table 1. Cultivar susceptibility to *Rhizoctonia solani* development of stems (stem canker) and seed tubers (black scurf). The values presented are the means of 1/8 and 1/10 inoculum level.

Cultivar	Stem Canker Incidence (%)	Stem Canker Severity (%)	Black Scurf Incidence (%)	Black Scurf Severity (Index)
Sante	54.7	37.9	36.2	0.5
Maris Piper	52.9	42.3	36.2	0.8
Pentland Dell	48.2	44.6	52.5	0.7
Desiree	57.7	46.1	46.2	1.0
Estima	59.6	46.5	56.2	1.1
Nadine	58.5	53.0	55.0	1.2
Osprey	55.6	48.5	58.7	1.5
LSD (5%)	8.95	7.24	13.47	0.49

Potato cultivars showed a range of susceptibility to *R. solani*. Although none of the cultivars showed complete resistance to the pathogen, some were more resistant than others. It may be possible to use the experimental protocol used in experiment 2 to test susceptibility of a wider range of cultivars for resistance to stem canker. Although cultivar resistance to *R. solani* is considered to be the most economical and efficient way to control the disease (Harrison, 1978), there was no evidence from the limited range tested that any one cultivar would be particularly effective in reducing disease at high inoculum levels.

ACKNOWLEDGEMENTS

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Inhibition of common cereal pathogenic fungi by clove oil and eucalyptus oil

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ABSTRACT

The work reported here aimed to examine the efficacy of two essential oils, eucalyptus and clove, to control *Fusarium culmorum* and *Alternaria alternata*, both common pathogenic fungi of cereals. Using an *in vitro* test spore germination test both treatments of showed some activity against *F. culmorum* and *A. alternata*, with eucalyptus oil showing a fungistatic effect and clove oil showing both fungistatic and fungicidal effects. Minimum inhibitory concentrations were obtained for each oil and fungus using the filter paper disc method and experiments conducted for effects of vapour and the phytoxicity of the oils on wheat and barley.

INTRODUCTION

The common pathogenic fungi *Fusarium culmorum* and *Alternaria alternata* have an impact on the yield of cereals. Furthermore *F. culmorum* may become active in the field at the end of the season and in storage, producing mycotoxins which are potentially highly damaging both to livestock and to humans when the contaminated grain is used as food. This paper describes work, investigating the efficacy of clove and eucalyptus oils for the control of these two fungi.

MATERIALS AND METHODS

The treatments used were clove oil and eucalyptus oil with olive oil, sterile tap water and saturated copper sulphate as controls. All plates were incubated in a black light box to promote sporulation (12h UV/12h darkness) at 25°C (Johnston & Booth, 1983).

Spore germination test

To test the spore germination, PDA plates were set up similar to a stitch plate. A fresh spore suspension was gently pipetted on either side of a long filter paper strip, saturated with a treatment in the centre of the plate. Then following 0, 5, 10 and 24h for *F. culmorum* and 0, 10, 24, and 48h. for *A. alternata*, the percentage of spores germinated was taken from counting 100 spores and noting how many germinated. The experiment was conducted for each oil and fungus in replicates of 4 and incubated as above. In addition microscope observations were made of each treatment after the time series.

Filter paper disc method

Using the filter paper disc method, a more detailed look into the effects of the essential oils on both fungi was carried out (Pattinaik *et al.*, 1996). Using an established fungal lawn (one day's growth), a 5 mm diameter sterile filter paper disc was saturated in the treatments, and placed in the centre of each plate. All treatments were pure oils, with 5 replicate plates, and incubated as

above for 14 days and measurement of the radial zone of inhibition and observations were taken every 2 days.

Minimum inhibitory concentration (MIC)

Once the effect of the oils on the fungi was established the MIC was determined using a similar technique to the filter disc method (Pattinaik *et al.*, 1996). The filter paper disc was saturated in the treatments at 5, 10, 15, 25, 35, 50, 65, 75 %, (diluted with olive oil) and pure oil with 4 replicated plates per concentration, per oil. The plates were incubated for 48h. as above and visual observations were taken.

Vapour action test

The effect of the vapour of the oils on the fungi was examined with one day's lawn growth, the method used involved gently placing a saturated filter disc in a warm drop of PDA on the lid of the petri dish, so the disc would stay firmly in place. Once in place, the plate was placed upright and sealed with parafilm to ensure that there was no escape of the volatile vapour. All oil treatments were used as pure oils and 5 replicate plates were made and incubated for 12 days, with measurement at day 1, 6, and 12.

Phytotoxicity test

Preliminary tests were carried out to observe any changes that might occur when the oils come in contact with the foliage of wheat and barley plants. To test the phytotoxicity the oils were gently sprayed on the plant at 25, 50 and 100% concentrations with controls of olive oil and sterile tap water. Observations for necrosis or change were taken after 4 days. Olive oil was used as a diluent as it was found not to inhibit germination or to be phytotoxic.

RESULTS AND DISCUSSION

Spore Germination Test

Table 1. The percentage of *F. culmorum* spores germinating in the presence of the essential oils in a timed series (n = 100)

	0h	5h	10h	24h
Clove Oil	0	20%	63%	87%
Eucalyptus Oil	0	7%	22%	60%
STW	0	67%	100%	100%

Table 2. The percentage of *A. alternata* spores germinating in the presence of the essential oils in a timed series

	0h	10h	24h	48h
Clove Oil	0	0%	0%	0%
Eucalyptus Oil	0	0%	0%	12%
STW	0	3%	78%	100%

After the microscopic count and noted observations there was a significant difference not only with the percentage of spores germinating but also with the appearance of the germ tubes. With *F. culmorum*, in comparison to the control, the germ tube took on a curly nature in the presence of the oils and had stunted growth. The details of how the spores and early germination are affected gives a better idea as to the mode of inhibition and the possible use of the oils as a seed treatment.

Filter disc method

Both essential oils were found to have an inhibitory effect on each fungus (see Figure 1). The observations showed that Eucalyptus oil on both *A. alternata* and *F. culmorum* gave a uniform fungistatic effect inhibiting both colony structure and aerial hyphae growth. The fungistatic actions made it impossible to measure the zone therefore observations were taken for the 14 days.

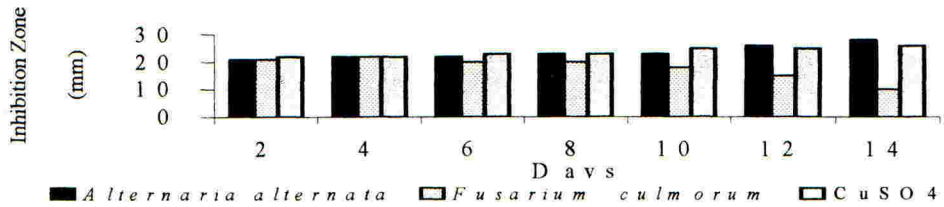

Figure 1. Average measurement of zone of inhibition with the application of clove oil.

The effect of clove oil on *A. alternata* gave a distinct fungicidal zone with average radii after 14 days of 26 mm. The zone gradually increased its diameter causing the established fungi to slowly curl back in the presence of the oil and its vapour. On *F. culmorum*, clove oil produced a zone of inhibition with average radii after 14 days of 10 mm. and did not have as strong an affect on the fungus as the outer edges of the zone were slowly allowing inward growth towards the filter paper disc.

Minimum Inhibitory Concentration

Table 3. Minimum inhibitory concentration test of *A. alternata* and *F. culmorum* against the essential oils with visual readings after 48h: ++ fungicidal inhibition, +, fungistatic inhibition and -, no inhibition (n = 4)

Treatment	Fungus	% of oil								
		5	10	15	25	35	50	65	75	100
Clove Oil	*A. alternata*	-	+	+	++	++	++	++	++	++
	F. culmorum	-	-	-	+	+	+	+	+	+
Eucalyptus Oil	*A. alternata*	-	-	-	-	+	+	+	+	+
	F. culmorum	-	-	-	-	-	-	-	+	+
Olive Oil (control)	*A. alternata*	-	-	-	-	-	-	-	-	-
	F. culmorum	-	-	-	-	-	-	-	-	-

F. culmorum was more susceptible to both the essential oils than *A. alternata* because it had a lower MIC concentration. This could be due to the faster germination rate of the *F. culmorum*.

Vapor Action Test

Table 4. The vapour affect of the essential oils on *F. culmorum* and *A. alternata* tested by a visual reading on inhibition of growth (n = 5)

	Fusarium culmorum			***Alternaria alternata***		
Day	1	6	12	1	6	12
Eucalyptus	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
Clove	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
Olive	Growth	Growth	Growth	Growth	Growth	Growth

From the visual readings there was a comparative difference between the essential oil plates and the control on each observation day. The inhibition of growth varied from fungistatic and fungicidal, to no inhibition at all (growth).

Phytotoxicity Test

Table 5. The percentage of necrosis found on wheat and barley leaves from the spraying of the essential oils (n = 3).

	% of leaves Necrosis (Day 4)	
Treatment	**Wheat**	**Barley**
100% clove oil	90	90
50 % clove oil	90	90
25% clove oil	50	50
100 % eucalyptus oil	75	80
50% eucalyptus oil	30	35
25% eucalyptus oil	5	5
100 % olive oil	0	0
STW	0	0

These data support the aim of this work in finding an inhibitory effect of clove and eucalyptus oils on the fungi and how inhibition takes place. The range of *in-vitro* experiments showed that there is both a fungicidal and fungistatic effect with the application of the oil and a distinct effect with the vapour of the oils. There is enough evidence to support a further assessment into the *in-vivo* application of these oils on wheat and barley seeds and plants.

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Biocontrol of canker on oilseed rape by reduction and inhibition of initial inoculum

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ABSTRACT

Leptosphaeria maculans the causal agent of canker on Brassicas cannot currently be consistently controlled by chemical means. *Cyathus striatus* shows potential as a biocontrol agent as it can reduce the production of the initial inoculum (pseudothecia) on rape stubble. Preliminary studies also show that metabolites produced by *C. striatus* have antifungal activity.

INTRODUCTION

Blackleg/canker caused by *Leptosphaeria maculans* is the second most significant disease of oilseed rape in the UK. The disease also affects other Brassica and Cruciferous crops such as cauliflower, broccoli and mustard. It is ubiquitous worldwide and the virulent strain is currently becoming an increasingly serious threat in Mexico (Moreno-Rico *et al.*, 2002). Previous research indicates that *Cyathus striatus* has potential as a biological control agent. It has been shown that *C. striatus* has a faster growth rate than *L. maculans* on a wide variety of media and therefore is a better competitor for nutrients. *C. striatus* also produces more cellulose and lignase enzymes, (Maksymiak & Hall, 2000).

The work reported in this paper focuses on the use of *C. striatus* to reduce/eliminate the initial inoculum of the disease, the pseudothecia containing asci, which are formed on infected rape stubble in between growing seasons.

Fungi in the genus *Cyathus* are also known to produce an antibiotic complex known as Cyathin, which may play a role in biocontrol. This complex is made up of 7 different organic compounds (Brodie, 1975). An attempt was made to produce and isolate Cyathin complex and this substance was used to inhibit the germination of *L. maculans* ascospores.

IN VIVO INHIBITION OF PSEUDOTHECIA PRODUCTION

Materials and Methods

Infected oilseed rape stems with blackleg lesions on them (but no pseudothecia), were collected from IACR – Rothamsted in September 2001. These were then placed in trays with drainage holes, on top of non-sterile soil. Six stems were placed in each tray and there were 2 trays for each treatment, giving 12 replicates in total. The trays were covered in a plastic mesh to ensure the stems were not blown away. The following treatments were applied to the infected stems:

1. 50mls of a saturated copper sulphate solution (to be certain of fungicidal activity), was sprayed onto the stems as a control.

2. A totally untreated control.
3. Water only was sprayed onto the stems as a control.
4. 50mls of *Cyathus striatus* hyphal suspension (made by scraping the mycelium off 10 2-week old *C. striatus* cultures into 100mls water), was sprayed onto the stems.
5. *Cyathus striatus* peridioles (30 per tray) were scattered on to the stems.
6. A 3cm layer of straw and cow dung with *Cyathus striatus* growing on it was placed on top of the stems.

The trays were then left exposed to natural weather conditions outside on a roof at the University. After 8 weeks, the stems were collected and cleaned and percentage pseudothecia cover was visually estimated. The density of the pseudothecia on the stems, (whether it was high, >300 per cm^2, medium, 50-299 per cm^2, or low, <50 per cm^2) was also recorded.

Results and Discussion

As shown in Table 1 the copper sulphate control almost completely inhibited the production of pseudothecia on the infected rape stems. Treating the stems with *Cyathus striatus* peridioles and *C. striatus* suspension demonstrated no reduction in percentage pseudothecia cover compared with the water only and no treatment controls, although the density of the pseudothecia was lower. However, the stems which had straw/dung mixture with *C. striatus* growing on it did indicate potential for reducing the disease inoculum production. This was indicated by the visibly lower density and percentage cover of pseudothecia. The value was not statistically significantly different from the controls, but it was decided to repeat the experiment *in vitro* and to mix the straw/dung/fungus mixture with the soil rather than just placing it on top of the stems. This was because the majority of the pseudothecia on the stems which had this treatment applied to them were on the side not in contact with the straw/dung/fungus mixture and this treatment showed the greatest potential for inhibition of pseudothecial development.

Table 1. Mean percentage pseudothecia cover on infected rape roots, incubated outside over winter (n=12).

Treatment	Mean % pseudothecia cover	S.E.	Pseudothecia density
Control – $CuSO_4$	2.3	± 1.67	Medium/low
Control – nothing	20.0	± 2.01	High
Control – H_2O	26.3	± 4.27	High
Cyathus peridioles	22.1	± 5.52	Medium
Cyathus suspension	31.3	± 6.03	High/medium
Cyathus/straw/dung	16.8	± 3.09	Medium/low

IN VITRO INHIBITION OF PSEUDOTHECIA PRODUCTION

Materials and Methods

Infected rape stems were buried in non-sterile soil in 20x15cm open boxes, 6 per box. The treatments applied were; saturated copper sulphate solution applied once, copper sulphate applied twice at bi-weekly intervals and no treatment as controls (2 boxes per treatment) and *Cyathus*/straw/dung mixture mixed in with the soil at an approximate ratio of 25:75 (4 boxes).

The boxes were then placed in an incubator at 11°C as this has been shown to be the optimum temperature for pseudothecia development in previous epidemiological studies (Pérès *et al.*, 1999). The soil was kept moist with collected rain water. After 8 weeks, stems were collected, cleaned and percentage pseudothecia cover estimated as before.

Results and Discussion

Table 2. Mean percentage pseudothecia cover on infected rape roots, incubated in controlled conditions at 11°C.

Treatment	Mean % pseudothecia cover	S.E.	Pseudothecia density
Control – $CuSO_4$ x1	3.8	± 1.52	Medium/low
Control – $CuSO_4$ x2	1.3	± 0.65	Low
Control – nothing	26.3	± 2.75	High
Cyathus/straw/dung	10.2	± 3.11	Medium/low

As can be seen in Table 2, mixing the *Cyathus*/straw/dung mixture with the soil, prior to burial of the stems, was far more effective at prevention of pseudothecia formation than merely placing it on the surface as in the *in vivo* experiment. This result is significantly different ($P<0.0001$) to the control with nothing added. All the stems which underwent this treatment were heavily colonised by *Cyathus*, which was visible as a characteristic white mycelium. In a practical situation, a *Cyathus*/straw/dung mixture could simply be ploughed into a field after rape harvest. As was expected, the stems treated with copper sulphate showed hardly any pseudothecial development. The mechanism by which the *Cyathus* mixture arrests pseudothecia development is likely to be superior nutrient capture by *Cyathus* and possibly production of the Cyathin antibiotic complex.

INHIBITION OF ASCOSPORE GERMINATION

Materials and Methods

This experiment was carried out as a continuation of the aim to reduce the initial disease inoculum using Cyathin, as *Cyathus striatus* was shown not to completely inhibit the production of pseudothecia and ascospores.

An attempt to produce Cyathin was made using the method of Allbutt, *et al.*, (1971). 800mls of a chemically defined liquid medium was made up and 200mls put into each of four flasks. These were inoculated with five 8mm plugs of *C. striatus* and incubated statically at 25°C for three weeks. The medium was then filtered through cheesecloth to remove fungal material and the organics extracted from the medium using an equal volume of ethyl acetate. After evaporation of the ethyl acetate, the resulting residue was re-suspended in 3mls of water to give a projected 10% w/v solution of Cyathin. As a control, 800mls of ethyl acetate alone was placed in a flask and evaporated and 3mls water added to pick up any possible residue.

Mature pseudothecia from the previous experiment, were picked off the rape stems and placed in 0.0075ml of sterile tap water on a glass slide. Under a microscope, using a needle, the pseudothecia were crushed to release the ascospores into the water. 0.0075mls of treatment was then added to each slide, 5 slides per treatment. This volume was used as it was found

that 0.015mls of liquid (the combined amount of water and treatment) was the volume which fitted under a coverslip. The treatments added to the ascospores were; water only, media only, dissolved ethyl acetate residue (if any) and copper sulphate as controls, liquid medium in which *Cyathus* had been grown and organic extract from *Cyathus* growth medium. The slides were placed in Petri dishes containing moist filter paper and a glass rod bridge to prevent them drying out and incubated at 25°C. After 24hrs, the slides were placed under a microscope and 50 spores were assessed as to whether they had produced germ tubes or not.

Results and Discussion

Table 3. Mean percentage germination of ascospores in water on slides, after incubation at 25°C for 24 hours (n=50).

Treatment	Mean % germination	S.E.
Control – water only	92.0	± 3.35
Control – growth medium only	92.0	± 3.03
Control – evaporated ethyl acetate	93.6	± 2.48
Control – $CuSO_4$	0.0	± 0.00
Cyathus growth medium	4.8	± 5.35
Extract from *Cyathus* growth medium	0.0	± 0.00

Table 3 clearly demonstrates that organic metabolites in the media, produced by *Cyathus striatus*, inhibit the germination of *L. maculans* ascospores. There is a significant ($P<0.0001$) reduction in numbers of spores germinating when media in which *Cyathus* had been growing was added to the spore suspension. When the organic compounds were extracted from this medium and added to the spore suspension, they inhibited germination as efficiently as the copper sulphate control. The fact that the media only and ethyl acetate controls do not inhibit germination, demonstrate that *Cyathus striatus* produces an organic metabolite which has antifungal properties. More work must now be carried out to analyse these potentially very useful organics.

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Impact of spectral cladding materials on the behaviour of glasshouse whitefly *Trialeurodes vaporariorum* and *Encarsia formosa*, its hymenopteran parasitoid

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ABSTRACT

The "spectral filters" plastic films XL Sterilite HDF, Anti-Botrytis and Solatrol (which absorb UV light) were compared with Visqueen film, UVI/EVA (non-UV absorbing), for their impact on the dispersal of both glasshouse whitefly, *Trialeurodes vaporariorum,* and its hymenopteran parasitoid, *Encarsia formosa.* The two insect species were released, in separate experiments, in the centre of four units covered with the selected films, leaving them with a choice of four different compartments in which to disperse. In replicated choice experiments, there was a high preference for both species to collect under the UVI/EVA film rather than under the spectral filters that absorbed UV light. Between 6 and 11 times as many whiteflies were caught in the control compartment, compared to the numbers trapped under each of the UV-blocking films. *T. vaporariorum* adults were also less flight-active under UV blocking films. Both XL Sterilite and Anti-Botrytis films attracted significantly fewer whitefly individuals than the Solatrol film. Approximately 2.5 times more parasitoids were trapped under the UVI/EVA film compared to the numbers trapped under each of the UV-blocking plastic films. *E. formosa* did not demonstrate any preference between the UV-blocking films.

INTRODUCTION

A new generation of plastic films that cause spectral modification has been commercialized for the production of protected crops. The use of these "spectral filters" is increasing around the world. At the same time, it is known that some insects utilize light in the UV and other spectral regions to locate their hosts. The main aim of this current study was to assess the impact of a range of commercial spectral filters on the biology of the glasshouse whitefly, *T. vaporariorum* and its hymenopteran parasitoid, *E. formosa*. The specific objectives were to design and construct an apparatus (choice chamber) that could measure dispersal/flight activity of both species under different light environments provided by 'control' and UV-absorbing plastic films.

MATERIALS AND METHODS

In order to satisfy the objectives of this study, a choice-chamber design was selected in which insects were released into the central compartment of an apparatus, designed in the shape of a cross. Each of the four compartments 1, 2, 3 and 4 were detachable from the central release chamber, so that the equipment could be reassembled with the different plastic films in different 90° orientations. The final experimental design was a Latin Square with 4 replicates of 4 treatments (different plastic films) and 4 orientations (East, West etc.). A total of sixteen

releases of each insect species was carried out between 19 June and 10 August 2001. Analysis of variance was used to analyse the numbers of insects recorded in the individual compartments, using Genstat version 5.

Each of the four compartments were constructed, with dimensions of 1.5x1x1m. Each wooden frame was covered with the preselected films; the film UVI/EVA, Anti-Botrytis, Solatrol (all three from Visqueen Agri, Stockton, UK) and XL Sterilite HDF (XL Horticulture, UK). For air circulation, insect mesh was attached to the base of the frames to a height of 30cm. Adhesive Velcro was used to seal the lower surface of the films to the upper surface of the insect mesh and those sides of the frames that would come in contact. The plastic film covering the central insect release chamber was black, to avoid any transmission of light from the central compartment to the rest of the units.

During the individual release experiments, the dispersal of insects into the individual compartments was measured from the numbers of insects recorded on plants, and a transparent sticky trap placed in each compartment. The plant used in the experimental programme was cucumber (*Cucumis sativus*). The experiments with whitefly were conducted with the cv. Telegraph Improved, while the experiments with *E. formosa* were carried out with the cv. Marketmore. Sticky traps (A4 size) were prepared from transparent acetate film, to avoid the use of traps (e.g. yellow or blue traps) that, by themselves might attract or repel insects. Each of the four compartments contained one sticky trap and two cucumber plants. The sticky traps were placed 20 cm away from the far end of the units. Half of the sticky surface was above the plant canopy and half at the same level, while the plants were placed in front of the trap. A pooter (Macaulay Entomology, Kimpton) was used to collect randomly 400 whitefly adults, from a culture supplied by BCP Ltd, Wye UK. A plastic box containing the whitefly was placed on the ground in the central compartment of the choice-chamber and the top cover was pulled away, to release the adult whiteflies. The releases were carried out at 10-10.30 a.m. Measurements of the numbers of whitefly on the sticky traps and plants were taken between 7 and 8 pm, on the day after each release. Access to the sticky traps and plants in each unit was obtained by detaching the Velcro holding in place the insect mesh and plastic films. Using large polythene bags, individual plants were covered carefully and were cut at the base. The bags were placed in a refrigerator, killing the whitefly and enabling the numbers of whitefly on each plant to be recorded without them flying away. Between each release experiment one Vapona strip (Sara Lee, UK) was placed on the pots remaining in each unit to avoid carryover of insects between individual replicate releases. In addition, a yellow sticky trap was placed in each of the four chambers to act as whitefly traps (Attgrow, UK).

For the studies on the dispersal of *Encarsia*, the procedure was similar to that used for the whitefly. For each release, tubes containing ≈2000 *Encarsia* (BCP, Wye, UK) were left in the appropriate box without their cap for 3-4 minutes to release 300-350 parasitoids. Measurements of the numbers of *E. formosa* trapped in each compartment were carried out in the next morning (i.e after 24 hours). After each release, any remaining insects were left to escape by placing the units upside down for a couple of hours.

RESULTS

Figures 1 and 2 clearly show that many more whitefly and *Encarsia* were trapped under the UVI/EVA film than under the UV-blocking spectral filters. The total number of whiteflies

recorded from traps and plants in the unit covered with the UVI/EVA film was, on average, 6-11 times higher than the numbers found under the spectral filters (Table 1). Statistical analysis of total numbers of whiteflies for each measurement indicated that there was a highly significant difference between the UVI/EVA film and the spectral filters ($P< 0.001$). Moreover, there was a significant difference in the total mean numbers of whiteflies recorded under the Solatrol film, compared with those under the XL Sterilite and Anti-Botrytis films ($P<0.05$).

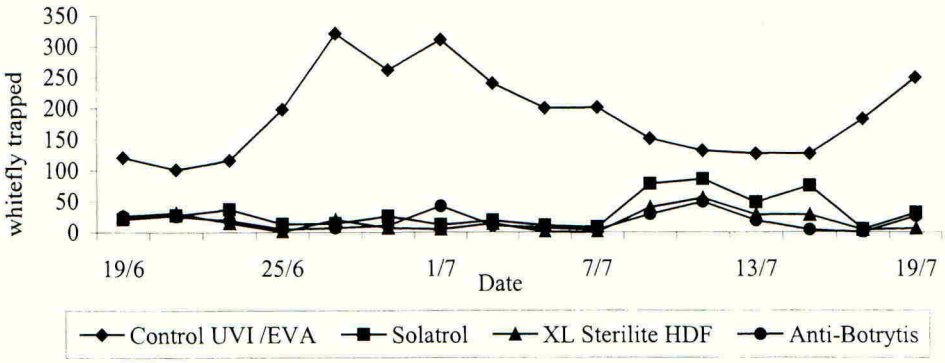

Figure 1. Numbers of whitefly on the sticky traps within each unit, for each of the sixteen experimental releases.

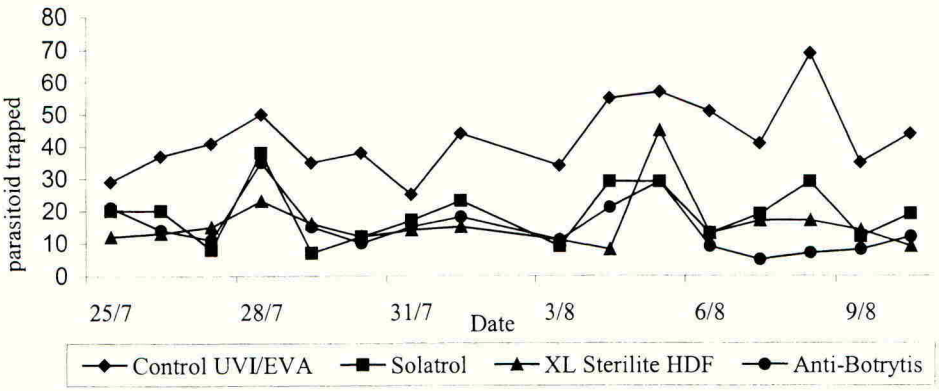

Figure 2. Numbers of *E. formosa* parasitoids counted on the sticky traps within each unit, for each of the sixteen experimental releases.

Table 1. Mean numbers of *T. vaporariorum* found under the plastic films

Film	Traps	Plants	Total
UVI/EVA	68.3	121.7	190.06
Solatrol	6.7	25.3	31.93
XL Sterilite HDF	3.6	13.9	17.56
Anti-Botrytis	4.3	13.5	17.81

Under the UVI/EVA film an average of 2.25-2.84 times more parasitoids were recorded than under the other films. Statistical analysis demonstrated a highly significant difference between the numbers of parasitoids trapped under the UVI/EVA film when compared with those under the spectral filters ($P<0.001$). However, there was no statistical difference at the 5% level between the numbers of parasitoids found under the different UV-blocking films. No parasitoids were found on the cucumber plants during the experiment.

DISCUSSION

As all three spectral filters were mainly UV-light blockers, the results suggest that *T. vaporariorum,* like many insects, utilizes UV radiation for its navigation. There was a consistent preference by the glasshouse whitefly to disperse into the compartments covered with the UVI/EVA film, regardless of the orientation of the compartments. Looking at the ratio between the numbers of whiteflies recorded on the plants and traps, it is very similar for all three spectral filters, with a higher proportion of whitefly recorded on the plants, but different to the ratio under the UVI/EVA film (Table 1). This suggests that, under UV blocking films, flight activity is reduced; otherwise, the numbers caught on the traps would be higher. Antignus *et al.* (1996) observed that *Bemisia tabaci* activity was greatly reduced when the insects were held under UV absorbing films. In the experiment with *E. formosa* releases, UV light transmission also clearly affected parasitoid dispersal. Significantly more parasitoids were found under the UVI/EVA film, but this difference was not so dramatic as with *T. vaporariorum*. One characteristic was that we could not find parasitoids on the plants. Stenseth (1985) reported that adult *E. formosa* principally lands to search for hosts only on whitefly-infested plants and seldom on uninfested plants. Probably, *E. formosa* does not prefer the environment beneath UV blocking films but, when an individual cannot immediately find an appropriate whitefly host then it appears to disperse more readily under UV-absorbing films, presumably in search of its host. Consequently, UV-absorbing films may work in favour of pest management, by discouraging whitefly colonisation, but also by sustaining some flight and searching activity by *E. formosa*. These results suggested that spectral filters may be promising as an alternative non-chemical insect control measure even under UK conditions.

ACKNOWLEDGEMENTS

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The effect of granular nematicides on the development of *Rhizoctonia solani* diseases and their interaction with *Globodera rostochiensis* on potato

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ABSTRACT

The nematicides fosthiazate, aldicarb and oxamyl significantly reduced the development of *Rhizoctonia solani* stolon infections ($P<0.002$) and the severity of black scurf ($P<0.05$) of potato in glasshouse conditions. A field experiment was undertaken to further investigate the effect of aldicarb on the development of stem and stolon canker and on its interaction with the cyst nematode *Globodera rostochiensis*. No significant reduction in stolon canker was observed in aldicarb treated plots. However, in plots left untreated a strong and significant relationship ($P<0.001$) was found between the proportion of stolons infected and the number of juveniles found in the roots.

INTRODUCTION

The fungal pathogen *Rhizoctonia solani* (AG3) is found globally and is known to cause two distinct diseases in potatoes, stem canker and black scurf. Stem canker is a destructive disease of young stems and stolons occurring at and prior to plant emergence. In comparison black scurf is the non-invasive deposition of sclerotia upon tuber surfaces that takes place predominantly during plant senescence. Tubers infected with black scurf can introduce *R. solani* diseases to subsequent crops and therefore infected seed stocks are downgraded and lose marketable value. Previous research has indicated that *R. solani* diseases may be further enhanced during co-infections with potato cyst nematode *Globodera rostochiensis* (Mazurkiewicz-Zapalowicz & Waker-Wójciuk, 1994; Back *et al.*, 2000).

In the UK, granular nematicides are applied to approximately 28000ha of potato cyst nematode (PCN) infested land each year to reduce the number of juvenile nematodes invading and damaging the roots of potato plants (Evans & Haydock, 2000). The effect of nematicides upon soil-borne fungi, in particularly *R. solani* has been examined by several workers. However, *in-vitro* experiments using nematicide-amended media have shown that nematicides may increase or decrease the radial growth of *R. solani* (Ruppel & Hecker, 1982; Hofman & Bollen, 1987), whilst field experiments measuring *R. solani* disease development have been largely inconsistent (Scholte, 1987; Hide & Read, 1991). In this study, combined field and glasshouse experiments were used to explore the effects of nematicides upon *R. solani* diseases and the *G. rostochiensis-R. solani* disease complex (Back *et al.*, 2000).

MATERIALS AND METHODS

Glasshouse experiment

Eighty pots (height: 22cm, diameter: 25cm) were filled to a depth of 5cm with sterilised peat based loam (John Innes No.2) before potato tubers (cv. Pentland Dell, grade: 35-45, sprout

length: 5mm), with their rose ends facing upwards were gently pressed into the centre of each pot. Each seed tuber was inoculated with 25g of sand-maize medium colonised by *R. solani* (Back *et al*, 2000), applied to the top of the seed tuber. The potting medium required to fill each pot by a further 15cm was either amended with the manufacturers recommended rate of Nemathorin ® (10% wt/wt a.i fosthiazate, 30kg ha^{-1}), Temik ® (10% wt/wt a.i. aldicarb, 33.6 kg ha^{-1}), Vydate ® (10% wt/wt a.i. oxamyl, 55kg ha^{-1}) or left unamended. This was achieved by mixing the calculated weight of potting medium and nematicide for each experimental pot for 2 minutes in a cement mixer, which was cleaned thoroughly between treatment groups. Twenty replicate pots were produced for each of the four treatments. The experimental pots were transferred to a polythene tunnel and arranged in a randomised block design. Watering was undertaken every two days and plant emergence was assessed on a daily basis for 14 days.

Ten pots from each treatment were harvested at 4 and 12 weeks after planting. At both harvest dates, assessments of stem and stolon canker were made together with measurements of plant fresh and dry weight. In addition percentage black scurf coverage on daughter tubers and tuber weights were recorded on the second harvest date.

Field experiment

The effect of the nematicide aldicarb on the development of stem and stolon canker and on the interaction between *R. solani* and *G. rostochiensis* was examined under field conditions at Harper Adams University College (HAUC). Prior to planting, experimental plots were sampled for PCN initial population densities (Pi) and species were confirmed using PCR assays undertaken under the guidance of the PCN species determination service at HAUC. Plots (single flat beds, 2 metres long, three planting rows) were either treated with full, ¾, ½, ¼ of the manufacturers recommended rates of aldicarb or left untreated. Each treatment was replicated 10 times and plots were arranged in a randomised block design. In each plot, 3 rows of 9 tubers (cv. Desirée, grade: SE2, size: 35-55, sprout length: 10mm) were hand planted at 20cm intervals to a depth of 15cm (25/05/01). The middle row was inoculated with 50g of sand/maize meal media colonised by *R. solani* as described for the glasshouse experiment. At 6 weeks after planting (06/07/01), 5 plants from the middle row of each plot were harvested and assessed for stem and stolon canker diseases. Furthermore, the number of juvenile nematodes that had successfully invaded the roots of each potato plant were determined using the methods described by Hooper (1986).

RESULTS

Glasshouse experiment

Potatoes grown in soil treated with either aldicarb or fosthiazate were found to take a significantly longer time (c. 2 days) to emerge ($P<0.05$) than those untreated, whilst oxamyl did not affect emergence significantly. No significant differences were found in any of the measurements taken at 4 weeks (harvest 1) after planting. However, at 12 weeks (harvest 2) after planting the percentage of stolons infected by *R. solani* was significantly reduced ($P<0.002$) in all of the nematicide treatments (Figure 1). In addition the assessment of daughter tubers for black scurf revealed that each nematicide treatment had significantly less tubers with black scurf occupying more than 5 % of the tuber surface ($P<0.05$) than the untreated control (Figure 1).

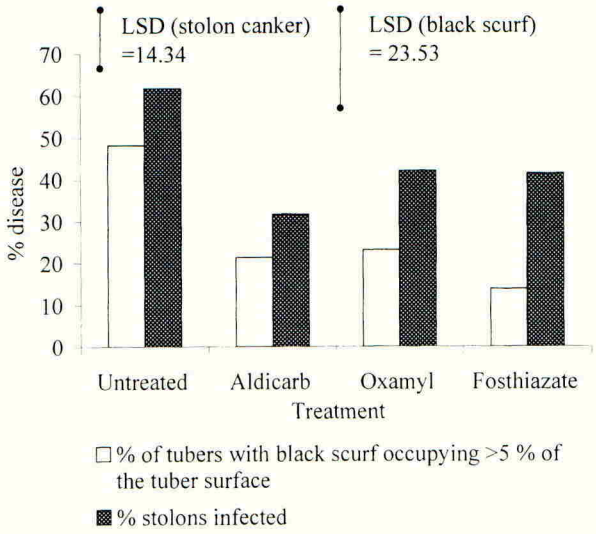

Figure 1. The effects of nematicides on the infection of potato stolons and on the development of black scurf on the surfaces of potato tubers (cv. Pentland Dell) by *Rhizoctonia solani* observed 12 weeks after planting.

Field experiment

Generally, initial population densities of *G. rostochiensis* (confirmed by PCR) ranged from 20-40 eggs/g soil over the experimental site and did not differ significantly between treatments. Although reductions in stolon canker were observed in nematicide treated plots, these were not found to be statistically significant and neither were additional measurements of *R. solani* infections. In plots left untreated a strong and significant relationship ($P<0.001$) was found between the percentage of stolons infected and the number of juveniles found in the roots. However, in plots treated with nematicide no relationship was found (Figure 2).

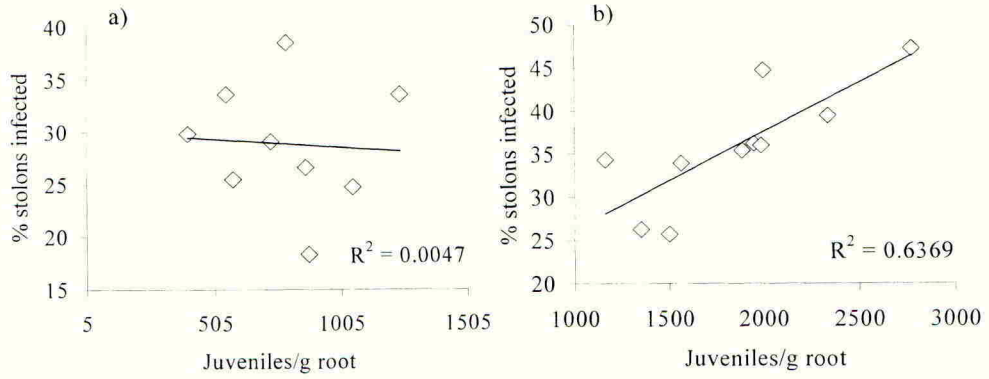

Figure 2. Relationships between potato stolons (cv. Desirée) infected by *R. solani* and invasion of roots by *G. rostochiensis* juveniles in plots either treated with full rate aldicarb (a) or left untreated (b).

DISCUSSION

The glasshouse experiment showed a reduction in both stolon canker and black scurf severity during the application of each nematicide tested. Similarly Hide *et al.* (1991) observed a decrease in black scurf development during field investigations with oxamyl. In contrast Scholte (1987) found increases of stem canker in field plots treated with aldicarb or oxamyl, and Ruppel & Hecker (1982) recorded increased root rot (*R. solani*) of sugar beet following aldicarb treatments. The differences observed between these investigations could be attributed to any number of factors. In controlled glasshouse and *in-vitro* experiments a large number of potentially important abiotic and biotic factors are excluded. For example, Hofman *et al.* (1991) recorded lower stem canker severity in the presence of the mycophagous nematode *Aphelenchus avenae* and the springtails *Folsomia fimetaria* and *Tullbergia krausbaueri* in fields in the Netherlands. However, experimental plots treated with granular nematicides caused a reduction in the population densities of springtails and in particularly *A. avenae*.

Our field experiment showed no significant differences in *R. solani* diseases between aldicarb treated and untreated plots. However, aldicarb prevented significant numbers *G. rostochiensis* juveniles from invading the potato roots and thus disrupted interactions with *R. solani*.

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A malathion-specific esterase in a highly resistant strain of the red flour beetle *Tribolium castaneum* Herbst

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ABSTRACT

A resistant strain of *Tribolium castaneum* was kept under repeated selection pressure with malathion to give high levels of resistance. General esterase, malathion-specific carboxylesterase, glutathione *S*-transferase and target site sensitivity were compared in this and a susceptible strain. Resistance in the highly resistant strain (resistance ratio 2812) was highly specific for malathion and was greatly suppressed by non-toxic carboxylesterase inhibitors. Acetylcholinesterase and esterase activity (α-naphthyl acetate, β-naphtyl acetate or α-naphthyl butyrate substrates) was significantly lower in the resistant strain. However, the rates of both *in vitro* and *in vivo* malathion hydrolysis, using [^{14}C-methoxy] malathion, were many times greater in the resistant strain. *In vitro* inhibition studies suggested that resistance was due to a carboxylesterase unique to the resistant strain and not to acetylcholinesterase, general esterase or glutathione *S*-transferase mechanisms.

INTRODUCTION

Malathion resistance is widespread among stored-product insects. *Tribolium castaneum* Herbst, the red flour beetle, is one of the best-known and most serious pests of food stuffs and has developed resistance to insecticides especially malathion. The most prevalent biochemical mechanism of pesticide resistance in insects is through detoxification by enzymes such as esterases, mixed-function oxidases (P450) and glutathione *S*-transferases (Plapp & Wang, 1983). Of these enzymes, esterases are the most important for the development of resistance to organophosphate insecticides. Beeman and Schmidt (1982) investigated the biochemical and genetic nature of malathion resistance in a *Plodia interpunctella* strain. Using whole body homogenates, there was a three-fold lower activity toward the general esterase substrate α-naphthyl acetate and a 33-times increase in malathion carboxylesterase activity in resistant insects. There was reduced non-specific esterase activity in the malathion resistant strain of the rust red grain beetle, *Cryptolestes ferrugineus*, with resistance due to hydrolytic breakdown of malathion by a malathion specific carboxylesterase (Spencer *et al.*, 1998).

The present project investigated the biochemical mechanisms of malathion resistance in a strain of *T. castaneum* (PH-1) which was kept under repeated selection pressure resulting in a very high level of resistance as compared to the susceptible strain.

MATERIALS AND METHODS

The malathion resistant (PH-1) and susceptible (FSS-II) strains of *T. castaneum* were

maintained at the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne. The resistant strain was further selected with malathion by topical application to the adults to give around 90% mortality and the survivors were used to produce the next generation. This treatment was repeated for every third generation.

For toxicity and synergism studies a range of the insecticide or insecticide plus synergist concentrations (5-7) in acetone were applied topically on the ventral side of the adult beetles at the rate of 0.5 µl/beetle with a microapplicator. The treated insects were kept under rearing conditions and the mortality was observed after 24 hours and LD50 values were calculated. General esterase and carboxylesterase activities were measured in strain PH-5 by the method of van Asperen (1962) using α-naphthyl acetate, β-naphthyl acetate and α-naphthyl butyrate as substrates. To measure carboxylesterase activity, eserine (10^{-4} *M*) and p-hydroxy mercuribenzoic acid (10^{-4} *M*) were included in the incubation mixture to inhibit cholinesterases and arylesterases, respectively.

Acetylcholinesterase activity was measured with acetylthiocholine as substrate (Ellman *et al.*,1960). Glutathion *S*-transferase activity was measured with 3,4-dichloronitrobenzene (DCNB) as substrate (Yu, 1982).

For *in vitro* malathion metabolism whole body homogenates of resistant and susceptible adult insects were incubated with ^{14}C-malathion and the malathionase activity and its inhibition by TPP were observed (Spencer *et al.*, 1998).

RESULTS AND DISCUSSION

The response of the malathion resistant (PH-1) strain to the selection by the insecticide is shown in Table 1. The results indicate the potential to increase the resistance factor (RF_0) of the original resistant strain to further selection pressure. Gopalan *et al.* (2001) found 2726 fold resistance to malathion in a strain of mosquito larvae after constant selection pressure up to 25 generations. Similarly Wang *et al.* (2001) reported the 29035 fold resistance against fenvalerate in a strain of *Aphis gossypii* after 17th generations of selection with the insecticide.

Table 1. LD50 values (with 95% confidential limits) of strains of *T. castaneum* following treatments every 3 generations with malathion

Strain	LD50 (µg/beetle)	Slope ±SE	RF_0
PH-1	6.4 (5-8)	2.76 ±0.32	133
PH-2	75 (65-94)	4.81 ±0.61	1562
PH-5	135 (112-170)	4.84 ±0.55	2812
PH-10	162 (142-191)	5.15 ±0.57	3375
FSS-II	0.048 (0.037-0.059)	3.78 ±0.48	1

RF_0: LD50 of R strain divided by LD50 of S strain

The effect of synergists on the levels of resistance in the original strain (PH-1) and the strain following five selection challenges (PH-5) is shown in Table 2. No synergism was seen with oxidase and transferase inhibitors in either resistant or susceptible strains but both esterase inhibitors indicated strong synergism in the PH-1 strain. This increased in the subsequent selected strain.

Table 2. Toxicities of malathion + synergist (1:5) combinations to the strains of *T. castaneum*

Strain	Synergist	LD50[a] (µg/beetle)	Slope ±SE	SF	RF_1	RF_2	RF_2/RF_0
PH-1	DEF	0.058 (0.04-0.07)	4.06 ±0.51	110	1.2	1.4	0.01
PH-1	TPP	0.083 (0.05-0.15)	1.93 ±0.26	77	1.7	1.9	0.01
PH-1	PBO	10 (7.6-13.4)	1.48 ±0.19	0.64	208	208	1.6
PH-1	DEM	14 (10-19)	1.36 ±0.18	0.46	291	237	1.9
PH-5	TPP	0.16 (0.12-0.23)	2.45 ±0.26	843	3.3	3.6	0.004
FSS-II	DEF	0.042 (0.03-0.07)	3.21 ±0.31	1.1			
FSS-II	TPP	0.044 (0.03-0.05)	3.88 ±0.48	1.1			
FSS-II	PBO	0.048 (0.03-0.06)	2.59 ±0.37	1			
FSS-II	DEM	0.059 (0.04-0.07)	3.97 ±0.46	0.8			

DEF: tributyl phosphorotrithioate, TPP: triphenyl phosphate, PBO: piperonyl butoxide, DEM: diethyl maleate. SF: LD50 without synergist divided by LD with synergist, RF_1: LD50 R strain with synergist / LD50 S strain without synergist, RF_2: LD50 R strain with synergist / LD50 S strain with synergist. [a] 95% confidence intervals

Esterase activities (Figure 1) were high in the species and carboxylesterases account for about two thirds of the total activity. For both enzyme types the susceptible FSS-II strain consistently

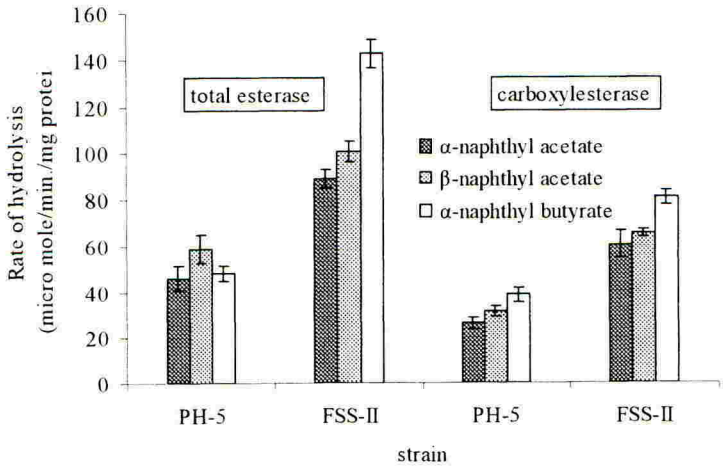

Figure 1. General esterase and carboxylesterase activity in the malathion resistant and susceptible strains of *T. castaneum*

had significantly ($P = 0.01$) higher activities than the resistant PH-5 indicating that adaptation favoured a more specific degradation route (Beeman & Schmidt, 1982; Spencer *et al.*, 1998). Using ^{14}C-malathion as a substrate the resistant strain had an increase of activity about 24 times that of the susceptible (Figure 2). The specificity of this was confirmed using the carboxylesterase inhibitor TPP which at 150nM completely inhibited this enzyme *in vitro*. Acetylcholinesterase activities were decreased in the PH-5 strain (1.67 ±0.09 for PH-5 and 2.52 ±0.14 nmole/min/mg for FSS-II) while GST values were similar (0.52 ±0.03 for PH-5 and 0.50 ±0.04 nmole/min/mg for FSS-II). These studies suggested that resistance was due to a carboxylesterase unique to the resistant strain and not to acetylcholinesterase, general esterase or glutathione *S*-transferase mechanisms.

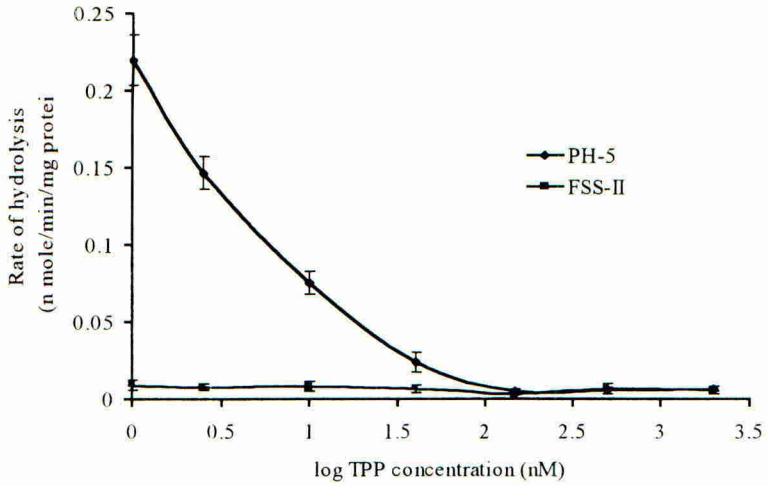

Figure 2. Malathion specific degrading activity *in vitro* in homogenates of the two strains and inhibition by TPP

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