POSTER SESSION 4B

PEST AND DISEASE MANAGEMENT IN HORTICULTURAL CROPS

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The investigation of the biology of the rose Blackspot fungus, *Diplocarpon rosae* leading to improved methods of control

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ABSTRACT

Isolates of *Diplocarpon rosae* derived from a variety of rose types (climbers, ramblers etc.) were obtained from the UK and overseas. The morphology of the fungus was analysed, comparing colony colour and spore length and width. The genetic diversity of the fungus was also studied using molecular analysis techniques. Epidemiological studies were also carried out. Three distinct variations in colony colour between isolates were found: yellow, pale/dark brown and olive green. Conidial lengths among isolates varied between 11.1µm and 30.7µm. There was also a relationship between colony colour and conidial size, the longer conidia being associated with a lighter yellow colonies and the smaller conidia with dark colonies. Preliminary analysis of the results suggests that there is a correlation between colony colour, conidial size, molecular type and geographical origin but not between the rose type and geographical location within the UK.

INTRODUCTION

Roses are among the most important horticultural crop in the UK, with exports alone worth £669,000 in 1997. One of the most severe diseases of field grown roses is Blackspot, caused by the host specific facultative fungal parasite *Diplocarpon rosae*, a disease that is confined only to the genus *Rosa* and is found worldwide. Symptoms are seen as dark brown-black lesions of 2-12mm in diameter and appear on the upper surface of the leaf. Leaf tissue surrounding the spots turns yellow and chlorosis extends throughout the leaflet until defoliation (Horst, 1983). The pathogen is actually present only in the lesion itself; the yellow tissue is caused by pathogen metabolites (Horst, 1983). During the growing season, conidia of *D. rosae* are dispersed by rain splash (Cook, 1981). The conidia consist of 2 cells that are formed in acervuli. These conidia are released from the acervuli when the cuticle ruptures and are seen as a white slimy mass on the leaf surface (under a hand lens). The fungus overwinters on infected dead leaves, thorns and also the stems of the bush (Cook, 1981). In the spring, new infections are initiated from conidia formed in acervuli (Cook, 1981). The most common method of controlling the disease is by regularly spraying infected bushes with fungicides such as captan, triforine or penconazole. Some commercial producers spray up to 40 times a year and many amateurs spray fungicides with great frequency. The differentiation into the physiological races of the fungus has been investigated (Debener *et al.*, 1998, Yokoya *et al.*,

2000) using pathogenicity studies. However, no work has been carried out to find different pathotypes using genetic analysis and studying morphological characteristics of the fungus.

This paper presents the morphology of a number of *Diplocarpon rosae* isolates, from infected leaves worldwide which have been isolated from a range of rose varieties (Hybrid tea, floribunda, damask etc.) and presents the preliminary results of molecular analysis of these isolates.

The overall aim of the MAFF project is to determine the number of pathotypes of *Diplocarpon rosae* occurring worldwide and on different rose types. The study seeks to find differences in isolates molecularly, morphologically and epidemiologically and so gain a better understanding of how to improve the management and control of the disease.

MATERIALS AND METHODS

Collection of infected leaves

Infected leaves were collected from wide geographical sources and also from a broad variety of rose species (Climbers, teas, patio roses etc.)

Isolation and storage of *Diplocarpon rosae* and morphological characteristics

Using a dissecting microscope, acervuli of *D. rosae* were observed within the necrotic lesions on the upper leaf surface. A sterile needle was inserted into an acervulus, which was not obviously oozing spores. The spores were then directly transferred onto 1/4 strength PDA (3.9 g PDA : 6 g Agar in 400mls) . The plates were sealed with Parafilm and incubated in the laboratory at approx. 20°C (12hr light/12hr dark) on a bench surface. Isolates were stored *in vitro* as agar plugs in sterile tap water at 4° C. The colony colour and hyphal characteristics of each isolate was assessed using a Standard colour chart (Ridgway, 1912). A conidial suspension was prepared and 50 conidia from each representative isolate were measured (length and width). Slide cultures were prepared and the hyphal characteristics of the fungus observed and the presence or absence of microconidia noted.

Molecular studies

DNA was extracted from 53 selected isolates and its variability investigated by simple sequence repeat (SSREP), and amplified fragment length polymorphism (AFLP). Genomic DNA was extracted from colonies on PDA plates (younger growth from edges), using the Promega Wizard DNA purification kit.

SSREP and AFLP

For the SSREP sequence repeat $(GACA)_4$ was amplified with 100 pmol of primer 5'-(GACAGACAGACAGACA)-3' in a 50 µl PCR reaction mixture, which consisted of: 5 µl of 10x buffer, 0.5 µl of Tth enzyme (5 U/µl) (HT Biotechnology), 500 ng of DNA, a final

concentration of 200 µM for each dNTP, final concentration of 2.5 mM of $MgCl_2$ and distilled water. The amplification program comprised of an initial denaturation cycle at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C/30 sec, annealing at 45°C/30 sec, extension at 72°C/1 min 30 sec, and an additional cycle including a 8-min extension at 72°C. The cycles were carried out in a Hybaid Express Thermal Cycler. 25 µl of the PCR products were electrophoresed in 1.5% SeaKem LE agarose at 80V for 2 hr, after which the gel was stained in ethidium bromide (0.5 µg/ml) and photographed. The banding pattern was then analysed using the GelCompar II software. For the AFLP the Genomic DNA was digested both with a regular-cutting (*Hpa*II) and a rare-cutting (*Eco*RI) restriction enzyme and adapters were ligated to the resultant fragments (modified from Vos *et al.*, 1995). Long primers, which anneal to the adapter sequence, were then used to PCR-amplify the entire population of restriction fragments (annealing temperature 56°C). Sub-populations of the DNA fragments were then amplified from this pre-amplified material using primer pairs which overhang the adapter by two or three bases. The selective primers are usually named by their enzyme followed by the selective nucleotides. For example, one of the 6 pairs of primers used was HPA-CAT (5'-GATGAGTCCTGAGCGGCAT-3') + ECO-AT (5'-GACTGCGTACCAATTCAT-3'). The primer specific to the rare-cutting enzyme adapter was labelled, and the PCR products were visualised on polyacrylamide gel on a 4200 Li-Cor DNA Analysis System.

Epidemiological studies

Air temperature, relative humidity and leaf surface wetness was monitored using data loggers (Gemini Tinytag Plus and Delta-T DL2 Data Loggers). Spore traps (adapted from Cook, 1979) were designed to catch rain splashed *D. rosae* conidia from infected rose bushes at the Gardens of the Rose, St Albans to see if there was a correlation between any of the environmental factors and spore release. Disease development was also monitored on naturally infected bushes.

RESULTS AND DISCUSSION

Over 100 isolates within Europe, the UK and India and from a variety of rose types were collected. Of these isolates, 53 were then chosen in a stratified manner to represent isolates from different geographical locations and also from different rose types. The results of 14 of the 53 selected isolates are presented with only one non-UK isolate represented (DR76) in Table 1.

Morphological characteristics

D. rosae is a slow growing fungus (0.5cm a month). The youngest colonies have a white mycelium with a feathery margin. However, as the colonies matured, a difference in colony colour was observed between isolates. Three distinct variations in colony colour between isolates were found; yellow, pale/dark brown and olive green (Table 1). Within these colour differences some isolates possessed striations within the colony. Conidial length between

isolates varied between 11.1μm and 30.7μm. There was a relationship between colony colour and conidial size with longer conidia associated with lighter yellow colonies and smaller conidia associated with dark colonies (Table 1).

SSREP and AFLP

The AFLP procedure proved to be more reproducible than SSREP. The latter has bias for high copy number sequences in the genome, and in some cases another PCR reaction from the same genomic DNA would not produce the same number of bands. Also the annealing temperature is quite low, which allows unspecific amplification. The number of bands produced in the AFLP was considerably higher than the bands obtained using SSREP, demonstrating that AFLP is a much more robust and sensitive molecular technique. Final conclusions on the isolate clustering can only be drawn when the AFLP analysis is complete for all strains selected, but initial results (14 samples out of 48) show that there are probably two main clusters of strains (Figure 1). The general dendogram obtained using the SSREP $(GACA)_4$ for all the strains selected show that there was no distinct clustering for the rose variety, and the geographical origin of the isolate in the UK. However, the clustering based on the relatively simple banding pattern obtained from the SSREP amplification remains to be confirmed and clarified with the AFLP banding pattern.

Table 1. Morphological, geographical and host group for isolates selected for molecular studies

Rosa variety	Host group	Location	Spore length μm	Standard colour	Group colour
Mme Alfred/ DR60	Noisette	Castle Howard (UK, North)	19.26 ±0.94	Olive brown XL 17'''k	Dark brown
Dream Waltz/ DR127	Floribunda	RHS (UK, South)	17.86 ±0.26	Deep olive buff XL 21'''b	Light Brown/yellow
DR38	?	Castle Howard (UK, North)	29.26 ±0.56	Deep olive buff XL 21'''b	Light Brown/yellow
Boys Brigade/ DR13	Patio	RBG-Kew (UK, South)	17.87 ±0.28	Dark olive buff XL21''	Olive
DR166		A. Roberts isolate	20.82 ±0.29	Olive brown XL 17'''k	Olive
Schneewittchen/ DR76	Floribunda	Germany (Bonn)	15.8 ±0.28	Sayal brown XXIX 15'''I	Brown
DR31	?	Castle Howard (UK, North)	26.52 ±0.86	Deep olive buff XL 21'''b	Light Brown/yellow
Agnes/DR23	Rugosa	Castle Howard (UK, North)	21.76 ±0.23	Deep olive buff XL 21'''b	Light Brown/yellow
St Cecilia/DR117	English rose	Castle Howard (UK, North)	23.42 ±0.39	Deep colonial buff XXX21''b	Yellow
Chianti/ DR89	Species	Castle Howard (UK, North)	29.48 ±0.66	Olive ocher XXX 21''	Deep cream/ yellow
DR167		A. Roberts isolate	20.90 ±0.57	Dark olive buff XL21''	Olive

A preliminary examination of both morphological and molecular data suggests that there may be some correlation between the two sets of data, in particular similar sets of isolates can be observed in the two clusters.

Selective primers: CAT-AT+CAT-GC+C TC-AT+CTC-GC+ACC-AT+ACC-GC

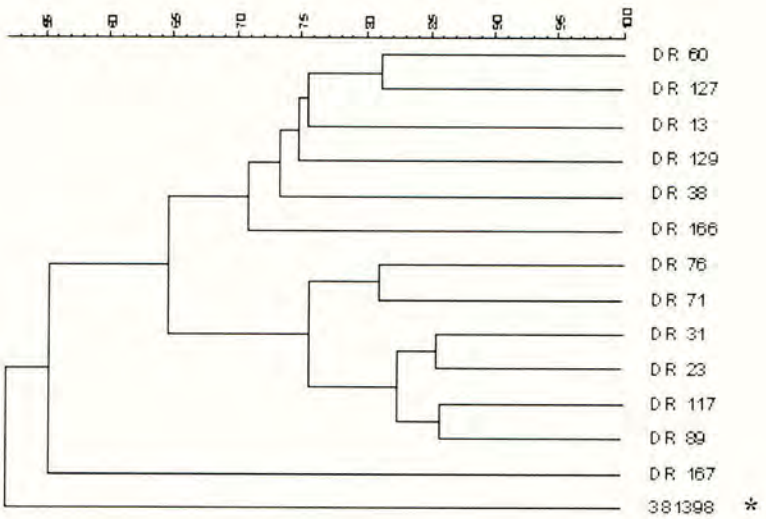

Figure 1. Dendogram showing 2 main clusters of strains using AFLP analysis from the selected 14 isolates of 53 (* related fungal species: control). The dendogram shows the combined results from the 6 combinations of selective primers, which have nucleotides overhanging at the 3'-end (E.g. HPA-CAT + ECO-AT, HPA-CAT + ECO-GC, and so on.

Epidemiological studies

D. rosae spores were caught in traps after rainfall suggesting that rain is an important factor influencing spore release. However, the number of spores collected in the traps was insufficient to draw a positive correlation between spore release, leaf wetness and temperature. Preliminary results showed that there was a positive correlation between leaf wetness and disease as shown by an increase in lesion development.

Future work

The correlation between *D. rosae* morphological characteristics, e.g. spore size, and banding pattern will be further investigated. Representative isolates from the AFLP clusters could be used for amplification of ITS regions, followed by sequencing and alignment analyses to determine stretches of the genomic DNA for use as primers for identification of pathotypes. Pathotype testing will also be carried out using the same isolates as above as well as those identified by Debener (1998) and Yokoya (2000).

The characterisation of molecular and physiological variability will provide markers that can be used to type isolates on incoming rose imports. A greater understanding of the cultural and environmental factors favouring disease progress may enable the development of an integrated control program for this disease based on pathotype.

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Influence of several surfactants on various asexual stages in the life cycle of two *Phytophthora* species

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ABSTRACT

Hydroponic forcing of chicory roots has severe disease problems caused by the oomycete fungus *Phytophthora cryptogea*. Several surfactants were tested for effects on his pathogen and some were found to inhibit zoospore release at a concentration of 20 µg/ml. However, the surfactants had little or no effect on mycelial growth or formation of sporangia except at very high concentrations.

INTRODUCTION

In recent years, hydroponic forcing of chicory roots has had to deal with severe disease problems caused by the oomycete fungus *Phytophthora cryptogea*. The hydroponic system is an ideal environment for the development and distribution of this pathogen, so once introduced, control is often very difficult. Recently, the efficacy of the non-ionic surfactant Agral 90 for the control of root rot of cucumber caused by *Pythium aphanidermatum* was reported (Stanghellini & Tomlinson, 1987). Agral 90 was demonstrated to rapidly lyse zoospores, which were implicated as the primary and most important lifecycle stage responsible for spread of the pathogen (Stanghellini *et al.*, 1996; Stanghellini & Miller, 1997). This paper evaluates the effect of Agral 90 and other surfactants on the life cycle of *Phytophthora cryptogea*. To test the selectivity of these chemicals, the effect on *Phytophthora cinnamomi* was also tested.

MATERIALS AND METHODS

Stock cultures of *P. cryptogea* (isolate PD 91/2009) and *P. cinnamomi* (isolate 845) were maintained on V-8 juice agar. The surfactants tested were Agral 90, Tween 20, MBA 13/8, SCS 6131, SCS 4730, SCS 2941 and G3780A.

Mycelial growth in the presence of surfactants

A 5 mm mycelial disk cut from a 3-day-old agar culture of *P. cryptogea* or *P. cinnamomi* was placed in the middle of a 9 cm Petri dish containing V-8 juice agar amended with a test surfactant at 10, 20, 100, 500, 1000 or 5000 µg/ml and incubated at 25°C. The same medium without surfactants was used as a control. Colony diameters were measured after 48 hr. There were three replicate plates of each surfactant concentration.

Sporangia formation and zoospore release in the presence of surfactants

To induce formation of sporangia, 5 mm mycelial disks were cut from the margin of a 3-day-old agar culture and placed in the middle of a 9cm Petri dish containing 20 ml of a sterile mineral salts solution (Erwin & Ribeiro, 1996) amended with a surfactant at 1, 5, 10 or 20 µg/ml. The sterile mineral salts solution without a surfactant served as a control. Sporangia formation was observed microscopically (x10) after 3 days incubation at room temperature and continuous light. Release of zoospores from the sporangia was then stimulated by incubation of the Petri dishes at 4°C for 1 hr followed by 1 hr incubation at room temperature. The mineral salts solution containing the zoospores was then centrifuged for 5 min at 3500 rpm and the pellet containing the encysted zoospores dissolved in 1ml water. The concentration of zoospores in the pellet was determined using a haemacytometer. Each Petri dish contained 10 mycelial disks and each surfactant concentration was replicated twice.

RESULTS

Mycelial growth in the presence of surfactants

The surfactants tested only affected mycelial growth at concentrations >100 µg/ml (Tables 1 & 2). Similarly, Stanghellini & Tomlinson (1987) did not observe any effect on mycelial growth of several *Pythium* sp. when Agral 90 was used at 15, 20 and 25 µg/ml. Normally, Agral 90 is used at 60-100 µg/ml to improve the wetting or spreading properties of chemical sprays applied to crop foliage (Stanghellini & Tomlinson, 1987).

Table 1. Radial growth of *Phytophthora cinnamomi* 845 (cm/24hr) in the presence of several surfactants (nt = not tested).

	Surfactant concentration (µg/ml)						
	0	10	20	100	500	1000	5000
Agral 90	2.67	nt	nt	2.00	1.56	1.38	0
Tween 20	2.67	nt	nt	2.00	2.00	2.7	1.7
G3780A	2.67	2.67	2.67	0.88	0	0	0
MBA 13/8	2.67	2.67	2.67	2.00	1.62	0	0
SCS 6131	2.67	nt	nt	2.00	2.00	1.78	0
SCS 2941	2.67	2.67	2.67	2.82	0.94	0	0
SCS 4730	2.67	2.67	2.67	2.00	1.82	1.0	0

Table 2. Radial growth of *Phytophthora cryptogea* 2009 (cm/24hr) in the presence of several surfactants (nt = not tested).

	Surfactant concentration (µg/ml)						
	0	10	20	100	500	1000	5000
Agral 90	1.65	nt	nt	1.43	1.31	1.11	1.0
Tween 20	1.65	nt	nt	1.87	1.87	1.57	1.13
G3780A	1.65	1.83	1.72	0.68	0.09	0	0
MBA 13/8	1.65	1.60	1.52	1.58	1.41	1.12	1.00
SCS 6131	1.65	nt	nt	1.77	1.72	1.46	1.41
SCS 2941	1.65	1.60	1.78	1.87	1.44	0.65	0.27
SCS 4730	1.65	1.79	1.81	1.80	1.66	1.22	0.83

Sporangia formation and zoospore release in the presence of surfactants

The effect of surfactants on sporangia formation of both *Phytophthora* sp. was similar as for mycelial growth: only high concentrations (100 – 1000 µg/ml) of G3780A, SCS 6131 and SCS 2941 inhibited sporangia formation (results not shown). However, the surfactants G3780A and MBA 13/8 still inhibited sporangia formation of *P. cryptogea* at 20 µg/ml (Table 3 & 4). At high concentrations, zoospore production was completely inhibited by all surfactants except Tween 20 (results not shown). Sensitivity to 20 µg/ml or less varied with the *Phytophthora* species involved. For example, *P. cryptogea* formed zoospores in the presence of the surfactant SCS 6131 at a concentration of 10 µg/ml while *P. cinnamomi* did not. Only surfactant SCS 6131 inhibited zoospore formation of *P. cinnamomi* at a concentration of 5 µg/ml. The lytic effect on zoospores (structures surrounded by a plasma membrane), suggests that the mode of action of the surfactants may reside in alteration of the integrity and/or permeability of the plasma membrane (Stanghellini & Tomlinson, 1987). This hypothesis is supported by the fact that the surfactants had no or little effect on mycelium or sporangia, structures that are surrounded by a cell wall that apparently provides protection.

Table 3. Sporangia formation and zoospore production of *Phytophthora cinnamomi* (845) in the presence of different concentrations of surfactants (nt = not tested).

		Surfactant concentration (µg/ml)				
		0	1	5	10	20
Sporangia formation	Agral 90	+	+	+	+	+
	SCS 6131	+	+	+	+	+
	MBA 13/8	+	+	+	+	+
	SCS 4730	+	+	+	+	+
	SCS 2941	+	+	+	+	+
	G3780A	+	+	+	+	+
Zoospore production (no/ml)	Agral 90	$9.75x10^4$	$6.25x10^4$	$3.00x10^4$	$5.00x10^3$	-
	SCS 6131	$9.75x10^4$	$4.50x10^4$	-	-	-
	MBA 13/8	$9.75x10^4$	$1.00x10^4$	$5.25x10^4$	$5.00x10^3$	-
	SCS 4730	$9.75x10^4$	nt	nt	$10.00x10^6$	20
	SCS 2941	$9.75x10^4$	nt	nt	$5.00x10^3$	-
	G3780A	$9.75x10^4$	$5.00x10^3$	$2.75x10^4$	-	-

Table 4. Sporangia formation and zoospore production of *Phytophthora cryptogea* (2009) in the presence of different concentrations of surfactants (nt = not tested).

		Surfactant concentration (µg/ml)				
		0	1	5	10	20
Sporangia Formation	Agral 90	+	+	+	+	+
	SCS 6131	+	+	+	+	+
	MBA 13/8	+	+	+	+	-
	SCS 4730	+	+	+	+	+
	SCS 2941	+	+	+	+	+
	G3780A	+	+	+	+	-
Zoospore production (no/ml)	Agral 90	$9.75x10^4$	$1.48x10^5$	$1.18x10^5$	$5.00x10^3$	-
	SCS 6131	$9.75x10^4$	$2.00x10^4$	$5.00x10^3$	$5.00x10^3$	$5.00x10^3$
	MBA 13/8	$9.75x10^4$	$1.23x10^5$	$5.00x10^3$	$5.00x10^3$	-
	SCS 4730	$9.75x10^4$	nt	nt	$2.75x10^4$	$2.50x10^4$
	SCS 2941	$9.75x10^4$	nt	nt	$2.33x10^5$	$7.25x10^4$
	G3780A	$9.75x10^4$	$6.75x10^4$	$5.00x10^3$	-	-

The results of this investigation show that other surfactants as well as Agral 90 such as G3780A, MBA 13/8, SCS 6131 and SCS 2941 inhibited zoospore production by *P. cinnamomi* and *P. cryptogea* even at low concentrations. Surfactant SCS 6131 is most effective against *P. cinnamomi* while G3780A appears to be the most effective against *P. cryptogea*. Surfactants could therefore provide an inexpensive and biologically safe alternative to fungicides. Further research will investigate if these chemicals also inhibit these pathogens in a mini-commercial system.

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Activity of the new BASF strobilurin fungicide, BAS 500 F, against *Plasmopara viticola* on grapes

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ABSTRACT

BAS 500 F is the new, broad-spectrum strobilurin fungicide being developed by BASF. The compound provides excellent control of *Plasmopara viticola*, the pathogen which causes downy mildew of grapevines. Field trials, under practical conditions, have shown that BAS 500 F controls this disease effectively on leaves and berries. Microscopic studies revealed that this good control is due to high activity of the compound against several developmental stages of the pathogen. The zoospores are extremely sensitive to BAS 500 F and react to contact with lysis. If zoospores escape lysis, the germination of encysted zoospores is stopped effectively by a preventative treatment. After curative application, the compound stops further development of the mycelium in the leaves.

INTRODUCTION

The new broad spectrum strobilurin, BAS 500 F (proposed trade mark of the active ingredient, *F 500*), shows a higher degree of activity than previously known strobilurins against a wide range of fungal pathogens (Ammermann et al., 2000). In grape, one of the main targets of BAS 500 F will be downy mildew, caused by *Plasmopara viticola*. Since its first appearance in France in 1878, downy mildew has been a destructive pathogen of grapevine (Large, 1940). It can infect both leaves and clusters. Leaf infections reduce the production of photosynthate needed for, among other things, maturation of the fruit. Severe infection of leaves can even lead to defoliation. Infection of clusters directly reduces the quantity and quality of the fruit.

Although grape varieties vary in their sensitivity to downy mildew, current commercially desirable varieties do not possess adequate resistance and, under climatic conditions favourable for downy mildew, require frequent applications of fungicides to protect leaves and developing fruit. This paper describes the field performance of BAS 500 F against downy mildew and the preventative and curative properties that bring about a high level of efficacy.

MATERIALS AND METHODS

In all studies, BAS 500 F was applied as a 250 g/l EC, azoxystrobin as Quadris (250 g/l SC), fosetyl-Al plus folpet as Mikal (75% WP) and cymoxanil als Curzate (50 WP).

BAS 500 F was evaluated in more than 150 field trials in important European vine growing areas to determine its efficacy against downy mildew in comparison to commercial fungicides. In this paper, the results of two trial series carried out in Germany and France are described. Field trials were conducted according to EPPO (European Plant Protection Organisation) guidelines. Trials were laid out as randomised blocks with 3 to 4 replications. All applications were made in 1000 l water/ha at rates given in the results section. Evaluations were made 14-21 days after the final application by estimating the percent leaf or fruit cluster area diseased or at intervals during the trial by assessing the disease incidence.

In trials to evaluate effects on zoospore release, sporangia suspensions were prepared by washing sporulating leaves, adjusting the suspension to about $4x10^5$ sporangia/ml and immediately adding fungicide suspensions to the sporangia suspension. 20 µl drops were transferred to slides and the effects were assessed under the light microscope by the evaluation of 4x50 sporangia per treatment 3.5 h after fungicide addition. To assess zoospore mobility and lysis, a zoospore suspension of $2x10^6$ zoospores / ml was prepared by chilling a suspension of sporangia to 4 °C for 3 h, then re-warming. Fungicide suspensions were added immediately after zoospore release. 20 µl drops were transferred to slides and the effects on zoospore motility, lysis, encystment and germination were assessed microscopically in 10 fields per treatment at various intervals.

To assess preventative and curative activity, rooted grape cuttings, cv. Mueller-Thurgau, grown in the glasshouse to the stage of 3 fully developed leaves were used. The plants were treated to run-off in a spray chamber to achieve coverage of all plant parts (4 plants per treatment). Applications were made either 1 day preventative or 1, 2 or 4 days post inoculation. Inoculation was made with a zoospore suspension, prepared as described above, using a spray gun. Assessment was made 7 days after inoculation by estimating the percent leaf area necrotic and/or sporulating. Germination of zoospores on leaves was assessed 24 hours after inoculation by epifluorescence microscopy following staining with diethanol (Leinhos et al, 1997). Mycelium development was evaluated at daily intervals by both epifluorescence and confocal laser microscopy after staining (Hood & Shew, 1996).

RESULTS

Activity in the field

In the first series of field trials with a 14 day spray schedule, BAS 500 F showed greater activity against *P. viticola* in comparison to azoxystrobin (Figure 1). In the untreated control, downy mildew began to increase about 6 weeks after the trials were initiated and reached an average incidence of 62 % on leaves and 72 % on clusters by the end of the trials. BAS 500 F gave nearly complete protection for the entire season, with an average incidence of only 5.6 % on the leaves and 7.4 % on the clusters. This was better than that attained with azoxystrobin. In the second trial series, BAS 500 F showed its high activity on

leaves and especially clusters in comparison to the combination of fosetyl-Al and folpet (Figure 2).

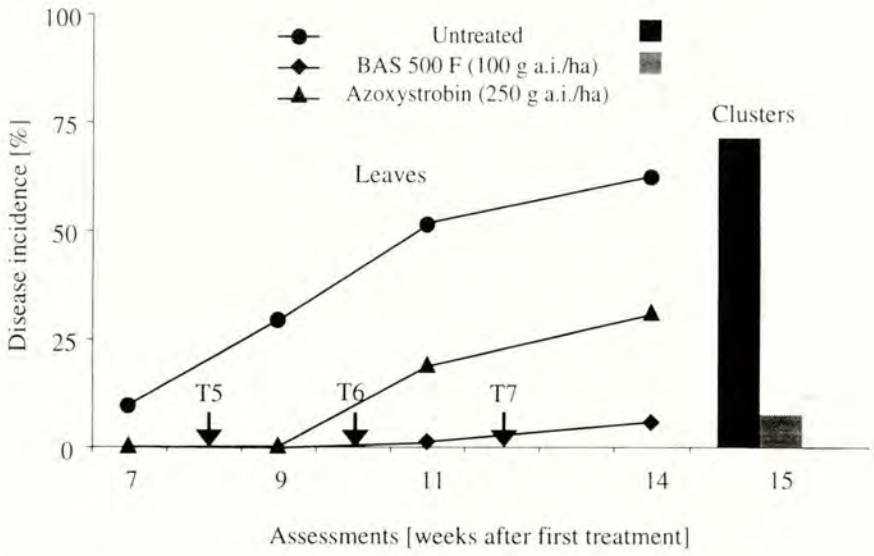

Figure 1. The effect of BAS 500 F and azoxystrobin against *P. viticola* on leaves and clusters. There were 7 applications (T1-7) at 14 d intervals (means of 3 field trials, Germany, SEM for all means < 4.5 %).

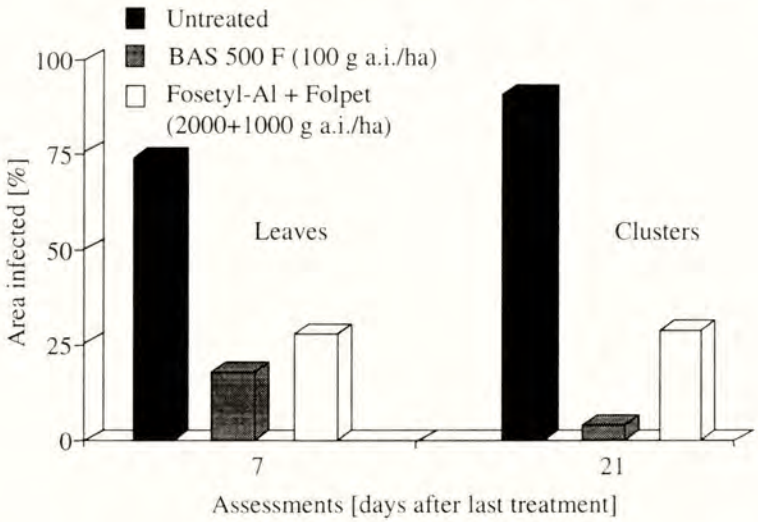

Figure 2. The effect of BAS 500 F and fosetyl-Al + folpet against *P. viticola* on leaves and clusters. There were 5-7 applications at 12-14 day intervals (means of 4 field trials, France, SEM for all means < 4.8 %).

Preventative activity

To identify the properties of BAS 500 F responsible for disease control in the field, its activity against the different developmental stages of *P.viticola* was studied both *in vitro* and *in vivo*. When BAS 500 F was added to a sporangia suspension of *P.viticola*, the release of zoospores was greatly inhibited at concentrations of 1 ppm and lower (Figure 3). Complete inhibition was observed at 10 ppm and higher. Similar but less suppressive effects were observed with azoxystrobin. Cymoxanil had no apparent effect on zoospore release.

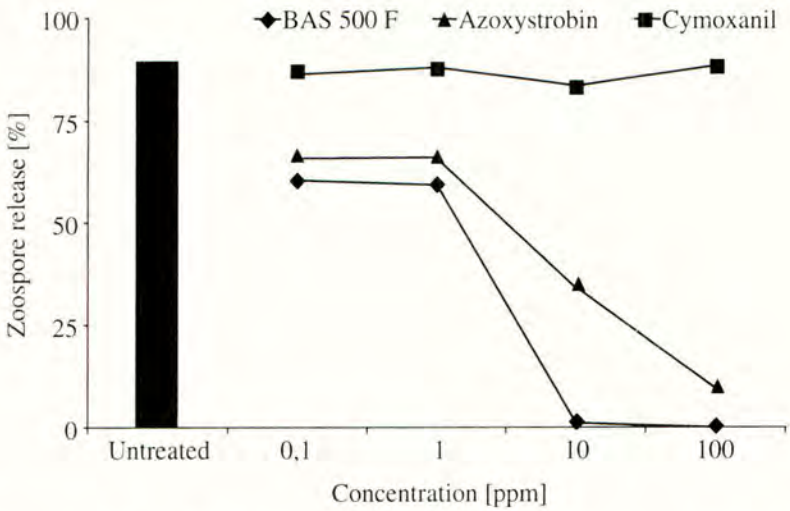

Figure 3. Effect of BAS 500 F and other fungicides on the release of zoospores from sporangia of *P. viticola* in vitro. Assessment was 3.5 h after suspension of sporangia (means of 4 replications, SEM for all means < 3.4 %).

After addition of BAS 500 F at concentrations of 0.1 ppm or more to zoospore suspensions, most zoospores ceased swimming movements within two minutes (Table 1). The zoospores then began to swell and within six minutes most had burst. In the few cases where zoospores exposed to BAS 500 F escaped bursting, encystment appeared normal but germination was almost completely inhibited. These effects were also observed with azoxystrobin, but to a lesser extent and only at higher concentrations. Cymoxanil had no detectable effects on the mobility or integrity of the zoospores and only a moderate effect on germination.

After application of a zoospore suspension onto leaves, a reduction in the number of encysted zoospores on leaves treated with BAS 500 F compared to untreated leaves was observed, but this effect was not as great as observed in the suspension tests. Germination of spores which escaped the effect of bursting and which were able to encyst was suppressed. After a 1-day preventative application of 4 ppm BAS 500 F, not a single germinated spore could be detected on treated leaves, whereas, on untreated leaves, 45 % of the spores germinated after 24 h.

Feeding preference bioassays

None of the chemicals reduced feeding in the preference tests and none appeared to act as a feeding deterrent.

DISCUSSION

This work shows that the CSI insecticide lufenuron has a useful degree of activity against adult vine weevil, resulting in significant adult mortality, reduced oviposition and greatly reduced egg viability. Adults fed readily on treated leaves. Other compounds in this group should also be evaluated to determine whether this activity is a property of the group as a whole. Experiments are also required to determine whether CSI insecticides are active in the field. If so, use of CSI insecticides as foliar sprays against adults to reduce oviposition and egg viability would provide a useful alternative strategy for control of this important pest. Good spray cover may be required as insecticides in this group are generally non-systemic.

ACKNOWLEDGEMENTS

This work was funded by MAFF. We would like to thank Sarah Gurnsey for technical assistance. We would also like to thank the companies who provided the compounds.

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Dose-response bioassays

Diflubenzuron, experimental compound A and fenoxycarb did not affect adult mortality although lufenuron did increase mortality (33% for 0.03-0.1, 50% for 0.3-1, 90% for 3-10 times the standard, versus 16% for the control). For all treatments, feeding decreased naturally over the time period, and especially once egg laying had started. Diflubenzuron, fenoxycarb and experimental compound A did not affect the amount of leaf eaten, although lufenuron reduced feeding at doses greater than 0.03 times the standard. Egg laying began during the third week of the experiment and laying and viability were low during the first 2-3 weeks of laying as has been previously reported by Moorhouse *et al.* (1992). The data on egg laying were very variable although they suggest that diflubenzuron, fenoxycarb and experimental compound A had no effect but that lufenuron reduced egg laying (Table 4). The % of hatched eggs after 10 days was also variable between replicates, but there were marked differences between treatments (Table 5). No eggs hatched from lufenuron at concentrations of 0.1 to 10 times the standard though a few hatched at lower concentrations. By the 17 day assessment, a large proportion of the remaining eggs had fungal infections.

Table 5. Mean % egg hatch after 10 days at 20°C, for eggs laid by vine weevils fed on leaves sprayed with different concentrations of IGRs in 1999.

Treatment	Weeks from onset of laying	Concentration (multiple of standard)							
		0	0.01	0.03	0.1	0.3	1	3	10
Lufenuron	2	56	37	7	0	0	0	0	0
	3	63	6	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0
	5	4	0	10	0	0	0	0	0
	6	16	0	0	0	0	0	0	0
Diflubenzuron	2	16	-	26	43	5	12	5	0
	3	65	-	68	51	66	41	18	2
	4	66	-	48	30	71	14	1	29
	5	5	-	82	72	79	74	21	6
	6	22	-	22	59	42	46	40	16
Fenoxycarb	2	19	-	40	51	30	43	42	-
	3	66	-	79	47	67	40	47	-
	4	40	-	45	33	17	52	32	-
	5	65	-	24	63	21	43	53	-
	6	28	-	19	42	24	22	38	-
Experimental compound A	2	13	-	34	26	35	36	34	30
	3	76	-	42	44	60	61	40	81
	4	50	-	51	6	25	23	25	46
	5	68	-	31	24	30	17	28	56
	6	24	-	39	31	39	28	34	51

Table 3. Mean % egg hatch after 10 days at 20°C of eggs laid by vine weevils fed on leaves dipped in different concentrations of IGRs in 1998

Treatment	Dose	Duration of exposure (weeks)							
		1	2	3	4	5	6	7	8
Lufenuron	standard	6	2	0	0	-	-	-	-
	¼ standard	49	11	2	0	0	0	-	-
Tebufenozide	standard	29	15	36	28	40	43	58	50
	¼ standard	34	39	63	22	32	56	52	43
Fenoxycarb	standard	40	34	34	24	34	43	59	47
	¼ standard	32	32	55	58	35	63	58	55
Diflubenzuron	standard	44	8	28	11	10	19	43	28
	¼ standard	24	40	36	4	38	38	43	29
Untreated	-	39	18	44	28	45	50	62	60

Table 4. Mean number of eggs laid per live vine weevil adult per week after feeding on leaves sprayed with different IGRs in 1999.

Treatment	Weeks from onset of laying	Concentration (multiple of standard)							
		0	0.01	0.03	0.1	0.3	1	3	10
Lufenuron	1	0.6	0.1	0	1.7	0	0	0.1	0.1
	2	2.7	0.7	0.9	9.2	1.9	0.4	1.7	0
	3	6.2	6.2	6.6	5.9	0.4	0.2	0.1	0.4
	4	4.1	4.5	2.5	6.1	0.2	0.8	0	0
	5	4.7	2.2	3.1	4.7	0.1	1.1	0	0
	6	9.9	1.1	2.1	2.3	1.0	0.3	1.0	0
Diflubenzuron	1	1.6	-	0.5	0.7	1.1	1.3	0.7	0.7
	2	6.3	-	6.0	7.6	7.3	10.6	11.3	3.4
	3	5.5	-	7.8	15.8	8.4	11.2	14.8	13.7
	4	3.6	-	10.8	9.7	9.8	10.0	6.0	10.7
	5	3.7	-	7.2	5.1	6.1	5.7	6.8	6.6
	6	5.3	-	1.6	8.2	8.7	11.7	6.3	8.1
Fenoxycarb	1	1.3	-	0.1	3.8	5	4.8	0.2	-
	2	8.0	-	13.0	11.7	13.7	10.5	1.4	-
	3	14.9	-	12.8	19.3	8.4	10.8	6.0	-
	4	10.0	-	14.7	14.3	10.4	9.3	6.3	-
	5	10.6	-	10.3	8.0	6.8	7.3	8.7	-
	6	10.1	-	12.8	7.4	4.3	4.5	4.6	-
Experimental compound A	1	1	-	0	1.1	4.5	2.2	0.1	1.0
	2	8.7	-	5.7	8.3	21.3	11.4	7.5	12.3
	3	16.2	-	2.9	6.1	12.8	5.0	3.6	4.7
	4	9.4	-	28.5	7.5	10.4	7.9	7.4	12.6
	5	6.0	-	10.2	4.4	7.6	8.4	4.3	10.9
	6	7.3	-	9.3	3.9	11.3	4.6	7.0	8.6

transferred to a clean Petri dish lined with damp filter paper, wrapped in Parafilm and maintained at 20°C with a 16:8 h photoperiod. Egg collections were continued for six weeks after the start of laying. The sable-hair paint brush (size 000) used to handle the eggs was first dipped in a dilute bleach solution (0.1 %) to minimise the establishment of pathogens. The number of hatched and unhatched eggs was assessed at 10 and 17 days after collection. The number of dried or mouldy eggs was also recorded, but excluded when calculating the % hatch. The data for the bioassays were not analysed statistically due to the large variability between replicates, though mean values were calculated.

Feeding preference bioassays

In 1999, a similar experiment was done to assess the feeding preferences of adult weevils. Leaf discs were sprayed as above with diflubenzuron, lufenuron, experimental compound A or fenoxycarb at the standard concentrations. Two discs were attached to each filter paper in each Petri dish. Comparisons were made between either two discs of the same treatment, one treated and one water control disc, or two water control discs. There were twenty replicates per treatment. One adult weevil was introduced per dish, and feeding activity was assessed after 3 days at 20°C. Data were analysed using Chi-squared tests.

RESULTS

Preliminary experiment

The control, fenoxycarb, diflubenzuron and tebufenozide treatments had <50% adult mortality by the end of the experiment but chlorpyrifos and cypermethrin gave complete adult mortality within three weeks. Lufenuron also gave 100% mortality after 6–8 weeks at both concentrations. It also reduced the amount of leaf material eaten and the number of eggs laid (Table 2). The smallest % of eggs hatched from the lufenuron treatments (Table 3). Diflubenzuron at the standard rate also reduced hatch. The fenoxycarb and tebufenozide treatments had little effect on egg viability, amount of leaf material eaten or number of eggs laid. Egg hatch at 10 and 17 days followed a similar pattern.

Table 2. Mean number of eggs laid per week by each live vine weevil fed on leaves treated with different IGRs in 1998.

Treatment	Concentration	Duration of exposure (weeks)							
		1	2	3	4	5	6	7	8
Lufenuron	standard	17.6	3.2	1.0	1.8	0	-	-	-
	¼ standard	8.2	12.6	4.6	3.6	2.1	0.3	0.5	-
Tebufenozide	standard	13.3	24.1	12.1	13.3	8.3	20.5	26.5	18.3
	¼ standard	6.6	19.0	7.6	6.6	9.5	16.5	31.9	10.3
Fenoxycarb	standard	13.3	27.2	7.1	16.5	10.1	9.5	27.6	13.5
	¼ standard	9.1	17.9	14.2	18.0	10.4	11.8	26.6	9.8
Diflubenzuron	standard	7.3	13.3	6.1	16.6	5.3	8.0	12.1	10.1
	¼ standard	11.5	13.5	7.3	8.6	2.0	4.6	14.0	10.7
Untreated	-	10.0	20.1	12.1	14.2	16.7	19.9	15.3	14.2

start of the experiment. In 1999 newly emerged weevils were collected in June prior to the start of egg laying.

Preliminary bioassays: In 1998, adult vine weevils were fed on strawberry leaves dipped in two dilutions of the insecticides chlorpyrifos, cypermethrin, diflubenzuron, fenoxycarb, tebufenozide and lufenuron in water (Table 1). Standard concentrations were based on the recommendation on the product label for a water volume of 1000 l ha^{-1} which approximates to 'run-off' spray application. Tests were done at the standard and at ¼ of the standard concentration. On 11 September, 10 or more replicate 9 cm Petri dishes, each containing a single vine weevil adult, were set up for each insecticide/concentration to be tested, plus 25 replicate dishes for the water only control. Each dish contained a damp filter paper to prevent desiccation. Leaves were dipped in the test solutions, air-dried and one leaflet was placed in each dish. Dishes were then placed in a controlled environment room at 20°C with a 16:8 h photoperiod. The leaves were replaced weekly with fresh treated leaves. Weevils were assessed weekly for mortality and feeding activity and the amount of leaf material eaten was estimated to the nearest 0.25 cm^2. The eggs laid were counted and transferred to a Petri dish containing damp filter paper, and held at 20°C. Egg hatch was assessed 10 and 17 days later.

Table 1. Insecticides and their standard concentrations used for bioassays.

Chemical	Product	Formulation	Standard concentration a.i.	
			1998	1999
			mg a.i. / l	µg a.i. / cm^2 leaf
chlorpyrifos	Dursban 4	480 g l^{-1} EC	720	-
cypermethrin	Ambush C	100 g l^{-1} EC	30	-
diflubenzuron	Dimilin Flo	480 g l^{-1} SC	144	0.316
fenoxycarb	Insegar	250 g l^{-1} WP	150	0.316
tebufenozide	Mimic	240 g l^{-1} SC	288	-
lufenuron	Match	5 % w/v EC	30	0.316
Compound A	-	240 g l^{-1} SC	-	1.440

Dose-response bioassay

In 1999, leaf bioassays were done using computer controlled spraying apparatus (Burkard Manufacturing Co. Ltd, Rickmansworth, Herts, UK) to apply 1.5 µl of insecticide per cm^2 of leaf surface. Discs of strawberry leaf (5 cm^2) were sprayed with the insecticides diflubenzuron, fenoxycarb, experimental compound A or lufenuron in distilled water. A range of concentrations from 0.01 to 10 times the standard, spaced logarithmically (see Table 4), and a water only control were tested, with 24 replicates per treatment.

Leaf discs were sprayed and changed twice weekly from 24 June, and weekly from 20 July (as feeding decreased). Sprayed leaf disks were air-dried and transferred to separate 9 cm Petri dishes lined with damp filter paper. One adult vine weevil was introduced into each dish. The Petri dishes were wrapped with Parafilm to prevent the leaf from desiccating and maintained at 20°C with a 16:8 h photoperiod. Weevils were assessed for mortality and feeding activity before each new leaf disc was introduced. The amount of leaf material eaten was assessed into categories as 0, ≤1.25 cm^2, ≤2.5 cm^2, ≤3.75 cm^2 or ≤5 cm^2 and the old leaf was then removed. Eggs that had been laid were counted and

Effects of insect growth regulators on vine weevil (*Otiorhynchus sulcatus*) egg production and viability

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ABSTRACT

Vine weevil (*Otiorhynchus sulcatus*) adults were exposed to IGR insecticides with three different modes of action: the chitin synthesis inhibitors (CSI) lufenuron and diflubenzuron, the ecdysone agonists tebufenozide and an experimental compound A, and the juvenile hormone analogue fenoxycarb, by leaf feeding bioassays in the laboratory. The CSIs reduced egg production and viability, especially lufenuron, but the juvenile hormone analogues and ecdysone agonists had no effect. Evaluation of a wider range of benzoylurea CSI insecticides in the laboratory and field is recommended with the aim of developing a new approach to control of the pest.

INTRODUCTION

Insect growth regulator (IGR) insecticides, especially modern highly active compounds, may have useful effects on vine weevil reproduction that have not been investigated and could be exploited for control of the pest in the field. Chitin synthesis inhibitors (CSI) affect many insect orders, including Coleoptera. The CSI diflubenzuron, an older compound with comparatively low activity, has already been shown to adversely affect vine weevil oviposition and egg viability when adults feed on treated foliage, but does not cause adult mortality (Zepp *et al.*, 1979; Sol, 1985). Since then a number of new benzoylurea CSI insecticides have been developed, but have apparently not been investigated for their effects on vine weevil. Juvenile hormone analogues (JHA), such as fenoxycarb, are also known to affect vitilinogenesis in adult insects and are ovicidal in some insects (Grenier & Grenier, 1993). Ecdysone agonists (EA) are mainly toxic to lepidopteran pests (Dhadialla *et al.*, 1998). However, tebufenozide has been shown to inhibit egg laying, but not egg viability in the coccinellid *Leptinotarsa decemlineata* (Smagghe & Degheele, 1994). In the work reported here, laboratory bioassays were done to investigate the effects on egg production and viability following exposure of adult vine weevils (*Otiorhynchus sulcatus*) to the three different classes of IGRs. The aim of the work was to investigate the possibility of an alternative approach to vine weevil control.

MATERIALS AND METHODS

Representatives of each class of IGR were chosen for the tests as follows 1) CSI - diflubenzuron, lufenuron 2) JHA – fenoxycarb 3) EA – tebufenozide, experimental compound A. For all experiments, weevils were collected from blackcurrant plots in Kent. In 1998 adult weevils were collected in August after the start of egg laying, and maintained on *Euonymus* sp. at 21°C with a 16 h light: 8 h dark photo-period before the

The prototype electrical barriers tested, each enclosing a small area (0.3 x 0.3 m) around individual courgette plants, were effective in protecting plants from severe slug damage that killed almost all plants not protected in this way. In this case, the majority of damage appeared to be caused by slugs that were not present in the soil within the 0.3 x 0.3 m square centred on the plant when barriers were inserted. The plants outgrew the barriers after a few weeks, but by then the plants were sufficiently robust to withstand slug attack. Where courgette plants were surrounded by electrical barriers, additional protection was provided by nematode treatment and this was just as effective when applied to the small area of soil within each barrier as when applied as an overall treatment to soil. Such targeted application to areas within barriers could considerably reduce the cost of nematode treatment.

Results of the experiment in The Netherlands indicate that treatment with common salt could be of potential value for control of slug damage in asparagus, a crop that tolerates high salt concentrations in the soil. The combination of nematodes with salt could be particularly useful as it could permit a lower concentration of salt to be used.

ACKNOWLEDGEMENTS

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Nematodes for control of slugs in lettuce, Switzerland

Application of nematodes resulted in a significant reduction of *D. reticulatum* over a period of three weeks (Fig. 3). By contrast, the early nematode treatment had no significant effect on numbers of *A. lusitanicus* (Fig. 3) or *A. distinctus* and had no effect on feeding damage to lettuce (results not shown).

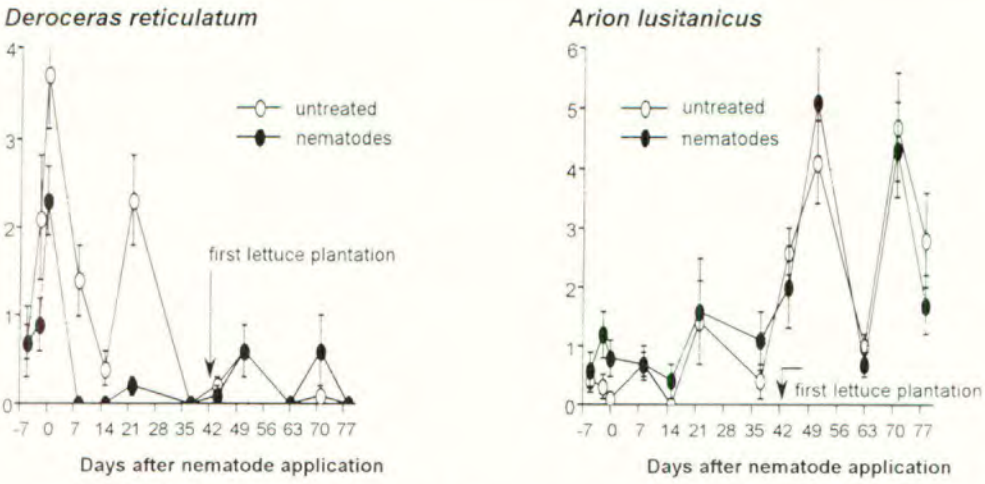

Figure 3. Numbers of *D. reticulatum* and *A. lusitanicus* trapped in plots with early nematode application and in the untreated plots (mean ±SE), Switzerland.

Salt and nematodes for slug control in asparagus, The Netherlands

Applications of salt at 1000 kg/ha (Treatment 2), salt at 500 kg/ha + nematodes (Treatment 3) and metaldehyde (Treatment 4) resulted in a significantly lower percentage of shoots damaged by slugs (6.2%) than the untreated shoots (14%). Salt applied four times at 500 kg (Treatment 1) did not show any difference from the untreated plots. Product quality was not different between treatments 1 to 4, but the untreated had significantly more shoots in the lowest quality class.

DISCUSSION

Slug-parasitic nematodes gave positive results for control of slug damage in a range of crops in The Netherlands, north west Spain, and the UK, and for control of snail damage in strawberries (southern France), showing that this nematode is a versatile biocontrol agent that can be used successfully throughout Europe. Previous field studies have shown that this nematode is effective in the UK (Wilson *et al.*, 1994a,b, 1995), The Netherlands (Ester & Geleen, 1996) and Switzerland (Speiser & Andermatt, 1996). It was particularly interesting that the present work has demonstrated that *P. hermaphrodita* was capable of reducing damage under field conditions in north west Spain and in southern France, despite warmer conditions than in previous field tests carried out in northern Europe. However, in Switzerland nematodes did not reduce numbers of *A. lusitanicus* and *A. distinctus* and, in Spain, manure application a few days before soil was treated with nematodes appeared to render them ineffective.

($P < 0.05$). For the first two weeks there were no significant differences, but from week three to week five, damage was significantly lower on nematode-treated than untreated plots. Overall, 22 of 24 plants protected by electrical barriers survived to produce fruits. In contrast, only 2 of 48 plants without barriers survived and only 1 of 24 plants surrounded by wool fibre matting survived. Because only three plants without electrical barriers survived to produce fruit, analysis of the effect of nematode treatment on slug damage to courgette fruit was confined to only those plants surrounded by electrical barriers. On average, slugs damaged significantly fewer courgette fruits on nematode-treated plots (1.9% on overall nematode treatment, 2.5% for patch nematode treatment) than on untreated plots (4.2%).

Nematodes for control of slug damage in Brussels sprouts, North West Spain

Results are summarised in Figure 2. Plants in the untreated plots were damaged significantly more than plants in the metaldehyde treatment and the nematode surrounding-3 treatment, on days 1, 3 and 7. Nematode centre-3 was significantly different from untreated plots on days 1 and 3, but not on day 7. On day 14, only metaldehyde was significantly different from the untreated, and on day 21 and 28 no treatment was significantly different from the untreated. From day 14 onwards, the most severely damaged plants were in the manure + nematode plots, and significant differences in damage existed between this treatment and nematode centre-3 and nematode surrounding-3 on days 21 and 28.

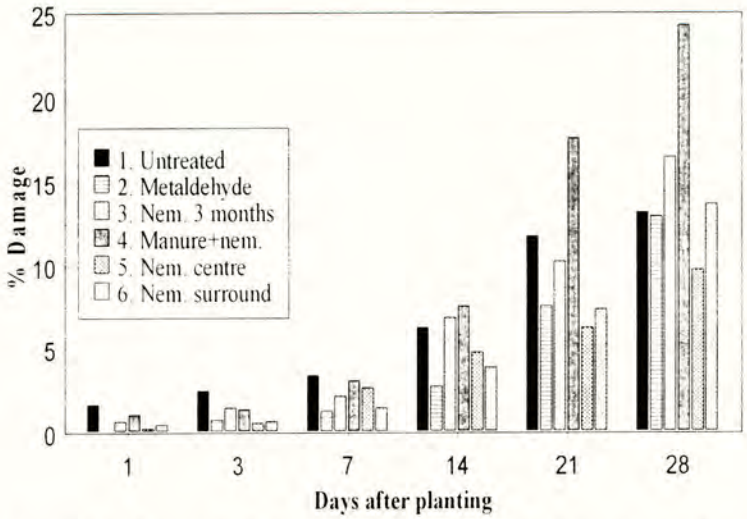

Figure 2. Mean % leaf damage to Brussels sprouts in field trial in north west Spain.

Nematodes for control of snail damage in strawberry cultures in southern France

When damage was assessed at the immature fruit stage, both the nematode treatment and the metaldehyde treatment were found to be effective in protecting the fruit and were not significantly different, with 4.9% and 3.5% of fruit damaged, respectively. In the untreated plots, significantly more fruit were damaged (41.5%), some fruit were destroyed and many showed some deformity, indicating previous snail feeding.

common species found near the experimental fields, were added and allowed to acclimatise for 6 hours. The plots were then treated and covered by the polythene tunnel. Treatments were assessed after 3 weeks, when the fruit was semi-matured.

Nematodes for control of slugs in lettuce, Switzerland

A field experiment with lettuce planted on 12 May 1999 was set up in Frick to test effects of early nematode application (31 March) on numbers of *Deroceras reticulatum*, *Arion lusitanicus* and *Arion distinctus*. There were nine replicates of treated and untreated plots. Slugs were trapped before and after nematode application on 12 occasions from 26 March to 16 June, using flowerpot saucers baited with cucumber.

Salt and nematodes for slug control in asparagus, The Netherlands

This field trial was done in a green asparagus crop at Oudkarspel. Each plot consisted of one row of 5 m, with five replicates of each treatment: (1) salt (NaCl) (500 kg/ha) applied four times, (2) salt (1000 kg/ ha) applied four times, (3) salt (500 kg/ha) + nematodes (150,000/m^2), each applied twice. (4) metaldehyde pellets (7kg/ha) applied four times, and (5) untreated. Treatments 1, 2 and 4 were applied on 9, 15, 22 and 29 April. In treatment 3, salt was applied on 9 and 22 April and nematodes on 15 and 29 April 1999.

RESULTS

Use of nematodes in combination with electrical barriers to protect courgettes

Damage to leaves and stems was considerably less ($P < 0.001$) on plants surrounded by electrical barriers than on plants surrounded by fibre mat or plants without barriers (Figure 1).

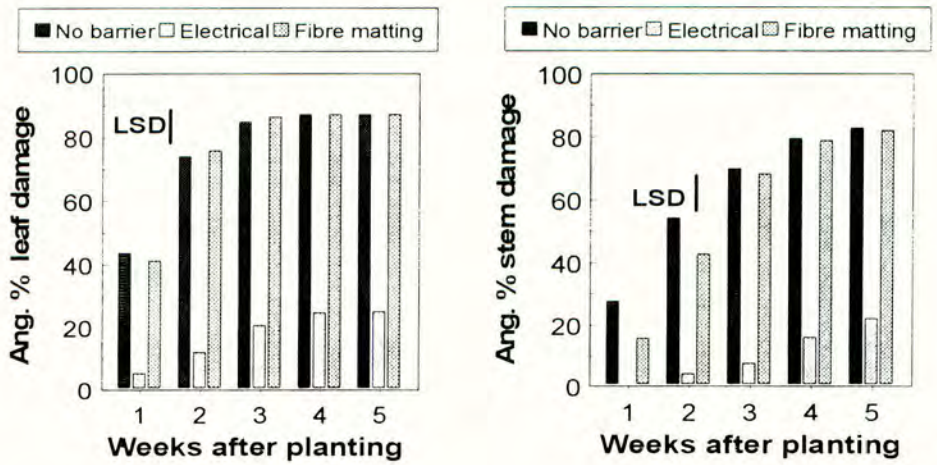

Figure 1. Slug damage to courgette plants without barriers, or surrounded by electrical barriers or fibrous collars (LSD = Least Significant Difference).

Stem damage was influenced by an interaction between nematodes and time after treatment

control, which may be of potential value to horticultural producers, especially organic growers who are unable to use molluscicidal pellets. The work described here is part of an EU project.

MATERIALS AND METHODS

Unless stated otherwise, nematodes were applied at the standard recommended rate of 300,000/m^2 (3 x 10^9/ha) and the metaldehyde pellets used were Metarex (De Sangosse). Damage by slugs or snails was estimated to the nearest 5%.

Use of nematodes in combination with electrical barriers to protect courgettes, UK

This experiment in a polythene tunnel at IACR-Long Ashton, had a total of 24 plots arranged in four randomised blocks. Each plot (1.75 m x 2.5 m) was separated by barriers. Four courgettes were planted per plot on 8 April 1999. In three plots per block, each plant was surrounded by a physical barrier of dimensions 0.3 x 0.3 m. Two plants per plot were surrounded by a square mat of wool fibre (Soltech Ltd). The other two plants were each surrounded by prototype electrical barriers (Snailaway Ltd). Each barrier was inserted into the soil to a depth of 10 cm and extended to a height of 10 cm above ground level. The upper part of the outer wall of each electrical barrier consists of two horizontal conducting bands, with the upper band overhanging the lower band, but not in contact with it. Each band was connected to one of the terminals of a 9 volt battery. Under normal circumstances, no current flowed between the negative and positive terminals. However, when a slug or snail attempted to cross the bands, it completed the electrical circuit and the resulting 9-volt charge was sufficient to kill it or at least make it turn back. Each plot received one of three nematode treatments: (1) none (2) nematodes as an overall drench, (3) nematodes applied only to the 0.3 m x 0.3 m square centred on each plant.

Nematodes for control of slug damage in Brussels sprouts, North West Spain

This trial, near Santiago de Compostela, was done in 36 plots arranged in a completely randomised design, each 4 m x 4 m, with a central cultivated area 2 m x 2 m surrounded by grass sward. Nine Brussels sprouts seedlings were planted on 19 August 1999. Treatments were: (1) untreated; (2) metaldehyde pellets (Caraquim, Massó Chemical Industries; 5% a.i.) broadcast at recommended rate (30 kg/ha); (3) nematode 3 months: nematodes applied in the central plot area 3 months before planting; (4) manure+nematode: 2 litres/m^2 of cattle manure (20 m^3/ha) applied to the central area of the plots seven days before planting, then three days before planting, nematodes applied to the same area; (5) nematode centre-3: nematodes applied 3 days before planting to the central plot area; and (6) nematode surrounding-3 nematodes applied 3 days before planting to the grass area around the planted area.

Use of nematodes to control snail damage in strawberries, southern France

A field trial was established in early May 1999 in a commercial strawberry farm near Maugio, Languedoc Roussillon, to investigate the efficacy of nematodes applied at the time when plants were covered with polythene tunnels. Experimental enclosures were fabricated by enclosing a 1 m length of polythene tunnel at each end with a snail fence. Enclosed plots were examined for the presence of living snails, which were removed. The experimental treatments were: (1) nematodes, (2) metaldehyde pellets (10 kg/ha) and (3) untreated. Each treatment was replicated four times. In each plot 20 juvenile *Theba pisana* (10 mm), the most

The use of slug-parasitic nematodes and other techniques for control of slug and snail damage in horticultural crops

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ABSTRACT

Results in 1999 are described, of a study to establish principles for using nematodes, *Phasmarhabditis hermaphrodita*, alone and with other methods, for control of slug and snail damage in horticultural crops. Nematodes gave positive results in asparagus, Brussels sprouts, courgettes and strawberries in The Netherlands, north west Spain, the UK and southern France, respectively. Failure of nematode biocontrol in lettuce in Switzerland was associated with the presence of *Arion lusitanicus*. Novel electrical barriers and, for asparagus, application of common salt appear to be promising control techniques, either alone or with nematodes.

INTRODUCTION

Slug and snail pests are difficult to control in a wide range of horticultural crops. Many crops are extensively damaged despite intensive use of molluscicides, but no adequate control measures are currently available. Damage can result in seedling losses. However, in crops such as asparagus, lettuce, Brussels sprouts and strawberries, slug damage to harvested produce and/or the presence of slugs or faeces in the harvested crop, result in loss of product quality. A nematode, *Phasmarhabditis hermaphrodita*, discovered as a slug parasite in the UK (Wilson *et al.*, 1993), has been developed as a biocontrol agent for slugs (Glen & Wilson, 1997). This nematode attacks only slugs and snails. It is produced in liquid fermenters, infective juveniles are harvested and formulated, and remain viable for several months under refrigeration. It is sold as a biological molluscicide in several European countries. The nematode formulation is mixed with water and applied to the surface of soil, as a drench or through standard spraying equipment. The work described in this paper focuses on experiments in 1999 on the use of nematodes in five European countries, alone or in combination with other novel methods of

programmes where the product can be used to control damaging pests such as caterpillars, thrips and dipterous leafminers, and still allowing the grower to use biological control methods and pollination by bumble bees. The exact mechanism for the selectivity to each predator type and the bumble bee has not been fully investigated, but reasons could include penetration, intrinsic lack of toxicity to a particular species or group, behaviour, and uptake.

Two key attributes of spinosad include a) exceptional safety to plants and b) low toxicity to many commercially important beneficials, natural predators and pollinators. This makes spinosad ideal for use in Integrated Pest Management (IPM) systems within glasshouses.

CONCLUSION

It can be concluded that spinosad is highly selective to beneficials and pollinators making it an ideal insect control product for use within glasshouse IPM programmes.

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The effects of spinosad on bumble bees under realistic conditions are summarised in tables 2a to 2c.

Tables 2a-2c The effects of spinosad on the foraging behaviour, mortality and brood development of the bumble bee (*Bombus terrestris*) tested under semi-field conditions.

2a) Reduction in foraging activity compared to the control*

Treatment	Day 2	Day 4	Day 6
Untreated	-	-	-
Spinosad	65%	19%	13%
Imidacloprid (systemic)	-57%	-21%	18%
Imidacloprid (foliar)	80%	74%	42%

2b) Mortality at the end of the seven day exposure period

Treatment	Mean Day 7	SD	Abbott	N
Untreated	21%	8%	-	4
Spinosad	20%	13%	-1%	4
Imidacloprid (systemic)	32%	6%	14%	2
Imidacloprid (foliar)	24%	17%	4%	2

2c) Brood development

Treatment	New immature off spring		Total off spring	
	Per bee	Reduction*	Per bee	Reduction*
Untreated	2.0	-	2.3	-
Spinosad	1.2	40%	1.7	27%
Imidacloprid (systemic)	0.8	61%	1.2	49%
Imidacloprid (foliar)	1.3	36%	2.0	15%

*Compared to the control. Negative values indicate better performance than the control.

At two days after application a reduction in flower visiting was observed, however at day 4 and day 6 the spinosad treatment was similar to the control. Mortality in the spinosad treatments was similar to the control treatment throughout the trial. A slight reduction in brood development was noted for spinosad, but this was not statistically significant. Similar safety has also been observed to honey bees (*Apis melifera*). In a large cage study on a flowering *Phacelia* crop conducted to EPPO guideline 170, dry product residues of spinosad applied at rates up to 36 g as/hL in 1500 L/ha of water (equivalent to 540 g as/ha) were harmless to foraging workers and queen and brood (Vinall, 2000).

A high margin of safety was observed for spinosad on a wide range of commercially available natural enemy species, including predatory mites, bugs, lacewings and lady birds. Parasitoids appear to be at risk from applications of spinosad. However by careful use of introduction periods both spinosad and parasitoids may be used in the same IPM programme successfully. Once dry spinosad is safe to foraging bumble bees. This level of selectivity makes spinosad an ideal product for use within glasshouse IPM

Table 1 The effects of spinosad on range of beneficial arthropod species (laboratory, extended laboratory and semi-field tests)

Species	Test	Conditions	Rate	Result
Phytoseiulus persimilis	Semi-field	Adults	4.8 g as/hL 9.6 g as/hL 19.2 g as/hL	Harmless class 1
Amblyseius californicus	Semi-field	Adults	19.2 g as/hL	Harmless class 1
Typhlodromus pyri	Field	Field studies in vines	24 g as/ha 48 g as/ha 96 g as/ha	Safe to weakly toxic
Orius insidiosus	Semi-field	L1/L2	19.2 g as/hL	Harmless class 1
Orius laevigatus	Semi-field	L1/L2	19.2 g as/hL	Harmless class 1
Macrolophus caliginosus	Ext lab	Adults	9.6 g as/hL 36 g as/hL	Harmless class 1 Slightly harmful class 2
	Semi-field	Adults and nymphs Direct spray	9.6 g as/hL 36 g as/hL	Harmless class 1 Recovered to control levels in 14 days
Chrysoperla carnea	Ext lab	L2	36 g as/hL	Harmless class 1
Chrysoperla rufilabris	Lab	Larvae	200 ppm	Harmless class 1
Hippodamia convergens	Lab	Larvae	200 ppm	Harmless class 1
Coccinella 7-punctata	Ext lab	L2	36 g as/hL	Harmless class 1
Encarsia formosa	Semi-field	Adults exposed to treated plants	9.6 g as/hL 36 g as/hL	Harmful class 4 (0DAA) Slightly harmful class 2 (7 DAA) Harmful class 4 (0DAA) Slightly harmful class 2 (7 DAA)
Aphidius colemani	Semi-field	Adults exposed to treated plants	9.6 g as/hL 36 g as/hL	Harmful class 4 (2DBA & 1DAA) Slightly harmful class 2 (7 and 14 DAA) Harmful class 4 (2DBA & 1DAA) Slightly harmful class 2 (7 and 14 DAA)
	Ext lab	Direct spray to mummified aphids	36 g as/hL	Moderately harmful class 3

to run-off and the bugs were fed with pollen. The number of bugs per plant was counted one, three, seven and 14 DAA.

Spinosad was evaluated against larvae of two lacewing species (*Chrysoperla carnea* and *C. rufilabris*) under laboratory conditions. *C. carnea* was exposed to treated pepper leaves and larval mortality and fecundity of the surviving adults was measured. *C. rufilabris* larvae were exposed to glass plates treated with spinosad and mortality assessed after 3 days. The ladybird *Coccinella septempunctata* was evaluated in a similar way to *C. carnea* and *Hippodamia convergens* in the same manner as *C. rufilabris*.

Effects on bumble bees were evaluated under semi-field conditions. Tomato plants were sprayed to run-off with spinosad at 36 g as/hL in the evening and bees allowed to forage on the treated plants the following day. Mortality, flower visiting and effects on queen and brood were recorded. Effects on colony development were determined 9 days after the end of the exposure period. Imidacloprid was applied as a foliar spray or a systemic (drench) treatment as a known harmful reference.

The findings were classified according to EPPO principles (EPPO, 1992) for the bumble bee studies and according to IOBC classifications (Hassan, 1992) for the predators and parasitoids.

RESULTS AND DISCUSSION

The effects of spinosad on a wide range of beneficial arthropods under realistic conditions are summarised in table 1.

Spinosad was shown to have no detrimental effect on *P. persimilis* or *A. cucumeris* at use rates up to 19.2 g as/hL and also to have limited effects on *T. pyri* at rates up to 96 g as/ha.

Rates of 19.2 g as/hL were harmless to the predatory bugs *O. insidiosus* and *O. laevigatus*. Spinosad was harmless to *M caliginosus* at 9.6 g as/hL and only slightly harmful at 36 g as/hL. Where spinosad was applied at 36 g as/hL directly to populations of *M. caliginosus* recovery took place within 14 days. Spinosad was harmless to the ladybird species *C. septempunctata* and *H. convergens* at rates of 36 and 20 g as/hL respectively. Lacewings were similarly unharmed, where 36 g as/hL was harmless to *C. carnea* and 20 g as/hL was harmless to *C. rufilabris*.

Direct application of spinosad at either 9.6 or 36 g as/hL was harmful to adult *A. colemani* foraging in a greenhouse crop as were one day old residues. However when these residues had been allowed to age under glasshouse conditions one and two weeks after application spinosad was only slightly harmful at the same rates. However direct application of spinosad at 36 g as/hL was harmful to pupal wasps within mummified aphids. Spinosad at either 9.6 or 36 g as/hL was harmful to adult *E. formosa* foraging on treated plants on the day of application. One week old residues of spinosad at the same rates were only slightly harmful.

with a variety of resistance mechanisms, giving spinosad an excellent fit in resistance management programs.

MATERIALS AND METHODS

A 480 g as/L SC formulation of spinosad (TRACER - Trademark of Dow AgroSciences LLC) was used as a representative formulation in all studies reported in this paper. Spinosad is typically produced as a suspension concentrate formulation.

A wide range of beneficial species were investigated. All studies were replicated and included an appropriate toxic and control references treatments.

In order to test effects on *Phytoseiulus persimilis* young bean plants infested with two spotted spider mite (*Tetranychus urticae*) were sprayed to run-off. The plants were trimmed to one leaf and infested with five adult female predatory mites. The experiment was conducted under glasshouse conditions. Live predatory mites were counted six days after infestation. For *Amblyseius californicus* the same method was employed. Two field trials in vines were set up in France to test the selectivity of spinosad to *T. pyri* and the number of mites per 25 leaves per plot was counted up to 41 days after application (DAA).

The aphid parasitoid (*Aphidius colemani*) was investigated under extended laboratory conditions by direct application of spinosad to mummified aphids on pepper plants. Wasp emergence and fecundity was measured. A semi-field trial on pepper plants under greenhouse conditions investigated the introduction times for *A. colemani* before and after applications of spinosad. Wasps were released to forage on plants infested with aphids two days before application and 1, 7 and 14 DAA. The performance of the parasitoids was measured by counting the number of parasitised aphids per plant seven to twelve days after wasp release. A semi-field trial on tomato plant under glasshouse conditions was employed for the white fly parasitoid *Encarsia formosa*. Plants infested with white fly were sprayed with spinosad and wasps released on the day of application and after one week. Two weeks after the release of the wasps the number of parasitised white fly per plant was counted.

Three species of predatory bug were investigated; *Orius insidiosus*, *O. laevigatus* and *Macrolophus caliginosus*. For the two *Orius* species the same method was used. Bean plants, approximately 20 cm tall, were sprayed to run-off. When dry, each plant was infested with five first or second instar nymphs and fed on pollen. The studies were performed in a glasshouse. The number of live *Orius* spp. was counted 4 DAA. Two studies were performed on *M. caliginosus*. In the first study mature pepper plants were sprayed to run-off and the plants were aged under glasshouse conditions and leaves were removed for bioassay with adult bugs on the day of application and 2 and 8 DAA. The bugs were fed on pollen and mortality was assessed after two days exposure. In the other study pepper plants (approximately 30-40 cm tall) were infested with a mixed population of adults and nymphs under glasshouse conditions. The plants were sprayed

Spinosad - a naturally derived insect control agent with potential for use in integrated pest management systems in greenhouses

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ABSTRACT

When used according to good horticultural practice, spinosad was shown to be compatible with predatory mites (*Typhlodromus pyri, Phytoseiulus persimilis* and *Amblyseius californicus*), predatory Heteroptera (*Orius insidiosus, O. laevigatus* and *Macrolophus caliginosus*), Coccinellidae (*Hippodamia convergens* and *Coccinella septempunctata*) and Neuroptera (*Chrysoperla carnea* and *C. rufibularis*). Parasitic Hymenoptera were sensitive to spinosad, however toxic effects were short lived due to the low persistence of spinosad. Consequently species such as *Aphidius colemani* and *Encarsia formosa* can be introduced to protected crops soon after application (1-2 weeks). Semi-field studies were also conducted on honey and bumble bees. Spinosad was applied when bees were not flying. Bees were allowed to forage on the treated plants when spray deposits had dried. The findings from these studies showed that spinosad was completely safe to foraging worker bees. No significantly adverse effects on queen or brood were observed on either species. It can be concluded that spinosad is highly selective to beneficials and pollinators making it an ideal insect control product for use within glasshouse IPM programmes.

INTRODUCTION

Spinosad is a novel insect control agent derived by fermentation of the Actinomycete bacterium, *Saccharopolyspora spinosa.* The active ingredient is composed of two metabolites, spinosyn A and spinosyn D (Thompson *et al.*, 1997). Spinosad controls many caterpillar pests in vines, pome fruit and vegetables (including tomatoes and peppers), thrips in tomatoes, peppers and ornamental cultivation and dipterous leafminers in vegetables and ornamentals. Application rates vary between 4.8 and 36 g of active substance per hectolitre (g as/hL) depending on the target pest (Bret *et al.*, 1997).

The mode of action of spinosad is completely novel, making it a useful resistance management tool. A novel mechanism of activity on the nicotinic acetylcholine receptors was identified as the primary cause of death (Salgado, 1997). Spinosad has additional effects on gamma-aminobutyric acid or GABA receptors, although it has not been shown that these effects contribute to insecticidal activity. The action of spinosad on nicotinic receptors is unique in comparison with traditional insecticides and is at a different site to nicotine and imidacloprid. Studies so far have found no cross-resistance

species can be utilized as natural, native plant protection resources. Their impact on TSM can be strengthened by releases of commercially available phytoseiids. However, many details still need to be studied for better understanding of the dynamics of the predator, prey and host plant. The effect of different phytoseiid species, prey preferences and interspecific competition, methods to enhance natural predators and the necessity, timing and amounts of the releases still need to be clarified in different conditions.

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covers (Genini *et al.*, 1991; Croft *et al.*, 1993). Irrigation and maintenance of vegetation in the alleys could diminish the effect of low humidity.

Predators were introduced twice when TSM infestations achieved 70-90 % of sampled leaves (16-49 mobile stages/leaf) (Table 1, cv. Heritage). Numbers of the generalist predator *A. cucumeris* increased more than those of the other introduced species. Together with naturally occurring phytoseiid predators, *A. cucumeris* reduced TSM numbers, but final TSM numbers were about the same as in the plots where no introductions were made.

The oligophagous *A. californicus* gradually increased after the introduction. Compared with the check block, TSM populations in plots with *A. californicus* decreased towards the end of the season. The specialised predator *P. persimilis* established and developed a small population. *A. californicus* and *P. persimilis* are useful species for augmentative release strategies. *P. persimilis* is a well-known TSM predator, but the release must be timed accurately to coincide with spider mite increase.

Switzerland

TSM populations in open organic plantations in 1998 and 1999 were too low to cause severe damage, with maximum densities of only 9-11 mobile mites/leaf in late summer. In both years, naturally occurring phytoseiids were able to prevent TSM populations increasing to harmful levels. *E. finlandicus* (76 % of the predatory mites identified) and *A. andersoni* (24 %) were found at higher density (max. 1 mobile stage/leaf) on summer-fruiting cv. Nootka than on the cv. Autumn Bliss (max. 0.2 mobile stage/leaf). In January 1999, overwintering TSM were found in splits of young primocanes. The covered IP-plantations were intensively fertilised and irrigated, resulting in higher numbers of TSM than at the organic farms. Naturally occurring *A. andersoni* (max. 2/leaf) were unable to prevent TSM increase (max. 70 mobile stages/leaf), especially at higher temperatures.

In 1999, the release of *P. persimilis* on cv. Autumn Bliss resulted in about 36 % reduction of TSM on the lower part of the plants, and 16 % reduction on the upper part. This could be due to the higher, and thus more favourable, humidity in the lower part of canes. After the release of *P. persimilis* and *A. cucumeris* on cv. Glen Ample, only low numbers of these predators could be found. *P. persimilis* releases resulted in better control (67 %) than those of *A. cucumeris* (31 %).

Conclusions

The number of mites in the terminal leaflet can be converted to the number on the whole leaf and the mite occupation percentage to numbers per leaf or leaflet. The percentage of the leaves or terminal leaflets occupied by adult TSM and phytoseiids can be used as a practical method of monitoring population density, as proposed by Mariethoz *et al.* (1994). However, it is likely that raspberry can tolerate high numbers of TSM without significant yield reduction, as no reductions in yield were detected in trials in Canada where mite numbers achieved 100-300/leaflet (Raworth, 1989).

The surveys revealed differences in the population dynamics of TSM and phytoseiids, and the species composition of naturally occurring phytoseiids between the geographic areas. Natural phytoseiid populations were the key factors in spider mite management in all areas, and several

Italy

All fields were infested by mixed populations of TSM and the yellow spider mite *Eotetranychus carpini* (YSM). YSM females started to lay eggs in April but caused less damage than TSM. YSM has a lower reproductive rate than TSM on raspberry, and its feeding induces symptoms less readily than does TSM feeding. *Amblyseius andersoni* was wide spread and the most abundant predatory mite (99 % of identified phytoseiids). *A. andersoni* and YSM started to colonise leaves on fruiting canes of cv. Tulameen soon after bud burst in April.

The ability of *A. andersoni* to exploit alternative food sources, e.g. YSM and pollen, is an important feature promoting control of TSM. When TSM females left the overwintering sites later in the season and started to colonise leaves, phytoseiids had already started to reproduce. *A. andersoni* was able to feed on TSM females before they started to lay many eggs. Phytoseiid numbers peaked in the middle of the season. The prey/predator ratio was <1 for almost the whole season and no damage was recorded at these low mite densities. The prey/predator ratio was >1 on new canes at this site, but predation was sufficient to suppress spider mite populations. Other predators (*Scolothrips* sp. thrips, *Feltiella* sp. midge larvae, *Orius* sp. bugs, *Stethorus* sp. beetles, and *Chysoperla carnea* lacewing larvae) completed the control at the end of the season. However, in the middle of the season, natural phytoseiid populations drastically decreased, probably due to the effect of high temperature and low humidity in July.

TSM developed more conspicuous populations on primocanes than on the fruiting canes. In response to the TSM infestation on primocanes, *A. andersoni* numbers increased more than they did on fruiting canes (Table 1, cv. Tulameen). Natural phytoseiid populations on primocanes also increased at the end of the season, probably because of migration from fruiting canes where populations decreased.

Table 1. Maximum numbers/leaf of mobile stages and eggs of all spider mites (SM) and introduced and native mobile phytoseiids (PH) in Italian experiments, 1999. On cv. Heritage, artificial introduction of TSM on 25 July, except the natural check blocks. Numbers of introduced phytoseiid species are in brackets.

Cultivar	Block/Introd.	Introd. dates	Max. SM(mobile)	Date	Max. SM(eggs)	Date	Max. PH(mobile)	Date
Tulameen	Fruiting canes	-	15.7	22.9.	38.1	3.8.	4.4	16.6.
	Primocanes	-	15.7	22.9.	37.9	3.8.	4.8	16.6.
Heritage	Natural check	-	25.5	28.9.	87.1	24.8.	3.3	28.9.
	Infested check	25.7.	52.9	13.9.	84.4	24.8.	6.2	13.9.
	A. cucumeris	18-25.8.	30.0	12.8.	53.5	12.8.	8.2 (3.4)	13.9.
	A. californicus	18-25.8.	30.0	12.8.	105.5	24.8.	9.2 (1.1)	28.9.
	P. persimilis	18-25.8.	53.9	12.8.	73.8	24.8.	8.0 (1.3)	13.9.

On cv. Heritage, TSM and *A. andersoni* colonised leaves soon after planting. The prey/predator ratio was >1, but in combination with predatory insects, *A. andersoni* suppressed TSM and YSM populations. Natural phytoseiid populations drastically decreased in the middle of the season, probably due to the effect of high temperature and low humidity under the polythene

Ample. *P. persimilis* (10/rm) was introduced on autumn-fruiting cv. Autumn Bliss in mid-July. Plantations were sampled from mid-May to end-October, and in January 1999 for overwintering sites of TSM and phytoseiids.

RESULTS AND DISCUSSION

Finland

In 1998, TSM overwintered in all plantations, but only low numbers were found during the season, due to cool weather. However, at one plantation, 100 % of the primocane leaves of cv. *Preussen* were infested by TSM at the end of the season, whereas 40 % of those of cv. *Muskoka* at the same site were infested. After the very cold winter, few TSM were found in 1999 before late June, even in the fields with high numbers in the previous autumn. During July, TSM numbers increased to 100-200 mobile stages and eggs/leaflet, causing severe leaf symptoms.

Released predator species were not found in any samples in 1998, even at sites with higher TSM populations. In half of the treated fields, the final TSM occupation was c. 30-70 % less than in control blocks, but no effect was found in three other fields. In 1999, predators were released in mid-June when TSM prey was not yet available. In spite of low TSM densities, 25-50 % control was observed at three *T. pyri* release sites, although only a few specimens of introduced phytoseiid species were found. Introductions later in the season might have been more successful. However, as low night temperatures or even frost often occur in Finland in late August, at least *P. persimilis* may not be able to reproduce enough then to control TSM.

In 1998, a native phytoseiid, *Phytoseius macropilis*, was found in higher densities than TSM in an unsprayed field (Fig. 1). *P. macropilis*, like many other phytoseiids can feed on pollen, and in spring 1998, birch pollen was richly available, allowing overwintered phytoseiids to reproduce on raspberry. In spring 1999, fewer predators had survived the hard winter, and birch pollen was not available because of poor birch flowering. TSM did not survive the winter and was not available for food (Fig. 1). The presence of native *P. macropilis* and alternative food may thus prevent harmful increase of TSM on unsprayed raspberry. Introduced foreign phytoseiids may not be effective enough in unprotected, open raspberry plantations.

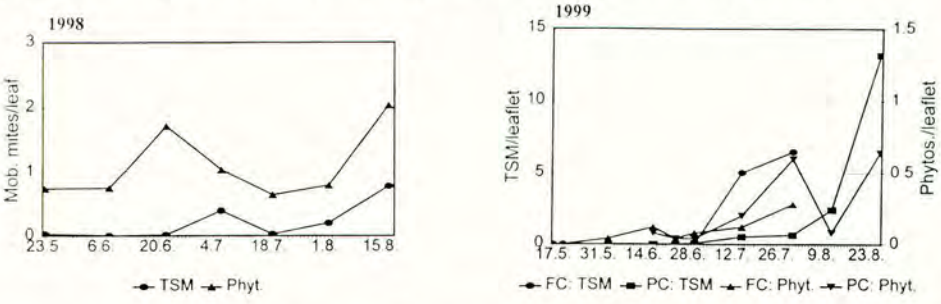

Figure 1. TSM and phytoseiid mites (Phyt.) on an unsprayed raspberry field. In 1998 first three inspections on whole leaves of fruiting canes, later on primocanes. In 1999 terminal leaflet inspections, with five parallel inspections on fruiting canes (FC) and primocanes (PC). *P. macropilis* covered 70 (1998) and 81 % (1999) of phytoseiids.

first' and 'banker plant' techniques, using phytoseiids and *Feltiella acarisuga* (Diptera: Cecidomyiidae) have been studied in protected raspberries in the UK (Labuschagne & Wardlow, 1999). Field experiments using *P. persimilis* have also been performed in Finland (Tuovinen, unpubl.).

TSM populations were monitored in growers' plantations in Finland, Italy and Switzerland. The same methods were used to evaluate the occurrence and effectiveness of phytoseiid predators, and to identify climatic and cultural practices influencing their effectiveness in these countries.

MATERIALS AND METHODS

Mobile stages and eggs of TSM and phytoseiid mites were counted separately on whole leaves in 1998 and on whole leaves or on terminal leaflets in 1999. Samples consisted of 20-50 randomly selected leaves or terminal leaflets per replicate, collected from nodes 1 m above ground level on fruiting canes, or < 0.5 m on primocanes. Separate fruiting cane and primocane leaf samples were collected at 2-4 week intervals. Phytoseiid mites were released either in spring, after the first TSM infestation was found, in early June or later in summer.

Finland

Trials with cvs Muskoka, Ottawa or Preussen were made in South and Central Finland, using eight plantations in 1998 and five in 1999, including one replicated blocks trial site. 1998 was cold and rainy, but in January a warm period broke dormancy in raspberry, resulting in severe winter damage at many sites. 1999 was dry and warm but a very cold period in January-February caused frost injuries. Predatory mites were introduced in May-June in seven plantations in 1998 and in four in 1999. The rates of released phytoseiid mites in 1998 were: *T. pyri*, 3/rowmeter (rm), *P. persimilis*, 50/rm and *A. cucumeris*, 100/rm, and 8-18/rm, 50/rm and 225/rm, respectively, in 1999. In 1998, *T. pyri* was released at lower rates because the number of live mites in cotton bands was less than expected. Leaves were sampled at c. 4 wks intervals.

Italy

Trials were conducted in two plantations with four replicates, 500 and 560 m above sea level, at Trentino, northern Italy. At both plantations rows were covered by polythene. The first site was planted in May 1999 with an autumn fruiting cv. Heritage. *P. persimilis*, *A. californicus* and *A. cucumeris* were released on two occasions at rates of 25/rm after an artificial introduction of TSM ('pest in first'). The development of natural populations of tetranychid and phytoseiid mites was followed in another tunnel. At the other site, the development of natural populations of tetranychids and phytoseiids was studied on summer fruiting cv. Tulameen. Whole leaves were sampled at c. 2 week intervals from April (cv. Tulameen) or June (cv. Heritage).

Switzerland

Two trials were conducted in north-eastern Switzerland in two uncovered organic plantations in 1998 and in 1999. Additionally, there were three trials in covered integrated production (IP) plantations in 1999. Predators were introduced in 1999 in two IP-plantations. *P. persimilis* (10/rm) and *A. cucumeris* (260/rm) were released twice in May on summer-fruiting cv. Glen

The role of native and introduced predatory mites in management of spider mites on raspberry in Finland, Italy and Switzerland

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ABSTRACT

Management of spider mites formed part of a recent EU project 'Reduced Application of Chemicals in European Raspberry Production'. Population development of spider mites and predatory mite species on raspberry was studied in 1998-1999 in Finland, Italy and Switzerland. The most important native phytoseiid species were *Phytoseius macropilis* in Finland, *Amblyseius andersoni* in Italy and *A. andersoni, Typhlodromus pyri* and *Euseius finlandicus* in Switzerland. The efficiency of introduced predatory mites (*Phytoseiulus persimilis*, *Amblyseius cucumeris*, *Amblyseius californicus* and *T. pyri*) depended on the initial spider mite population and conditions after their introduction. In Finland, the absence of spider mite prey for introduced predatory mites may have allowed the late population growth of spider mites. In Italy, introduction of *P. persimilis* and *A. californicus* succeeded better and provided long lasting control of spider mites. In Switzerland, native *A. andersoni*, *T. pyri* and *E. finlandicus* were able to provide effective control in an organic plantation.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae; TSM) is an important pest of raspberry, particularly in regions with warmer summers (Höhn *et al.*,1995; Gordon *et al.*, 1997). TSM was included in an EU CRAFT project 'Reduced Application of Chemicals in European Raspberry Production' (RACER), initiated by raspberry growers in six European countries to monitor key pests of raspberry and to manage them with reduced pesticide applications (Gordon & Woodford, 2000).

Control of TSM is largely based on acaricides, but biological control, by manipulating native phytoseiid mites (Acari: Phytoseiidae) or introducing foreign phytoseiids, has been tested in Canada (Wood *et al.*, 1994) and Switzerland (Höhn *et al.*, 1995; Baillod *et al.*, 1996). 'Pest in

resulting in "designer mixes" specially formulated for their pest control properties.

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geraniums a mix of nearly equal parts vermiculite and perlite with little or no coir would simultaneously reduce fungus gnat population growth and promote plant growth.

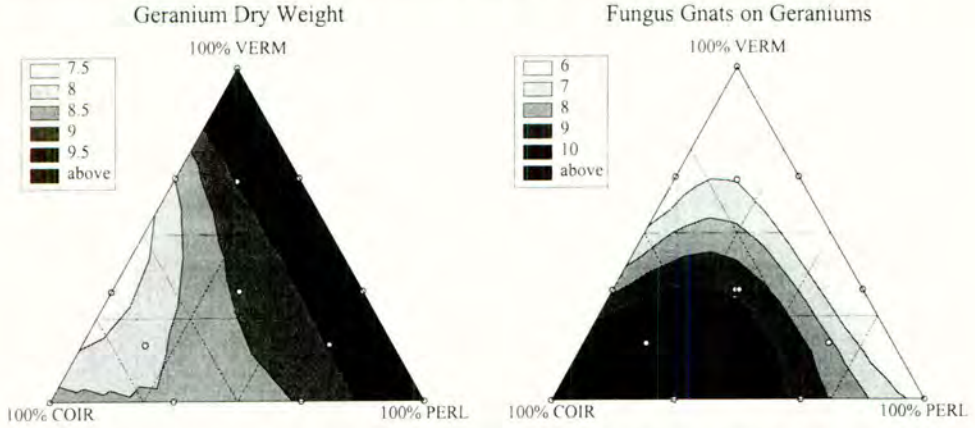

Figure 3. Plant dry weight (g) and number of adult fungus gnats caught on Geraniums

DISCUSSION

The goal of producing quality pest-free crops at a profit continues to present challenges for floriculture operations. Recent research has demonstrated the importance of having the correct *proportions* of nutrients for plant health and defence (Busch & Phelan, 1999; Herms, 2000). Our research extends this idea to include the supporting medium and (probably) its physical characteristics. Although we do not fully understand the causal relationship between fungus gnat population and plant damage, the empirical data we have obtained lead us to believe that survival and feeding behaviour of fungus gnats are in part determined by the water retention of media in the pots.

Our initial hypothesis was that physical properties of the medium, particularly water holding capacity would be important determinants of insect growth and plant health (given that plant nutrition is not a factor in our ebb and flood experimental setup). However, examination of the response surface of water holding capacity to the media components shows little correlation with either plant growth or population growth. To examine this question further we are currently investigating the effect of soil moisture on survival rates of western flower thrips (*Frankliniella occidentalis*) prepupae and pupae in different potting media. We have chosen to continue this work using western flower thrips because the prepupae and pupae are particularly vulnerable stages of the life cycle that spend only 3-5 days on or in the media, which can speed up the turnover of experiments.

Based on our work with fungus gnats, we believe that the potting medium can markedly influence the survival and subsequent population growth of insects with a soil inhabiting stage. The practical result of this work lies in its potential for reducing injury to plants during the vulnerable seedling phase by reducing pesticide use with the attendant reductions in worker exposure and pesticide costs. Application of this technique in practice should increase costs little or not at all because growers frequently mix their own media. We see this approach as

RESULTS

The results of mixture experiments are depicted as triangular graphs showing contours of the response surface. The graphs have three axes: each component (coir, perlite, vermiculite) is largest at the corner and smallest at all points along the opposite face. Any point in the triangle represents the response to the three-way combination of components represented by the perpendicular distances to each corner. Table 2 summarises the fits of the model to the data and Figs. 1-3 show the response surfaces obtained for plant dry weight and second generation fungus gnat adults caught on sticky cards.

Table 2.Response of plant (dry weight) and fungus gnat population growth to potting media

	Response Surface Equation	R^2	F	
Poinsettia	24.8x+21.7y+30.8z+35.1xy+18.4xz-22.5yz-6.72xyz	0.35	4.1	**
Fungus gnats	30.0x+4.27y+24.2z+274xy+259xz+201yz-1772xyz	0.54	8.9	***
Impatiens	1.93x+1.22y+1.25z+1.87xy+2.13xz+0.63yz-2.13xyz	0.24	2.6	*
Fungus gnats	13.3x+5.53y+5.59z+2.54xy+13.4xz-9.19yz+76.8xyz	0.35	4.4	***
Geranium	8.24x+9.78y+10.7z-3.22xy-8.54xz-3.48yz+18.8xyz	0.18	2.3	*
Fungus gnats	56.2x+8.08y+57.4z-77.8xy-42.0xz+14.9yz+134xyz	0.26	3.7	**

x = proportion of coir, y = proportion of perlite, z = proportion of vermiculite in the mixture.

The pattern of response of plant dry weight to the three constituents are qualitatively similar for impatiens and poinsettia but these differed markedly from geraniums. Surprisingly, the response of fungus gnat population growth differed markedly between the three trials, perhaps indicating a more complex relationship between soil condition, plant health and insect survival than our original hypothesis. For both impatiens and poinsettia the presence of coir in the mix apparently favoured plant growth; a mix comprising nearly equal parts of coir and vermiculite with about 15% perlite would minimise fungus gnat population growth while providing the beneficial properties of coir to plant growth. The presence of coir does little to assist geranium growth and its presence apparently favours fungus gnat population growth. For

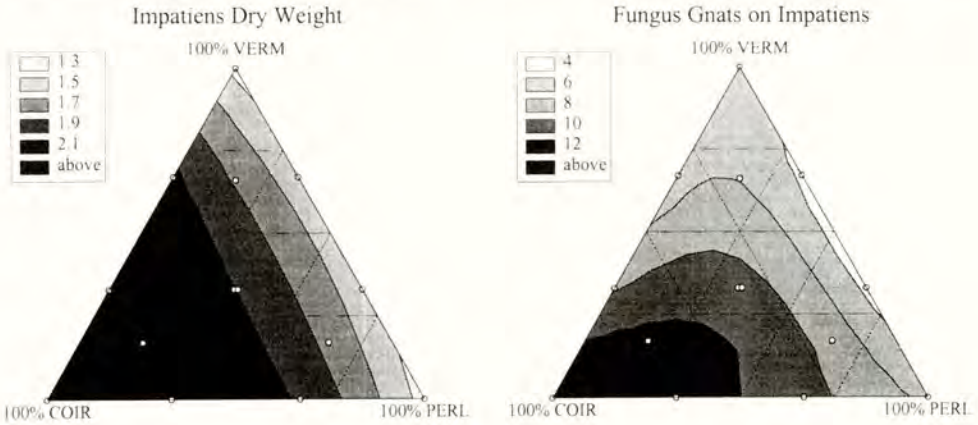

Figure 2. Plant dry weight (g) and number of adult fungus gnats caught on Impatiens

populated the caged pots. The second generation of adults were caught on yellow sticky traps introduced into each cage after 4 weeks. Two weeks later the number of fungus gnats on each card was counted and the plants were removed and their condition assessed by counting the number of leaves, measuring their height and their root and top dry weights. Data of adults caught and plant condition were analysed using the method of mixtures.

Table 1: Mixture combinations used in soil media trials

Combination	% Coir	% Perlite	% Vermiculite
1	100	0	0
2	0	100	0
3	0	0	100
4	67	33	0
5	67	0	33
6	33	67	0
7	0	67	33
8	33	0	67
9	0	33	67
10	66	17	17
11	17	66	17
12	17	17	66
13	34	33	33

A mixture experiment as described was conducted using rooted cuttings of poinsettia (*Euphorbia pulcherrima* var. 'Freedom Red') and another used cuttings of New Guinea impatiens (*Impatiens hawkeri* var. 'Anaea'). A trial using ivy geranium, (*Pelargonium peltatum* var. 'Sybil Holmes') differed from the preceding trials in three ways: the design had 5 blocks instead of 4 (total of 70 pots), adult sampling was started at week 2 and continued weekly for 5 weeks, and the plant condition was assessed by measuring dry weight of the roots, stems and foliage separately.

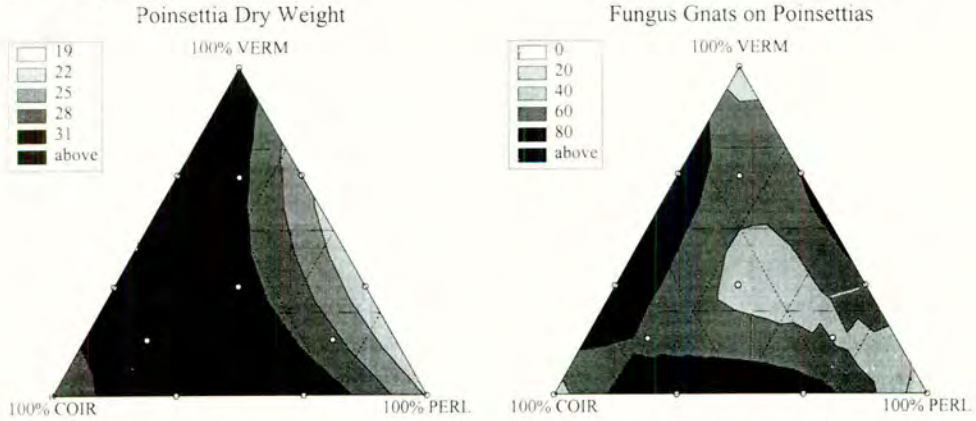

Figure 1. Plant dry weight (g) and number of adult fungus gnats caught on Poinsettias

also been shown that plant health can be manipulated via environmental factors to minimise the effects of insect attack (Herms, 2000). Applying too much fertiliser to young trees can increase their susceptibility to insect attack, apparently because they devote more resources to growth than defence.

The statistical method of mixtures is an experimental procedure for finding a combination of several components of a mixture that produces a desired response in another variable. The big advantage of the mixtures approach is that to determine the optimum combination of three constituents for a desired outcome, say crop yield, many times more experimental units would be needed using a factorial design than using a mixtures design. The method is described in detail by Cornell (1981). We investigated the hypothesis that there is a combination of media components that simultaneously minimises insect pest survival and maximises plant health. By blending potting media using different components it should be possible to construct a medium with desirable physical characteristics that simultaneously promote plant growth and inhibit insect pest population growth. In this paper we report on three studies employing the method of mixtures to investigate the effect of coir, perlite and vermiculite as components of potting mixes on fungus gnat (primarily *Bradysia coprophila* and *B. impatiens*) survival and plant health.

METHODS AND MATERIALS

The method of mixtures assumes the response to depend only on the proportions of the components in each blend, not on the total amount of the components. In practice, this condition is met by holding the total amount of the components in all blends fixed. The important features of mixtures experiments are the definition of total and that the components combine to produce the same total for all treatments. The independent variables or components are usually expressed as a percentage of the total. For example, if $A = B1 + B2 + B3$ where A is the total (100%) and $B1$, $B2$ and $B3$ are expressed as percentages ranging from 0 to 100% and if $B2$ is 100%, then $B1$ and $B3$ are each 0%. Clearly, in mixture experiments the predictor variables (components) are not independent. This is in contrast to a standard experimental design, for example a factorial experiment, requiring a different approach. Instead of analysing mixtures as factors, the approach is a modified polynomial regression in which a response surface is fitted to the dependent variable(s) in order to find peaks or troughs representing the optimum combination of components.

Mixture experiments were conducted using three horticultural crop plants. The methods for all three plants were essentially the same. Rooted cuttings were placed in 16.5 cm pots made up with 13 different mixtures (Table 1). Except as noted, each mixture was replicated 4 times, except the mixture with equal amounts of each media component which was replicated 8 times, for a total of 56 pots. The pots were arranged in a randomised block design on an ebb and flood irrigation table with a constant feed of macro- and micro-nutrients appropriate to each plant. Irrigation frequency was controlled by calibrated soil moisture tensiometers. Two days after establishment, 25 fungus gnat larvae were introduced to each pot and a tight-fitting screen cage was placed over it.

The larvae completed their development, pupated in the soil and emerged as adults that re-

Influence of potting media components on insect survival: a cultural pest control method

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ABSTRACT

The method of mixtures is an experimental procedure in which two or more components are blended together in a range of proportions and a response of interest is recorded for each blend. Using this method we investigated the influence of different combinations of coir, perlite and vermiculite on the survival of fungus gnat (*Bradysia* spp.) larvae in the media. We hypothesised that the water and heat holding capacity of the medium influences survival of the soil-inhabiting stages of insect pests and therefore the growth rate of their populations. Simultaneously, we examined the influence of the potting mix blends on plant condition. Crop plants were poinsettia, New Guinea impatiens and ivy geranium. Comparison of the fitted surfaces for pest survival and plant condition enabled us to determine optimum potting mix formulations with insect control properties for these plants. We found that there is an optimum combination of components for enhancing pest survival and that some combinations not favourable to pest survival were favourable to plant condition. Using potting media to reduce the growth rate of pest populations has obvious benefits in reducing pesticide use and worker exposure. Furthermore, application costs little or no extra as growers frequently mix their own media.

INTRODUCTION

Insect and mite species with one or more soil-inhabiting life stages can be affected by the properties of the medium - moisture, pH, physical structure, etc. Any such species might therefore be a suitable candidate for control by manipulating the physical properties of the medium by adjusting its composition. For example, potting mixes are known to vary in their attractiveness to fungus gnats (*Bradysia* sp.) (Lindquist *et al.*, 1992; Lindquist & Casey, 1994; Lindquist, 1997). Less attractive potting mixes may result in increased plant injury because larvae will feed on plant roots rather than on fungi or other resources. Conversely, more attractive potting mixes can produce large numbers of fungus gnats without any apparent plant injury (Lindquist *et al.*, 1985).

The potting medium also has an important role in plant health so that any manipulation of the medium for beneficial pest control properties must be balanced against potential deleterious influences on plant growth. In fact, the potting medium can have both direct and indirect impact on plant health. For example, there is strong evidence that nutrition influences pest status via plant health (Herms & Mattson 1992; Busch and Phelan, 1999). Very healthy plants can be much less susceptible to injury than slightly less healthy but still marketable plants. It has

based on the numbers of RB trapped before flowering. Although most RB were trapped before flowering at sites in Finland, RB phenology in the other countries was very variable and differed between sites and years (Fig. 2, cf. Sites GB1 and CH5 in 1998 and 1999). Crops will be particularly at risk where large numbers of RB are active during the flowering period. However, trap efficiency may decline when RB are attracted to the flowers and the traps become obscured by dense foliage.

Although fruit damage was positively related to RB numbers at most sites in Scotland and Switzerland, results from Finland were quite different. In 1998, the amount of damaged husks and berries in Finland was generally low, despite large numbers of RB at some sites, but most assessments were made by the growers themselves, and were based on saleable berries. In 1999, husks were sampled, as in the other countries, but unexpectedly large amounts of damage were found at some sites with small numbers of trapped RB (e.g. Fig 2, 1999, site FI/11 in central Finland). Most of the raspberry plantations in Finland were small and surrounded by forests in which wild raspberries are abundant. RB from these sources are thought to have migrated to the plantations when the cvs began to flower and traps were not then as attractive. The results from Switzerland and Scotland suggest that insecticide control will not be required for fresh market raspberries at sites where the total number of RB caught on single plate white sticky traps before flowering is <5/trap. For processed raspberries, a higher threshold of 5-20 RB/trap is suggested.

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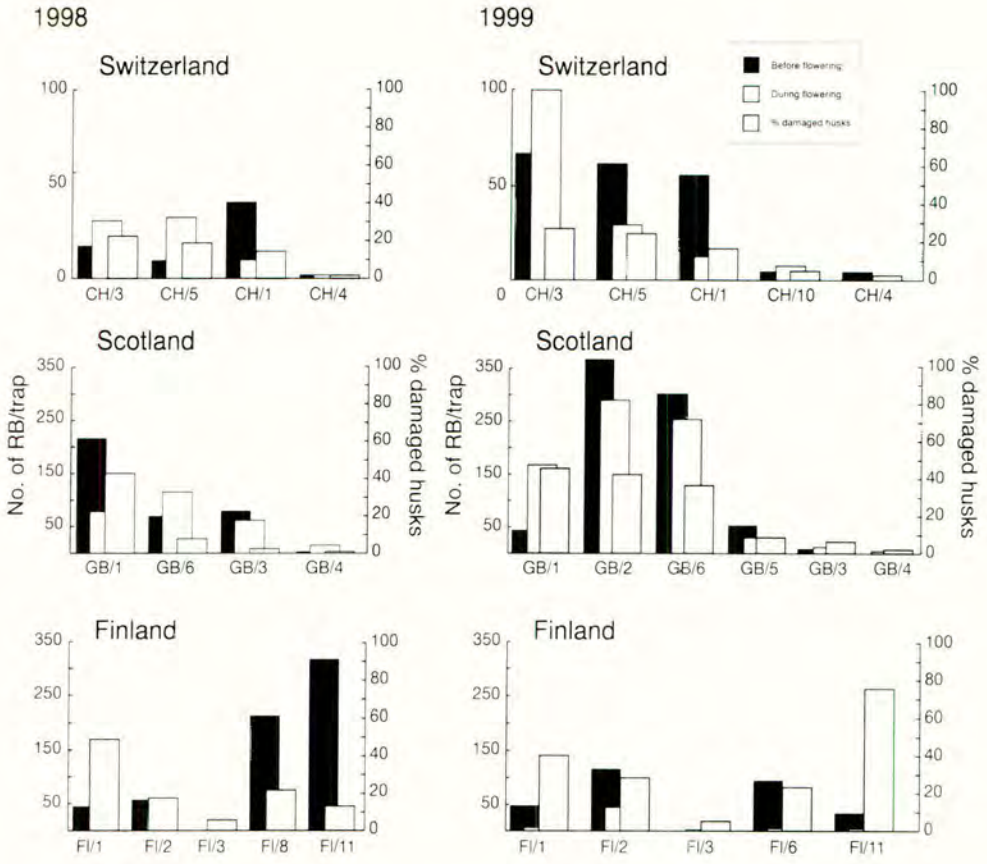

Figure 2. Mean numbers of RB trapped before and during flowering and percentage of damaged husks at sites in Switzerland (CH), Scotland (GB) and Finland (FI).

DISCUSSION

Höhn *et al.* (1995) suggested that there was little risk of infested fruit (<1%) in Swiss raspberry plantations in which the total number of RB caught on white sticky traps between April and mid-July was <5/trap, and that spraying was unnecessary at such sites. The single plates used in the RACER project had half the surface area of the crossed traps used by Höhn *et al.* (1995), and caught almost half as many RB (Schmid & Höhn, unpublished), permitting a direct comparison with control thresholds proposed by Höhn *et al.* (1995). RB lay their eggs during the flowering period, but insecticides must not be applied when pollinating insects are active in the crop. In many European countries, insecticide sprays to control RB are only permitted before flowering. Control thresholds would therefore be most useful if they were

The numbers of RB varied widely between sites and years. The largest numbers were trapped at sites in central Finland in 1998 (Table 2).

Damage assessments

There was a close relationship between damage to the husks (a network of larval channels, usually with one or more larvae/husk) and damaged fruits (drupelets browsed around the rim, near the fruit base) (Fig. 1; $r^2 = 0.928$). Larvae were found far more often in husks than in the detached raspberries. Provided sites were visited less than 1 day after picking, inspection of damaged husks was an efficient indirect method for evaluating fruit damage, and this method was used at all sites in 1999.

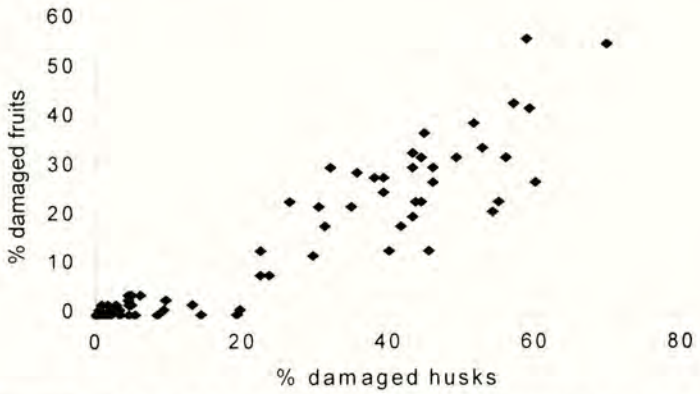

Figure1. Relationship between percentage of damaged fruits and husks in samples from 3 harvests at 7 sites in Scotland in 1999.

Relationships between numbers of adult RB and larval damage to ripe fruit

A preliminary analysis of individual replicate values showed that, within trials, there was rarely a significant positive relationship between the percentage of damaged husks, or number of larvae/husk, and numbers of RB trapped before, and during, flowering (8/20 trials in 1998; 2/19 trials in 1999). Plots of all the replicate values for every site per country showed positive relationships between damage and RB numbers in Scotland and Switzerland, but not in Finland (data not shown). However, the variation between the percentage of damaged husks and numbers of trapped RB was large, and relationships between damage and numbers of beetles before or during flowering varied between years and countries. The number of trapped RB could not, therefore, be used to predict the extent of fruit damage. Fig. 2 shows examples of the mean numbers of RB trapped before and during flowering, and the corresponding percentage of damaged husks. Despite the lack of a close association between numbers of trapped RB and larval damage, there was usually more damage at sites where large numbers of beetles had been trapped. Sites with fewer than 20 beetles/trap before and during flowering usually had less than 5% damaged husks at harvest (equivalent to c. < 0.5% raspberries containing larvae) (Fig. 2).

RESULTS

RB flight activity

The first RB were trapped in late April in Switzerland, and about 1-4wk later in Scotland, and 2-6wk later in Finland, where beetles were not trapped until mid-May or early June (Table 1). Crops in Switzerland and Finland were more widely separated by distance and altitude than those in Scotland, and the various cvs started to flower over a wider range of dates, but RB were usually trapped for at least 3wk before flowering started at each site. The maximum number of RB were usually trapped before flowers opened in plantations in Scotland and Finland, but large numbers were caught during the flowering period in Switzerland and Scotland (Table 2). At those sites in Finland where trapping was continued after flowers opened, few beetles were caught during the flowering period (<5% of the total number of trapped beetles, cf. >45% from sites in Switzerland and Scotland).

Table 1. Dates of the first RB caught on white sticky traps and the start of flowering at monitored sites in Switzerland, Scotland and Finland in 1998 and 1999.

	Switzerland	Scotland	Finland
1998			
First RB (wk ending)	23 April -1 May	5 May - 27 May	15 May - 3 June
Start of flowering	15 May - 8 June	25 May - 26 May	16 June - 4 July
1999			
First RB (wk ending)	26 April - 7 May	5 May -12 May	26 May - 9 June
Start of flowering	13 May - 11 June	25 May - 3 June	13 June - 20 June

Table 2. Ranges of the total number of RB/trap/site caught before and during the flowering period in Switzerland, Scotland and Finland in 1998 and 1999.

	Switzerland	Scotland	Finland
1998			
Before flowering	1.8 - 40.3	2.3 - 216.0	7.5 - 694.0
During flowering	3.8 - 101.8*	15.5 - 115.5	1.2 - 59.5
1999			
Before flowering	4.3 - 66.5	0.3 - 367.0	0.8 - 294.0
During flowering	0.8 - 100.0	2.8 - 289.0	0.5 - 6.0

*total numbers for Swiss sites in 1998 also include RB trapped after flowering.

and feed on the inner surface of ripening drupelets (Taylor & Gordon, 1975). There is one generation per year.

In Switzerland, Höhn *et al.* (1995) proposed spray thresholds for RB, based on relationships between the extent of fruit damage and the numbers of beetles caught on white sticky traps. The development of spray thresholds for RB was one objective in a recently completed 2-year EU project to initiate a pan-European approach to more sustainable production. 'Reduced Application of Chemicals in European Raspberry Production' (RACER) was a project involving raspberry producers in six European countries who joined with scientists to develop IPM methods for the major pests and diseases of this crop (Gordon & Woodford, 2000). This paper describes trials in 1998 and 1999 to monitor the flight activity of adult RB in summer-fruiting raspberry crops in Scotland, Switzerland and Finland. Damage thresholds are presented, based on relationships between numbers of adults trapped before flowering and subsequent larval damage to ripe fruit in monitored crops in widely differing environments.

MATERIALS AND METHODS

Monitoring adult RB flight activity

Modified Rebell® bianco crossed sticky traps (registered product of FAW, Wädenswil, and manufactured by BSZ Stiftung, Seewen, Switzerland) were used to trap adult RB. The white, non-UV reflective traps were shown to be the most effective colour for trapping RB in Switzerland (Höhn, 1991). In the present trials, single plates (20.5 x 15cm) of these traps were suspended from supporting wires 50-70cm above ground in spaces between canes, and facing the alleys between rows. Four traps/plantation were placed at least 20m apart, and avoiding outer rows, in areas of the plantation that were not sprayed with insecticides to control RB. They were put into plantations in late-April or early-May when the first flower buds were visible. Traps were changed at weekly intervals, usually until the end of the flowering period, but in 1998, trapping at most Finnish sites ceased when flowers opened because traps in Finland did not catch many RB after flowering had started. RB was monitored in cvs Glen Clova and Glen Ample at sites within 40km of Blairgowrie, the main raspberry-growing area of eastern Scotland; in IPM and organic plantations (several cultivars) at elevations of 409 to 1060m in Switzerland, mainly in the north east; and in cvs Ottawa, Muskoka and Preussen in Finland, mostly in central and eastern areas, but also at a few sites in southern Finland, c. 400km south of these areas.

Assessing damage to ripe fruit

Damage caused by RB larvae was assessed on two or three occasions (early-, mid- and late-season) by examining freshly exposed husks (receptacles) left on the canes shortly after harvests. Samples normally consisted of 250 fruits or husks/replicate from 10m lengths of the trap row (centred on the trap position) and two adjacent rows. Husks were inspected in the field, or collected and examined in the laboratory. Fruit samples were examined in the laboratory to check the accuracy of field assessments and to determine the numbers damaged or infested by RB larvae.

Monitoring raspberry beetle (*Byturus tomentosus*) with white sticky traps: the experience from three geographically distinct European areas

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ABSTRACT

Raspberry beetle (*Byturus tomentosus*) is one of the most important pests of cultivated raspberries in Europe, necessitating the application of prophylactic insecticide sprays to prevent larval contamination of the fruit. White sticky traps, first shown to trap adult raspberry beetles in Switzerland, were tested in Scotland, Switzerland and Finland in 1998-1999 as part of the EU project 'Reduced Application of Chemicals in European Raspberry Production (RACER)'. The main objective of this study was to develop control thresholds based on relationships between numbers of trapped raspberry beetles and amounts of fruit damage. The traps were placed in insecticide-free plots in raspberry plantations at 'first flower bud' stage and changed at weekly intervals for 6-10 weeks. The percentage of damaged raspberries, estimated by examining harvested fruit and husks for raspberry beetle larvae and feeding damage, or by inspecting freshly exposed fruit husks *in situ*, could not be accurately predicted from the total number of raspberry beetles caught before and during flowering. In Finland, raspberry beetles were not often trapped once flowers opened, and there was usually no correlation between trap catches and fruit damage. Provisional damage threshold levels were set for Scotland and Switzerland, where traps usually caught raspberry beetles before and during flowering.

INTRODUCTION

Raspberry (*Rubus idaeus*) is a high value crop, grown in many European countries. It is vulnerable to many pests and diseases, and growers rely on the prophylactic use of pesticides to produce high quality fruit (Gordon *et al.*, 1997). Raspberry beetle (RB) (*Byturus tomentosus*) damages raspberry flower buds, flowers and, especially, fruits, and there is a very low market tolerance for larval infestations or contamination of fruit. Consequently, most growers routinely apply one or two insecticide sprays before harvest to control this major pest (Gordon *et al.*,1997). Adult RB emerge from the soil in spring and feed on young raspberry foliage until flower buds develop. They fly readily at temperatures >15°C and feed and mate in raspberry flowers (Willmer *et al.*, 1996), and females lay eggs on stamens or styles. Newly hatched larvae feed initially on the surface of developing fruits, then tunnel into the receptacle

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Ambient conditions were very warm during this experiment, and levels of sciarid flies emerging from untreated paper pots were very high (>5 per plug). This high level of infestation resulted in severe damage to untreated poinsettia cuttings, especially in the paper pots, which generally supported higher fly populations than glue plugs. Compost treatment of paper pot plugs with chlorpyrifos or imidacloprid granules resulted in a highly significant increase in foliage weight and significantly decreased the number of flies emerging. The differences between untreated and insecticide treated glue plugs were less marked, probably reflecting the fact that significantly fewer ($P<0.05$) flies emerged from this type of plug compared with paper pots.

DISCUSSION

Commercial propagators of ornamentals such as poinsettias stick the cuttings sequentially over a long period, in this case from June to August. Most glasshouses used for this purpose are cleaned and sterilized between crops, so the population of sciarid flies at the start of propagation is usually low. However, sciarid fly populations tend to build up over the summer, often to very damaging levels. The continuous mist used in propagation tends to leach out any drench applications of insecticide, or insect parasitic nematodes such as *Steinernema feltiae*, so repeated applications are necessary, which are both time consuming and expensive. In addition, temperatures can exceed 30^0C for extended periods, which is detrimental to the nematodes.

These results have shown that incorporation of either chlorpyrifos or imidacloprid granules into the plug before sticking can give excellent control of sciarid fly, increasing both foliage weight and plug weight, thus improving quality. The active ingredient is located exactly where it is needed, and presumably diffuses out of the granule over the whole rooting period. Total persistence of effect is unknown, but further protection may be provided when the rooted plug is potted on into final pots.

The results also showed that the type of plug used in propagation can influence the level of attack by sciarid fly. Paper pots contained compost with a high air filled porosity, whereas the glue plugs were much denser. This may have reduced either the mobility of the sciarid fly larvae, or their ability to locate and feed on poinsettia roots, leading to significantly fewer flies emerging from glue plugs compared to paper pots. The type of growing media used, as well as the plug type, can have a significant effect on levels of sciarid fly and the subsequent damage caused by larval feeding. Lindquist (1998) found that media based on coir supported higher numbers of larvae than various peat or peat/bark mixes. Inert media such as rockwool can also support high numbers of larvae, but the proprietary material Oasis appears to be resistant (Fuller, pers. comm.).

Compost treatment with imidacloprid or chlorpyrifos granules to poinsettias at the plug stage provides a cost-effective method of controlling sciarid fly larvae. Compatability with biological control agents such as *Hypoaspis sp.* has not been evaluated, but it is probable that treated cuttings could be potted on into untreated compost, and an IPM programme successfully used on the finished crop (Lindquist *et al.*, 1994). This possibility will be investigated in future work.

Table 2. Levels of sciarid fly and relative plant weights in Experiment 1, Site 2 (plants stuck 14/07/98, assessed 24/08/98). Means in columns followed by the same letter are not significantly different at P=0.05.

	Treatment	Mean foliage weight (g)	Mean plug weight (g)	Mean number of flies/trap
Paper Pots	Untreated	2.40 a	4.95 a	16.3 c
	Chlorpyrifos	3.75 cd	5.30 b	0.8 a
	Imidacloprid	3.35 abc	5.90 c	2.3 ab
Glue Plugs	Untreated	2.68 ab	7.10 d	7.5 b
	Chlorpyrifos	4.39 d	8.01 f	2.0 ab
	Imidacloprid	3.50 bcd	7.50 e	0.5 a
S.E.D.		0.43	0.15	2.92

The second experiment at Site 2 was done later in the sequential sticking phase of poinsettia cuttings, when numbers of flies had increased. The results are shown in Table 3.

Table 3. Levels of sciarid fly and relative plant weights in Experiment 2, Site 2 (plants stuck 27/07/98, assessed 29/08/98). Means in columns followed by the same letter are not significantly different at P=0.05.

	Treatment	Mean foliage weight (g)	Mean plug weight (g)	Mean number of flies/trap
Paper pots	Untreated	1.36 a	4.60 a	63.0 a
	Chlorpyrifos	2.49 b	5.02 b	7.8 b
	Imidacloprid	2.49 b	5.06 b	1.3 b
Glue plugs	Untreated	2.58 b	6.96 b	13.8 b
	Chlorpyrifos	2.89 b	7.40 e	2.5 b
	Imidacloprid	2.82 b	7.38 e	1.0 b
S.E.D.		0.29	0.19	6.66

RESULTS

Incorporation of insecticide granules into the plug compost significantly ($P< 0.05$) increased both plug and foliage weight for both types of plug at Site 1 (Table 1). Numbers of emerging flies were also significantly reduced compared with untreated plugs, when the plug compost was not treated with a granular insecticide. Significantly more sciarid flies emerged from paper pots than from glue plugs, despite the greater volume of the latter. This indicated that the type of plug used in poinsettia propagation could significantly affect the incidence of sciarid fly.

In the first experiment at site 2, the total numbers of flies was generally lower than at Site 1, and again significantly more flies emerged from paper pots than from glue plugs. However, insecticide granular treatment of the latter still resulted in a significant increase in foliage weight and plug weight (Table 2).

Table 1. Levels of sciarid fly and relative plant weights at Site 1 (plants stuck 28/07/98, assessed 25/08/98). Means in columns followed by the same letter are not significantly different at P=0.05.

	Treatment	Mean foliage weight (g)	Mean plug Weight (g)	Mean number of flies/trap
Paper pots	Untreated	1.95 a	4.84 a	26.0 a
	Chlorpyrifos	3.35 c	5.34 b	14.8 b
	Imidacloprid	2.84 bc	5.48 b	3.3 c
Glue plugs	Untreated	2.26 ab	7.22 c	14.3 b
	Chlorpyrifos	3.27 c	7.86 e	7.3 bc
	Imidacloprid	3.05 c	7.51 d	3.8 c
S.E.D.		0.27	0.11	3.56

MATERIALS AND METHODS

Control of sciarid flies was studied at two commercial propagation nurseries in Warwickshire, UK (denoted Sites 1 and 2). Both nurseries produced poinsettias from cuttings rooted using overhead misting systems. The experiments were located within the commercial crop, using naturally-occurring sciarid fly infestations.

Three experiments were done (one at Site 1, two at Site 2). In each experiment, the following insecticide treatments were evaluated in two different types of plug: a) a 'paper pot'; and b) a 'glue-plug'. The growing media was a peat/perlite compost mix. The paper pot system was the proprietary Elle pot system. With this type of plug, the compost is wrapped in a permeable paper membrane and extruded, then cut off into individual plugs of approximately 50 mls volume. With the glue plug method, the compost is mixed with a liquid polymer to form a slurry, which is then poured into plug trays. The polymer binds the compost together and 'cures' making a durable plug. The volume of the glue plugs used was approximately 75 mls.

TREATMENTS USED IN THE TRIAL

1. Untreated

2. Controlled release 10% chlorpyrifos granules at 500g/m^3 (suSCon Indigo)

3. Granules of 5% imidacloprid at 280g/m^3 (Intercept)

Both types of granule were pre-mixed evenly into the compost before the plugs were formed. Plugs were inserted into a plastic plug tray containing 42 cells. Each treatment (one plug tray of plants) was replicated four times. All treatment trays were arranged in a randomised complete block design on the greenhouse bench, with the commercial crop alongside. Plugs were wetted up, and then poinsettia cuttings (cv. Sonora) were inserted the same day. Rooting under the mist system took three to four weeks.

When well rooted, the poinsettias were cut off at compost level and the fresh weight of the plant determined. After oven drying, the dry weight of the plug was also calculated. The control of sciarid fly was determined by taking a random sample of 12 plugs per replicate and placing them into open polystyrene boxes. A yellow sticky trap was placed horizontally over the plugs, and the entire box was then covered with fine mesh cloth. Boxes were left *in situ* in the greenhouse for two weeks to allow adult flies to emerge and become caught on the sticky trap. The total number of flies on the traps was then counted, using a hand lens to differentiate between sciarid flies and shore flies (*Scatella stagnalis*), which were also present at both trial sites.

Data were analysed using analysis of variance. Where significant F tests were found, differences between means were determined using Duncan's multiple range test.

Control of sciarid fly, *Bradysia paupera*, in ornamental plant propagation

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ABSTRACT

The use of insecticides to control damage by larvae of sciarid flies to cuttings of poinsettia, *Euphorbia pulcherrima* was evaluated under commercial glasshouse conditions at two sites. Damage was significantly reduced by the incorporation of chlorpyrifos granules or imidacloprid granules into the plug before sticking. Both these treatments gave a significant increase in fresh foliage weight and dry plug weight compared with untreated plugs. The level of sciarid fly activity increased during the season with sequential sticking of cuttings, but control from these insecticides was still maintained over a period of approximately a month. The type of plug also had an effect upon the incidence of sciarid fly. When no insecticide was incorporated into the compost, significantly more flies emerged from paper pots than from glue plugs, where a polymer was used to bind the compost together.

INTRODUCTION

Sciarid flies (*Bradysia* spp) are common and often abundant in ornamental greenhouses in much of Northern Europe, including the UK, and the feeding of their larvae damages the roots of a wide variety of plant species (Jarvis, 1990). Damage is often made worse by the dissemination of plant pathogens such as *Pythium* and *Thielaviopsis* by both adults and larvae (Goldberg & Stanghellini, 1990; Biddulph & Entwistle, 1996). Composts based on peat, coir, bark or other highly organic matter provide a favourable environment for larvae to thrive (Lindquist, 1998).

Established plants with vigorous root systems can tolerate low infestations of sciarid fly larvae, but newly stuck cuttings in plugs can be severely damaged (Buxton, 1993). Normally, feeding is confined to the newly formed roots, but in the case of poinsettias, larvae readily tunnel into the hollow stem, thus killing the cutting (Sanderson, 1998). Cuttings are normally rooted under mist with bottom heat from recirculated hot water, creating both high humidities and a temperature range of 20 - 35^{0}C. These conditions lead to a rapid build up of the sciarid fly population.

Spray application of chemical insecticides to control sciarid flies is difficult, particularly as leaching from the small volume of compost in the plug is a constant problem. Insecticide sprays may also cause phytotoxicity (Osborne *et al.*, 1985). To overcome these problems, the effect of incorporating granular insecticides into the plug was evaluated to determine if the control of sciarid fly larvae could be improved without adverse effects on the plants.

DISCUSSION

The *in-vitro* toxicity tests demonstrated that abamectin is toxic to bud and leaf nematodes at equivalent concentrations to those currently recommended against other pests. The plant experiments confirmed that abamectin is a potentially useful treatment in the short-term suppression of bud and leaf nematodes in hardy ornamentals, giving a level of nematode control comparable to that of the standard treatment, aldicarb. The two-spray abamectin treatments did not appear to confer any advantage over the application of a single spray. Furthermore, the double-rate abamectin used on the *Saxifraga* and *Cistus* did not significantly reduce nematode numbers to any greater extent than that of the label-rate applications.

Neither abamectin nor aldicarb should be viewed as eradicant treatments as small numbers of nematodes survive treatment to give rise to later attacks. An integrated programme of cultural hygiene measures is essential to eliminate the pest from hardy ornamental propagation cycles.

Other limited observations (Young & Maher, unpublished) suggest it is possible that the persistence of abamectin against bud and leaf nematodes may not be as robust as that of aldicarb. It is likely that repeat sprays of abamectin at approximately two to three monthly intervals may be required to maintain an acceptable suppression of nematode populations. Longer-term studies are required to investigate this issue. Therefore, in the context of the current work, abamectin sprays should be viewed as a potentially useful alternative to, rather than as a replacement for, the use of aldicarb granules.

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Table 3. The effect of aldicarb and abamectin on the numbers of *A. ritzemabosi* infesting *Saxifraga* cv. James Bremner (numbers/gram leaf tissue).

Treatment	Days after treatment				
	0 (pre- treatment)	7	28	35	63
Untreated	35	17	8	5	7
Aldicarb	14	23	0.1*	0.04*	0.1*
Abamectin label rate x1	14	13	2*	1*	1*
Abamectin label rate x2	11	4*	0.4*	0.3*	0.04*
Abamectin double rate x1	15	33	1*	0.4*	1*
Abamectin double rate x2	15	16	0.4*	0.03*	0.04*
SED (18 d.f.)	11.2	12.4	2.4	1.0	1.0

* Significantly different from untreated ($P < 0.05$)

In the *Cistus*, the untreated nematode population peaked at 28 days after initial treatment and then subsequently declined. All of the chemical treatments significantly ($P < 0.001$) reduced nematode numbers in the 28 and 35 DAT assessments (Table 4). By the time of the final assessment (63 DAT), none of the nematode counts in the chemical treatments were significantly lower than untreated but numbers were lowest in association with the aldicarb and double-rate abamectin treatments.

Table 4. The effect of aldicarb and abamectin on the numbers of *A. ritzemabosi* infesting *Cistus* cv. Corbariensis, (numbers/gram leaf tissue).

Treatment	Days after treatment				
	0 (pre- treatment)	7	28	35	63
Untreated	160	68	455	106	97
Aldicarb	209	132	9*	3*	14
Abamectin label rate x1	118	97	139*	6*	80
Abamectin label rate x2	115	91	43*	14*	62
Abamectin double rate x1	188	113	148*	14*	15
Abamectin double rate x2	163	168	80*	3*	4
SED (18 d.f.)	49.0	60.2	60.5	22.1	37.8

* Significantly different from untreated ($P < 0.001$)

Table 1. Calculated lethal concentration 50 (LC_{50}) and lethal concentration 90 (LC_{90}) values from *in-vitro* tests of abamectin against *A. ritzemabosi* (ppm abamectin).

Exposure time		Lethal conc. (ppm abamectin)	95% confidence range: Lower limit	Upper limit
1 hour	LC_{50}	51.8	39.6	70.9
	LC_{90}	na*	na*	na*
4 hours	LC_{50}	25.6	15.8	45.7
	LC_{90}	na*	na*	na*
24 hours	LC_{50}	0.2	0.1	0.3
	LC_{90}	5.7	3.9	8.8

* Calculated values not valid as they were outside of the range of results recorded

The post-treatment observations, in which nematodes were removed from the abamectin solutions and placed in water to recover, indicated that the majority of nematodes showed no sign of recovery after 24 hours and that they were irreversibly paralysed (Table 2).

Table 2. Percentage of non-mobile *A. ritzemabosi* following exposure to abamectin for 24 hours. Data are means of two tests.

Treatment	Post-treatment time in water	
	3 hours	24 hours
Untreated	5	10
Abamectin 0.36 ppm	83	73
Abamectin 2.8 ppm	100	98
Abamectin 9 ppm	100	100
Abamectin 45 ppm	100	98
Abamectin 90 ppm	98	95

Plant experiments

The untreated nematode population in the *Saxifraga* declined naturally during the course of the study (Table 3). However, all chemical treatments significantly reduced the numbers of nematodes in the assessments made 28, 35 and 63 days after initial treatment, compared with the untreated ($P < 0.05$). The numbers surviving were very low by the time of the final assessment (63 DAT) and there were no significant differences between any of the chemical treatments.

naturally infested plants containing *A. ritzemabosi* were obtained from commercial nurseries. The following treatments were applied:

1. Untreated. Plants sprayed with water only @ 3000 litres/ha.
2. Aldicarb (Temik 10G; 10% wt/wt aldicarb granules; RP Agric.). Applied to compost surface @ 80kg/ha.
3. Abamectin (1.8% wt/v EC). One spray @ 0.05% product in 3000 litres of water/ha.
4. Abamectin (1.8% wt/v EC). Two sprays @ 0.05% product, the first applied as in treatment 3, followed by a second application 28 days later.
5. Abamectin (1.8% wt/v EC). One spray @ 0.1% product in 3000 litres of water/ha.
6. Abamectin (1.8% wt/v EC). Two sprays @ 0.1% product, the first applied as in treatment 5, followed by a second application 28 days later.

All the abamectin treatments were applied with an Oxford Precision CO_2-powered sprayer. The aldicarb granules were applied by hand as single doses to individual pots. There were four replicates of each treatment. Each replicate consisted of four plants in the *Saxifraga* study (3 litre pots) and five plants in the *Cistus* study (1 litre pots). All of the plants exhibited visual symptoms of bud and leaf nematode attack at the start of the study. Each treatment was placed in a separate chamber measuring approximately 2 m × 1 m × 1 m, with a solid fibreglass base on which was mounted a tubular frame covered with clear polythene. The plants were watered and misted regularly to maintain damp and humid conditions within each chamber. The plant chambers were kept in a shade tunnel (47% shade) during the study period (mid-April to mid-October, 1999).

Plants were assessed for nematode infestation immediately prior to treatment and then at 7, 28, 35 and 63 days after treatment (DAT). For each assessment of the *Saxifraga*, four points were sampled within the foliar 'cushion' of each plant. For the *Cistus*, one leaf was taken from each plant. In each case, leaves exhibiting visual symptoms of nematode attack were selected wherever possible. Each sample was weighed and nematodes were extracted from leaf tissue using the same technique as detailed above for toxicity tests. The aqueous suspensions of extracted nematodes were then placed in Doncaster dishes and counted under a low-power microscope.

RESULTS

In-vitro toxicity tests

At the higher concentrations of abamectin tested, the immobilising effect of abamectin was visible within one hour of exposure as nematodes lost their normal swimming movements. However, maximum effects were not visible in all treatments until after 24 hours of exposure. The 24 hr LC_{50} and LC_{90} values were lower than their equivalent 1 hr and 4 hr values (Table 1). The 24 hr LC_{90} of 5.7 ppm corresponded closely with the spray concentrations recommended on the product label (for two-spotted spider mite and leaf miner) which range from 4.5 ppm to 9.0 ppm abamectin.

against bud and leaf nematode and to assess the activity of abamectin applied as a foliar spray against the pest on hardy ornamental hosts, in comparison with the standard treatment of aldicarb granules.

METHODS AND MATERIALS

In-vitro toxicity tests

The bud and leaf nematode species *A. ritzemabosi* was extracted from naturally infested lavender (*Lavandula* sp.) plants by placing chopped leaves into a nylon mesh bag and leaving them for 48 hours in a beaker of aerated tap water. The nematodes migrated from the leaf tissue into the surrounding water, and were collected on a 53 µm sieve and placed in a small volume of water immediately prior to use. The following concentrations of abamectin (Dynamec; 18 g/l abamectin EC; MSD AGVET) were prepared as aqueous solutions for use in each of three tests, all conducted at room temperature (*c.* 20–25°C) during 1998:

1. Untreated
2. Abamectin, 0.4 ppm (0.002% product)
3. Abamectin, 1.8 ppm (0.01% product)
4. Abamectin, 9 ppm (0.05% product)
5. Abamectin, 45 ppm (0.25% product)
6. Abamectin, 90 ppm (0.50% product)

Each test solution was made up to a total volume of 10 ml with de-ionised water and placed in a nematode counting dish (Doncaster dish). Freshly extracted and visibly mobile nematodes were transferred to the test solutions by hand using a mounted needle or eyelash, with the aid of a low-power microscope. In the first test, 30 nematodes were added to each test solution whilst in the second and third tests 60 and 50 nematodes were used, respectively. The nematodes were examined and counted under a low-power microscope at intervals of 1 hour, 4 hours and 24 hours after being placed in each test solution. On each occasion, the nematodes were classified as either mobile (visible swimming movements) or non-mobile (no visible signs of body movement).

Nematode recovery checks were made in two of the tests to ascertain if any of the immobilised nematodes were capable of recovery. This was done immediately after the completion of the 24-hour post-treatment counts by removing 20 immobilised nematodes from each abamectin solution and the untreated control and placing them in de-ionised water. The nematodes were then examined after 3 hours and 24 hours for signs of renewed movement.

The toxicity test data were analysed using probit models fitted to each of the exposure times tested, using the Generalised Linear Model part of GENSTAT (1993). The effective LC_{50} and LC_{90} values were subsequently calculated together with their 95% confidence limits.

Plant experiments

Abamectin sprays were compared with aldicarb granules for the control of bud and leaf nematode in *Saxifraga* cv. James Bremner and *Cistus* cv. Corbariensis. In each case, batches of

Evaluation of abamectin against bud and leaf nematode in hardy ornamentals

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ABSTRACT

The toxicity of abamectin against bud and leaf nematodes (*Aphelenchoides ritzemabosi*) was investigated using *in-vitro* toxicity tests and with spray treatments on infested plants. The 24 hour LC_{90} of abamectin was calculated to be 5.7 ppm, which corresponded closely with the spray concentrations (4.5–9.0 ppm) recommended against insect and mite pests. Abamectin caused irreversible paralysis of the nematodes at the higher concentrations tested. The efficacy of abamectin sprays was also compared with the standard treatment, aldicarb granules, for the control of bud and leaf nematode in naturally infested *Saxifraga* and *Cistus*. Abamectin was applied as one- or two-spray programmes at dose rates of 0.05% and 0.01% in 3000 litres of water/ha. Abamectin gave nematode control comparable to that obtained with aldicarb for up to three months after treatment. No significant additional control was gained from the second sprays or the higher rates of abamectin. Abamectin offers a potentially useful alternative treatment to aldicarb for the short-term suppression of bud and leaf nematode in hardy ornamentals. However, neither abamectin nor aldicarb can be viewed as long-term curative measures as small numbers of nematodes survive treatment to resurge at a later date.

INTRODUCTION

The increasing prevalence and awareness of bud and leaf nematode (*Aphelenchoides ritzemabosi & A. fragariae*) attacking hardy ornamental nursery stock is a cause for concern to growers. Chemical control gives only short-term suppression of this widespread and insidious pest. The nematodes often go undetected in the propagation cycle as unhealthy mother plants do not always exhibit obvious external symptoms of attack, which usually include angular leaf blotching and/or foliar distortion.

Abamectin belongs to a larger group of compounds known as the avermectins, which are known to possess anthelmintic properties and were originally developed as veterinary products against gastrointestinal worm parasites of domestic animals (Hotson, 1982; Stretton *et al.*, 1987). The avermectins are macrocyclic lactones derived from the actinomycete soil organism *Streptomyces avermitilis*; these compounds inhibit nerve transmission in motor neurons mediated by the neurotransmitter GABA (gamma-aminobutyric acid), resulting in paralysis of the target organism.

Aldicarb is the only chemical treatment currently used against bud and leaf nematode in hardy ornamentals. Abamectin is approved in the UK for use against insect and mite pests of protected and outdoor ornamentals. The use of abamectin as a foliar spray against bud and leaf nematodes could provide growers with an alternative to aldicarb and may be of use in suppressing outbreaks of this pest. The objective of this work was to investigate the *in-vitro* toxicity of abamectin

Despite being a strong compound on *Frankliniella occidentalis*, lufenuron possesses remarkable selectivity towards important commercially used beneficial arthropods such as *Amblyseius* sp. In a non-replicated trial in Spain in 1995, one application was made on sweet peppers in plastic houses using 4 rows (200 plants) per treatment. The plot was sampled taking 2 flowers and 2 leaves from 50 plants selected at random. Samples were taken 0, 3, 7 and 14 days after treatment. The predatory mite population was somewhat heterogeneous at the time of application and demonstrated a slight increase after treatment (Figure 7) This is possibly due to increased mobility of the predator searching for thrips prey that perished following application of lufenuron.
Experience in Spain has confirmed that these predatory mites may be used simultaneously with lufenuron with no negative impact on populations.

CONCLUSION

Lufenuron shows good activity on eggs and immature stages of *F. occidentalis* and has no adulticidal effect, although feeding adults are subject to a marked transovarial effect. The recommended dose rate is 10g a.i./hl and the product should be applied at 7-10 day intervals to obtain best results. Application timing needs to be early in order to prevent population build up and virus transmission. Lufenuron has good selectivity to predatory mites and bumblebees.

ACKNOWLEDGEMENTS

We thank Burkhard Sechser and Beat Reber for their trial work on bumblebees, Alfred Rindlisbacher and Peter Wyss for their laboratory studies and Federico Garcia of Novartis BCM for his field trial using *Amblyseius* mites.

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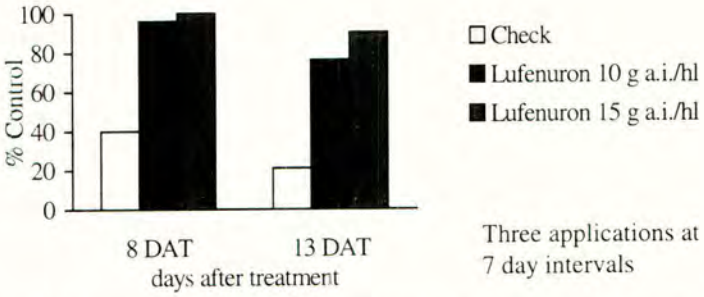
The difference in effect on adults and larvae is clearly visible in a replicated field trial on sweet peppers from Spain (see figure 5). On the 10th of July there is a clear difference in performance of lufenuron on larvae and adults. 11 days later there is already an apparent effect on the adults, which is probably due to the high larvicidal activity rather than a direct adulticidal effect. Although lufenuron will not kill adults, their feeding on treated leaves will result in a transovarial effect and subsequent control of the progeny.

Melons

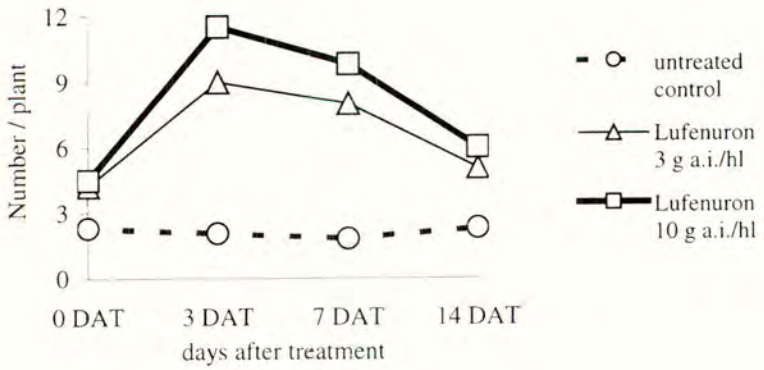
Figure 6. Reduction of *F. occidentalis* nymphs on melon (Spain, 1997)



The effect of lufenuron is not heavily dependent on dosage. In a replicated field trial on melons in Spain (figure 6), there was no significant benefit from increasing the dose from 10 to 15 g a.i./hl. The counts were made of larvae on 10 leaves or flowers. Performance declined 13 days after treatment. It is recommended to use lufenuron at 7-10 day intervals to maintain a high level of control. The reason for this is partly related to the fast growth of the plants in greenhouses, the lack of plant systemic activity of the compound (low water solubility) and the need to cover newly emerging plant parts to protect them.

Selectivity to predatory mites

Figure 7. Effect on *Amblyseius (Neoseiulus) californicus*



In a field cage test, 9 flowering *Phacelia acetifolia* plants were sprayed with 5 g a.i./hl lufenuron and placed in a mini greenhouse (3 m x 3 m x 3 m). Diflubenzuron and water were used as standards. Each treatment was replicated three times. Bumblebee hives were placed in each mini greenhouse and the population was evaluated 4 weeks after foraging started. Results are presented in Table 2. The bumblebee population developed normally in all treatments (Sechser & Reber, 1998).

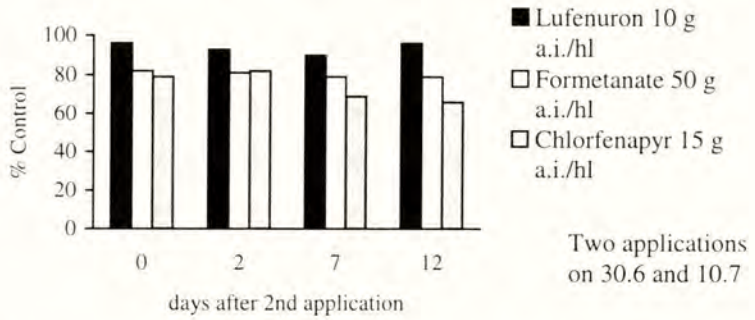
Since bumblebees are sensitive to the water sprayed during spray activities, it is recommended to close the hives during spraying and re-open them as soon as spray deposits and vapours have disappeared. In practical conditions this is usually defined as 'the next day'.

BIOLOGICAL PERFORMANCE UNDER FIELD CONDITIONS

Sweet Peppers

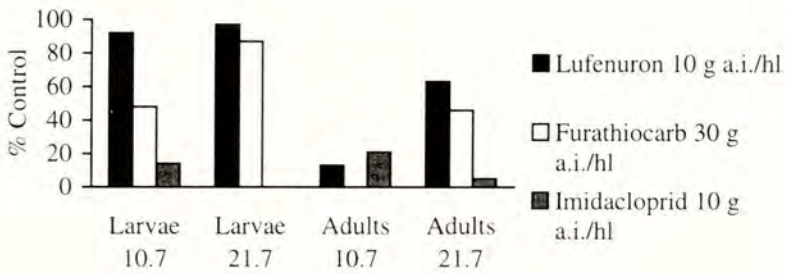
Lufenuron is particularly suited for the control of *Frankliniella occidentalis* on sweet pepper as demonstrated by the replicated field trial below in figure 4.

Figure 4. Reduction of nymphs and adults of *F. occidentalis* on sweet pepper (France, 1997)



Lufenuron showed that it was able to control populations of *F. occidentalis* as effectively as a traditional product like formetanate.

Figure 5. Control of *F. occidentalis* nymphs on sweet pepper (Spain, 1998)



In this experiment, bean leaf discs (diam. 50 mm) were dipped in compound solutions and air-dried on soft paper with the lower leaf surface upwards and gently placed in the same position onto Agar (5 ml, 2%) in Petri dishes (diameter 5 cm). 5 adult thrips were transferred to the leaf discs, dishes were covered with cotton cloth and closed with tight fitting plastic lids. After 2 days, adults were transferred to untreated leaf discs: Petri dishes with treated discs and adults were kept in reverse position over Petri dishes with untreated discs until adults had moved to untreated discs. After 2 more days adults were removed from the discs using a vacuum device. 8 DAT (27° C; 60 % RH; 12 h day / 12 h night cycles) the number of living larvae was assessed on treated discs and untreated discs. Numbers of larvae were transformed to % product efficacy using Abbott's formula.

The effect on treated leaf discs is a combination of transovarial activity and larval mortality after hatch. In contrast, the effect against eggs laid onto untreated discs, by adults which were kept for 2 days beforehand on treated discs, is only transovarial. As a consequence the activity on treated discs is higher than on untreated discs. However, the transovarial activity was high: at 10 and 100 ppm a.i. it was nearly as high as the combined transovarial/larvicide effect, pointing to an important contribution of transovarial effect in the control of this pest by lufenuron. The commercially effective dose of lufenuron is 10 g a.i./hl or 100 ppm.

Safety to pollinators

Table 1. Effect of lufenuron by topical application to bumblebee workers (*Bombus terrestris*)

Treatments	Rate g a.i./hl	% mortality days after application		
		1	2	5
Untreated control	-	0	5	5
Lufenuron	5	0	0	0
	50	0	0	0
	500	0	0	0
	5000	100	100	100

Bumblebees are important pollinators in greenhouse vegetable crops, and the safety of a compound must be considered before use in greenhouses. One of the benefits of lufenuron is its low contact activity on insects. Topical application of lufenuron to bumblebee workers (Table 1) only shows effects above 500 gai/hl, which is 50 times above the commercial dose for thrips control (Sechser & Reber 1998).

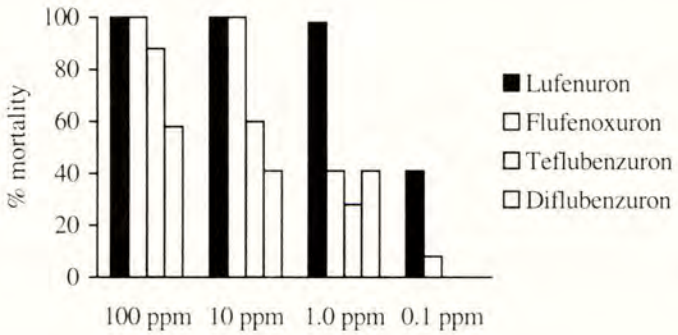
Table 2. Effect of lufenuron in a field cage test to bumblebees (*B. terrestris*)

	Workers		Males		Queens	
	Live	Dead	Live	Dead	Live	Dead
Untreated control	299	157	58	33	3	0
Lufenuron 0.005%	300	116	25	33	1	2
Diflubenzuron 0.01%	180	109	78	48	2	1

Bean leaf discs were sprayed using a Potter tower (Rindlisbacher & Horvath, 1995). Whilst lufenuron shows a high level of activity on the eggs, larvae and pro-nymphs of this target insect, it has no direct effect on the adults. This means that control strategies should be aimed at the developing population and the young life stages. Lufenuron cannot be used as a curative adulticide. Efficacy should be evaluated by monitoring larval populations of thrips.

Comparison with insecticides of similar mode of action

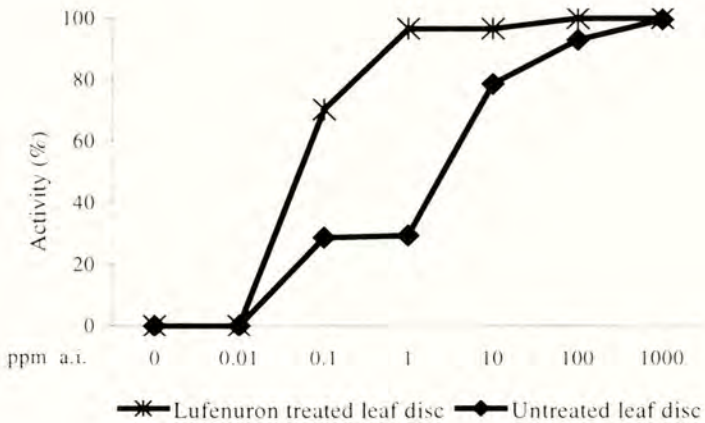
Figure 2. Effect of various acylureas against *F. occidentalis* L1



Laboratory studies comparing four similar compounds using leaf dip tests (Rindlisbacher & Horvath, 1995), revealed lufenuron to be more effective than other acylureas against *F. occidentalis*.

Transovarial activity

Figure 3. Lufenuron transovarial activity on *F. occidentalis*



Ovicidal, larvicidal and transovarial effect of lufenuron on the western flower thrips, (*Frankliniella occidentalis*)

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ABSTRACT

Lufenuron is the first and strongest acylurea insecticide to be used on *Frankliniella occidentalis* in vegetables and ornamentals. Lufenuron shows excellent control of *F. occidentalis* at 10 g a.i./hl and also possesses a good degree of selectivity to predatory mites and bumblebees. Data are presented on ovicidal, larvicidal and transovarial activity, as well as selectivity. Best spray timing is at the onset of infestation, early in the season, using 2-3 applications at 7 days interval.

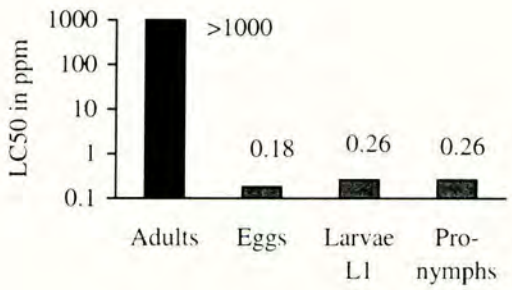
INTRODUCTION

Western flower thrips are important pests of vegetables, ornamentals and other plants. Damage is caused by larvae and by adults. They cause visual damage and transmit virus diseases. Control methods include insecticides, such as methiocarb, formetanate and acrinathrin, and also biological agents such as *Orius* sp. and *Amblyseius* sp. However, some compounds are losing efficacy and others have selectivity problems towards beneficials. Lufenuron, as an acylurea insecticide, offers a new mode of action against this pest in vegetables and ornamentals. In addition it was found that it can be safely integrated with beneficials and pollinators.

BIOLOGICAL PROPERTIES UNDER LABORATORY CONDITIONS

Activity on different life stages: ovicidal and larvicidal activity

Figure 1. Effect of lufenuron against development stages of *F. occidentalis*



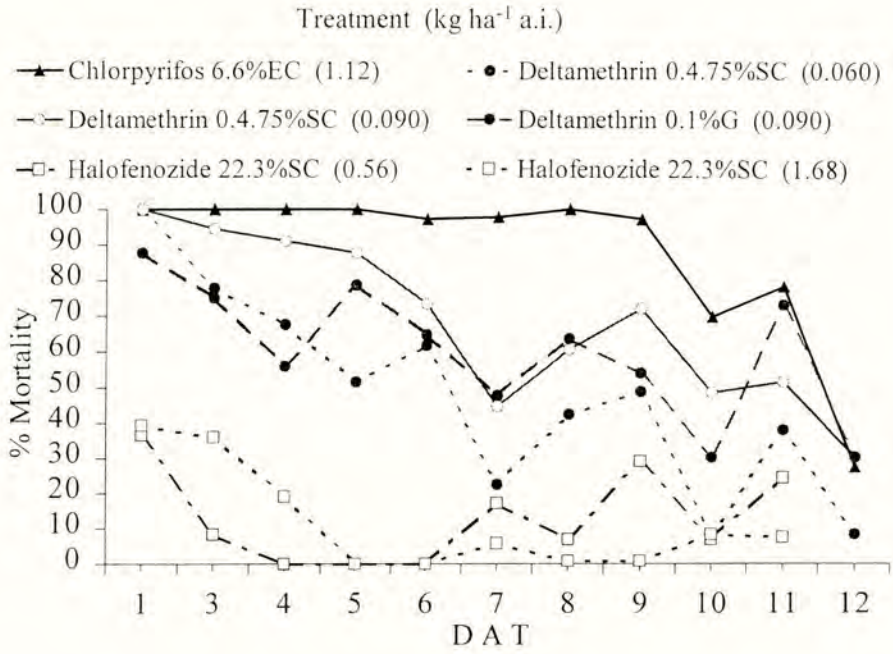


Figure 3. Percent mortality at DAT for *Spodoptera frugiperda* larvae on *Cynodon dactylon* from field plots treated with insecticides on 4 September 1996. Formulations: EC=Emulsifiable Concentrate; SC=Suspension Concentrate; G=Granular.

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period of >90% control was extended to 9 DAT, and it did not fall below 50% until 12 DAT. Based upon these results it appears that a longer residual control may be expected during the fall season when *S. frugiperda* annually reach their peak damaging levels.

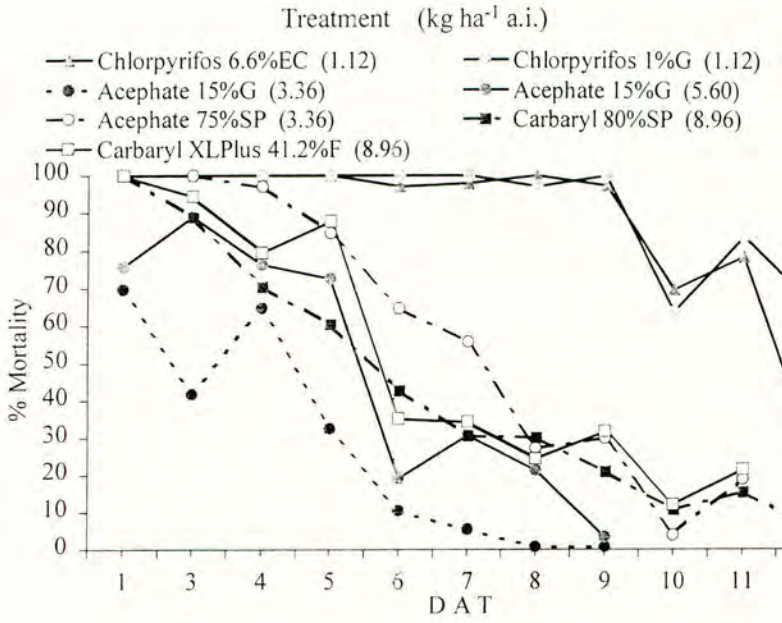

Figure. 2. Percent mortality at DAT for *Spodoptera frugiperda* larvae on *Cynodon dactylon* from field plots treated with insecticides on 4 September 1996.
Formulations: EC=Emulsifiable Concentrate; G=Granular; SP=Soluble Powder; F=Flowable.

Three synthetic pyrethroid insecticides (lambda-cyhalothrin, bifenthrin and deltamethrin) were evaluated. Each chemical provided a very different level of residual control. Lambda-cyhalothrin (formulated as a 9.52%WP) provided >95% residual control for at least 13 DAT. The deltamethrin SC formulation provided 100% initial control which gradually declined in time to <50% control by 12 DAT. Bifenthrin also provided 100% initial control, but the level of control dropped below 50% within 3 DAT. These results indicate that the choice of insecticide strongly influence the long term residual control potential. Additionally, changing environmental factors through the seasons may significantly change the residual potential for an insecticide treatment.

ACKNOWLEDGMENTS

Appreciation is extended to Mr D Hays and Ms S Haley for technical assistance.

efficacy over time, but provided >50% control for 6 DAT. Halofenozide 22.3%EC did not provide acceptable control at the rates evaluated. Both the EC and G formulations of chlorpyrifos at 1.12 kg ha^{-1} a.i. provided close to 100% control for 9 DAT, and >78% control for 11 DAT, but by 12 DAT, control dropped below 30% for the G formulation.

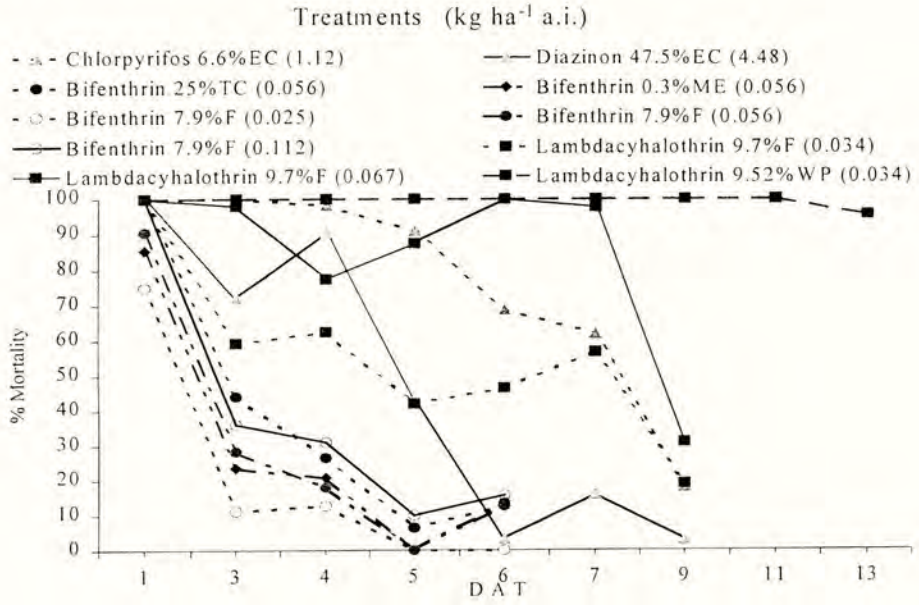

Figure 1. Percent mortality at DAT for *Spodoptera frugiperda* larvae on *Cynodon dactylon* from field plots treated with insecticides on 23 July 1996.
Formulations: EC=Emulsifiable Concentrate; TC=Termiticide Concentrate; ME=Micro Encapsulated; F=Flowable; WP=Wettable Powder.

CONCLUSIONS

This method of treating field plots and exposing the treated grass sequentially in time to larvae from a laboratory colony, provides an excellent means to evaluate the long term potential control provided by turfgrass insecticides. It additionally provides a good side-by-side comparison for different formulations of the same chemical. For several insecticides, a spray was compared side-by-side to a granule and the spray treatment usually provided better residual control. Even though litter at the soil line was harvested with the grass samples, probably a good portion of the granular treatment was unavailable to our method of analysis.

Chlorpyrifos 6.6%EC was evaluated in both experiments and provides an excellent comparison of the control potential during late summer and early fall and under the differing environmental conditions. During the summer treatments on 23 June with exposure to much higher solar radiation and higher potential biodegradation, the level of control remained above 90% for only 5 DAT, and fell below 50% by 9 DAT. For the experiment later in the year on 4 September, the

transported to the laboratory within a cooled ice chest. Samples from each plot were mixed thoroughly to ensure a representative sample was fed to larvae.

For each plot, and for each sample day, 10 larvae (divided among 3 dishes with 3, 3 and 4 larvae/dish) were caged in 9 cm diam. x 20 mm deep plastic petri dish feeding-chambers filled c. two thirds with the composite sample of grass from the respective plot. Each feeding chamber was first provided with two water saturated 7 cm filter paper discs, to maintain grass turgidity during the test.

Mortality of *S. frugiperda* larvae was evaluated after 24 and 48 h of exposure and feeding on each treatment for each test d. Only 48 h data are presented. Data were adjusted to the untreated check by Abbott's formula (Abbott, 1925) and analyzed using the General Linear Model procedure, and means separated by Waller-Duncan k-ratio t-test (k=100, P = 0.05) (SAS Institute, 1987). Analysis was performed on arc sine transformation of the percentage mortality data for each plot. Untransformed means are presented here.

RESULTS AND DISCUSSION

Experiment 1

Figure 1 presents results from the 23 July experiment. The highest rate of each insecticide evaluated provided 100% control at 1 DAT. However, significant differences in the level of larval mortality were produced by the different formulations of the same chemical. Lambda-cyhalothrin 9.52 WP (Wettable Powder) at 0.034 kg ha^{-1} a.i. provided 96 to 100% control for 13 DAT when the experiment was terminated due to an unavailability of additional *S. frugiperda* larvae. The same rate of an encapsulated formulation of lambda-cyhalothrin (9.7%F) (Flowable) fell below 50% control by 5 DAT while the 2X rate provided control longer before falling below 50% control at 9 DAT. Bifenthrin 7.9%F at the 0.112 kg ha^{-1} a.i. rate provided 100% mortality at 1 DAT. As the rate of bifenthrin was reduced from 0.112, 0.056 to 0.025 kg ha^{-1} a.i., the level of control also declined from 100, 90.5 to 74.9% at 1 DAT, respectively. By the 3 DAT evaluation, control provided by each formulation and rate of bifenthrin fell below 44%. Chlorpyrifos 6.6% EC (Emulsifiable Concentrate) provided >90% control for 5 DAT, while diazinon 4EC fell below 90% control one d earlier. Chlorpyrifos continued to provide >60% control for 7 DAT.

Experiment 2

Results of the 4 September treatments in Experiment 2 are presented in Figures 2 and 3. Chlorpyrifos EC is presented in both Figures for comparison, since it represents the industry standard. Up to 4 DAT, acephate 75%SP (Soluble Powder) provided >97% control while the 15G (Granular) formulation at the same 3.36 kg ha^{-1} a.i. rate provided its best control of 70% at 1 DAT. Carbaryl formulated as 80%SP and XLP 41.2%F, both evaluated at 8.96 kg ha^{-1} a.i. only provided 100% control at 1 DAT, and the level of control for each formulation in time was very similar each d but fell below 50% by 6 DAT. For the two formulations of deltamethrin applied at 0.090 kg ha^{-1} a.i., the 4.75%SC (Suspension Concentrate) provided 100% control while the 0.1G provided 88% control at 1 DAT. All three formulations of deltamethrin gradually lost

Depending upon the target pest, most efficacy data reported in the literature consists of samples of the target population at weekly, biweekly or monthly intervals to determine if the target population has been controlled or if the treatment is still effective. Few field tests are sampled frequently over a long enough interval to record control potential for the next generation of insects or the reinvasion by populations migrating from untreated adjacent areas. For many pests with 1-yr-life-cycles (white grubs and other soil insects), a good suppression of the target pest population within this time frame is adequate to last until the next annual generation. However, with multivoltine pests, reinvasion by ovipositing adults or migration of immatures and adults from adjacent areas is a primary concern and pesticides must be reapplied frequently to protect against recurring damaging populations throughout the season. Many of the primary turfgrass pests, including the Lepidopteran complex (cutworms, armyworms and webworms) and chinch bugs can reinvade the turf within 1 to 4 wk. With the southern chinch bug (*Blissus insularis*) (Reinert, 1974), and with sod webworms (Reinert, 1973; 1983), chemical control experiments were monitored frequently and over a long enough time period to determine when each insecticide had ceased to provide control, at which time reinfestation or reinvasion of the treated turf by the pest was documented.

The fall armyworm (*S. frugiperda*), is a destructive pest of over 50 species of plants including most of our commonly used turfgrasses (Luginbill, 1928). Annually, it only overwinters in the southern USA (Texas and Florida), Caribbean Islands and Mexico, and its northern migration progresses throughout the summer and into the fall throughout the eastern half of the USA and into NM, AZ and CA (Cobb, 1995; Vittum *et al.* 1999). Infestations are usually associated with lush turf in late summer and fall after populations have increased in field crops and pastures. Populations in turfgrass often are undetected until individual late instar larvae begin to consume as much as a hand-full of grass during a nightly feeding.

MATERIALS AND METHODS

Two experiments were conducted on a turf planting of *C. dactylon* cv. 'Common' at the Texas A&M University Research & Extension Center at Dallas, TX, USA. The first experiment was treated on 23 July 1996 and a second experiment was treated on 4 September 1996. For each experiment the turf area was divided into 1 x 1 m plots with a 30 cm wide buffer zone left untreated between plots to avoid cross contamination. Plots were assigned to 4 blocks for each experiment. Treatments were randomly assigned to plots within each block for each experiment. Fig. 1 gives the formulations and rates (kg ha^{-1} a.i.) for each treatment evaluated in Experiment 1 and Fig. 2 and 3 present treatments and rates applied in Experiment 2. Granular materials were dispersed with a hand shaker and washed into the turf with c. 4 liters of water per plot. Each of the other formulations were mixed with 1 liter of water and sprayed on the grass with a CO_2 charged sprayer.

For laboratory analysis of the treated grass, 4- to 5-d-old *S. frugiperda* larvae from a laboratory colony, maintained on cuttings of *C. dactylon*, were fed clipped samples from the treated plots. These samples were collected from each plot at the respective DAT as indicated in Fig. 1 to 3. Grass samples on each sample d were collected from 4 randomly selected sites (minimum of 25 cm from the plot border) within each grass plot. For each sampling, the grass in c. 5 cm^2 was cut at the soil line and combined in a plastic bag with the other samples from the same plot and

Residual control with insecticides of fall armyworm (*Spodoptera frugiperda*) on field treated *Cynodon dactylon*

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ABSTRACT

The objective of the study was to evaluate the residual control for fall armyworm (*Spodoptera frugiperda*) (Lepidoptera: Noctuidae), provided by field applications of insecticides. Field plots of bermudagrass (*Cynodon dactylon*) cv. 'Common' were treated either in late July or early September 1996 with various insecticides. Residual control was bioassayed by feeding 4- or 5-d-old *S. frugiperda* larvae in the lab on grass samples that were harvested sequentially in time from the field plots. Treatments with one or more rates of acephate, bifenthrin, carbaryl, chlorpyrifos, deltamethrin, diazinon and lambda-cyhalothrin provided 100% control of larvae within 48 h after exposure on the grass that was harvested 1 DAT from field plots. Maximum residual corrected control >90% was provided as follows; lambda-cyhalothrin (13 DAT or longer), chlorpyrifos (9 DAT), acephate (4 DAT), diazinon (4 DAT), deltamethrin (4 DAT), carbaryl (3 DAT), and bifenthrin (1 DAT) after the grass was treated. The longer residual control (>90% for 9 vs. 5 DAT) provided by chlorpyrifos in September than in July may have been due to seasonal or environmental conditions. This method of treating field plots and exposing the treated grass sequentially in time to larvae appears to provide an accurate estimate of the long term potential control for turf insecticides.

INTRODUCTION

Insecticides are extensively used as the standard control for insect and mite pest management in turfgrasses. A common assumption across the turfgrass industry is that once a treatment is applied, residual control will be present to protect the turf planting for 1 to 2 wk or even longer. When the turfgrass planting becomes reinfested during this assumed residual period, a common perception exists among landscape and golf course managers that the pesticide had not been applied correctly, or that resistance to the pesticide has developed in the invading pest population.

An earlier experiment (Reinert, unpublished data) showed that several turf pesticides applied to 'Tifway' bermudagrass (*Cynodon dactylon* X *C. transvaalensis*) had no residual activity after 3 d when treated grass was challenged with larvae of the tropical sod webworm (*Herpetogramma phaeopteralis*). None of the pesticides were effective against the larvae for more than 5 d following treatments. Additionally, previous work in a short duration experiment with *Spodoptera frugiperda* (Reinert *et al.*, 1996) showed several insecticides provided effective residual control for at least 3 d.

larvae as they moult. It therefore seems likely that although *M. gyrator* is certainly able to locate and parasitise this host species, *C. chalcites* is not a fully permissive host, and may only yield live parasitoids when superparasitised in laboratory conditions, and not in the glasshouse where superparasitism will probably be more infrequent.

From these results it appears that *M. gyrator* shows potential for the biological control of *L. oleracea*, but not for *C. chalcites*. These results demonstrate that potential in the laboratory may not always translate to potential in the glasshouse environment. *Meteorus gyrator* has the advantage over *Eulophus pennicornis* (Nees), another parasitoid that has shown potential against *L. oleracea*, due to its ability to attack all larval stages as opposed to only the later instars (Marris & Edwards, 1994) and is currently the subject of continuing research to further substantiate its viability as a biological control agent for *L. oleracea*.

ACKNOWLEDGEMENTS

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absence of the parasitoid.

Table 1. The effect of parasitism on the quantity and area of tomato leaf eaten by *C. chalcites*. Values followed by different letters are significantly different ($P<0.05$).

	Food consumption (g dry weight from III instar onwards)	Tomato leaf area consumption mm^2		
		3 days post para..	5 days post para.	7 days post para..
Control	0.666 ± 0.04 c	910 ± 54 a	1361 ± 317 a	2820 ± 265 a
Parasitised	0.121 ± 0.01 d	721 ± 54 b	351 ± 115 b	634 ± 107 b

Table 2. The effect of the release of *M. gyrator* into glasshouses containing tomatoes infested with either *L. oleracea* or *C. chalcites*. Figures show percentage change over control plants kept in the absence of the wasp. Significant differences from the controls are indicated.

	Effect of the presence of *M. gyrator* over controls	
	L. oleracea	*C. chalcites*
Weight of larvae / plant	- 68% P<0.01	+4% NS
No. of larvae / plant	-15% NS	+ 9% NS
% damaged leaves	-48% P<0.001	- 6% NS
Damage score	- 48% P<0.001	+ 4% NS
Weight of plant material	+ 44% P<0.01	N/A

DISCUSSION

Meteorus gyrator was seen to attack a range of noctuid pests that frequently occur in glasshouses. When given a choice of hosts, wasps showed a strong preference for *L. oleracea*, but they readily parasitised all of the hosts tested when presented in isolation (F. Smethhurst, unpublished data). Preference for *L. oleracea* may be, in part, due to the fact that the wasp is maintained in culture on this host. Notwithstanding this, it was clearly apparent that *C. chalcites* and *S. littoralis* were markedly less favoured for oviposition than the other hosts tested. The effect that the species of rearing has on the host preference of *M. gyrator* is currently the subject of further investigation.

In the laboratory, *M. gyrator* has already been demonstrated to have a marked effect on the growth and food consumption of *L. oleracea* (Bell *et al*, in press) and this was also found to be true for *C. chalcites*. Similarly, it was observed that leaf area consumption was also markedly reduced in parasitised larvae, although feeding continued throughout the period of parasitism and was never fully eliminated. While the effects on *L. oleracea* translated into significant reductions in crop damage this was not the case for *C. chalcites*, which were not parasitised by the wasp in the glasshouse. The reasons for this are unclear. However, it has been observed in the laboratory that a proportion of *C. chalcites* hosts which have been parasitised by *M. gyrator* subsequently eliminate live developing wasps by the process of cuticular encystment (Vinson, 1990), which allows resistant hosts to shed endoparasitoid

The effect of parasitism on *C. chalcites*

Parasitism by *M. gyrator* markedly reduced the growth of *C. chalcites* hosts, compared to similar, non-parasitised larvae (Figure 2). On average, parasitised larvae only reached 20% of the weight achieved by control larvae. Parasitoids emerged 10 -12 days after parasitism and weights of hosts are not shown after this point. Similarly, the quantity of food consumed was also reduced in parasitised larvae to approximately 20% of the dry weight consumed by control insects (Table 1).

The area of leaf consumed by parasitised or non-parasitised *C. chalcites* is presented in Table 2. Overall, consumption was reduced by 21%, 74% and 78% at three, five and seven days post parasitism respectively, although on day seven there was an increase in the total area eaten by the parasitised larvae over that eaten at the previous time points.

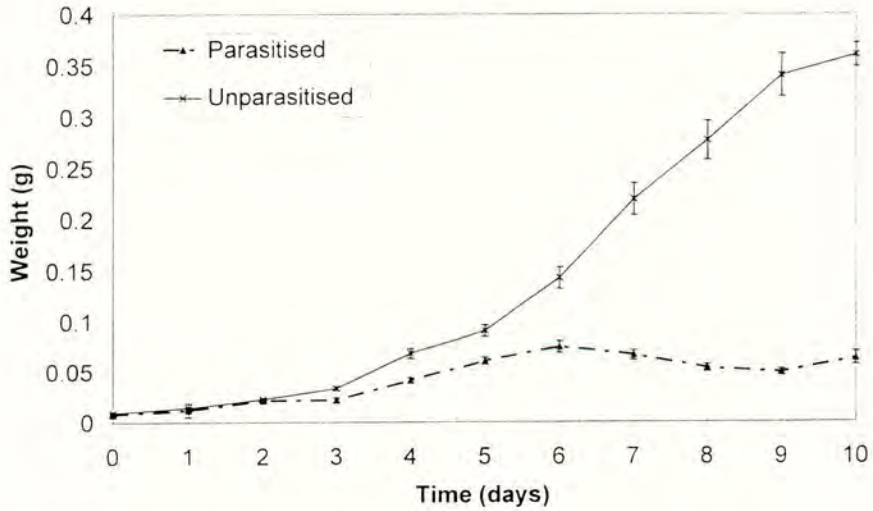

Figure 2. The growth of parasitised and unparasitised *C. chalcites*

Glasshouse investigations

The presence of an initial infestation of ten *C. chalcites* larvae per plant reduced the dry matter present by appproximately 27% over a 20 day feeding period when compared with uninfested controls ($P<0.05$). The effect of the presence of *M. gyrator* on semi-field populations of *L. oleracea* and *C. chalcites* is shown in Table 2. The parasitoid reduced damage caused by *L. oleracea* by almost 50% over the feeding period of the moth larvae (21 days), and reduced the weight of larvae per plant by 68%. The effect of the parasitoid on *C. chalcites* was negligible. Parasitism of *L. oleracea* was 50%, whereas no parasitism was recorded in *C. chalcites*. The presence of *M. gyrator* resulted in almost a 44% increase in the dry matter of tomatoes remaining at the end of the trial in the *L. oleracea* trial while, due to the negligible effect of the wasp on *C. chalcites*, no measurement of the biomass for the tomatoes infested with this moth was made. Furthermore, no reduction in the numbers of *C.*

Glasshouse trials

The effect of *C. chalcites* on tomato plants in the absence of control measures was determined by infesting 20 tomato plants with 10 larvae (III instars) and leaving for 20 days, harvesting the remaining plant material, drying it, and comparing the dry matter present with that of a similar number of control plants grown in the absence of the pest. Small scale glasshouse trials were set up to investigate the effect of *M. gyrator* on semi-natural populations of *L. oleracea* and *C. chalcites*. For each species, two glasshouses were set up with tomato plants infested with *L. oleracea* at a rate of 10 third instars per plant and *C. chalcites* at a rate of 15 third instars per plant. Three days post-infestation, *M. gyrator* females were released at the rate of 5 per infested plant into one of the glasshouses for each of the test species. The plants in each of the glasshouses were harvested at a point just before unparasitised larvae were about to pupate, and the number and weight of insects remaining on the plants was determined and the extent of parasitism recorded. Damage to the plant was also determined through counting damaged leaves and attributing scores to the severity of the damage such that leaves with greater than 50% eaten were given a score of 2, while lesser damage was given a score of 1. Glasshouse conditions varied with the prevailing weather such that night-time lows of around 10°C and daytime highs of >30°C were recorded.

RESULTS

Host Range

Meteorus gyrator parasitised all of the candidate host species presented to it (Figure 1). The wasp showed a significant preference for *L. oleracea* larvae over all of the other host species (ANOVA, $P<0.05$), although *M. brassicae* and *S. exigua* were also readily parasitised. *Spodoptera littoralis* and *C. chalcites* were parasitised much less frequently, with less than 0.5 hosts per wasp parasitised over the 48 h exposure period.

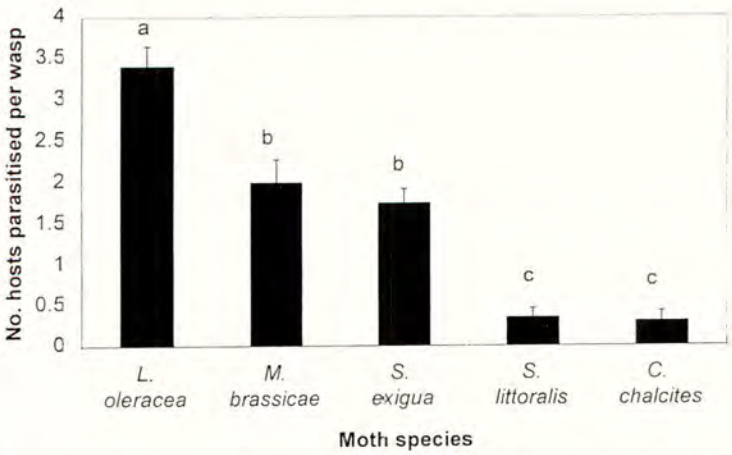

Figure 1. The host preference of *M. gyrator*. Columns labelled with different letters are significantly different.

we sought to document the effects of this wasp on the growth, development and food consumption of *C. chalcites* under laboratory conditions. Finally, small-scale glasshouse trials were conducted to provide a preliminary assessment of the performance of this wasp against infestations of *L. oleracea* and *C. chalcites* under simulated field conditions.

MATERIALS AND METHODS

Host preference

To determine the preferred host of *M. gyrator*, the parasitoid was exposed to a range of pest noctuid species. Early third instar hosts of *L. oleracea*, *C. chalcites*, *Mamestra brassicae*, *Spodoptera littoralis and Spodoptera exigua* were selected from laboratory cultures kept at 25°C, 70% r.h., L/D 16h/8h. Larvae of this developmental stage were chosen as previous studies had shown them to be the preferred host stage for *M. gyrator* (Bell *et al.* in press). Five insects of each species were placed in plastic boxes (150 × 150 × 57 mm) and supplied with maize-based artificial diet (Korano, La Balmes-les-Grottes, France). Into each box a single mated *M. gyrator* was introduced and allowed to forage for 48 h after which all larvae were recollected. The larvae were subsequently dissected and the number of each species parasitised by the parasitoid was recorded. The experiment was repeated 20 times, and was conducted at the conditions described above.

The effect of parasitism on *C. chalcites*

Forty third instar *C. chalcites* were taken from laboratory culture and exposed overnight to 10 *M. gyrator* females, a number of wasps sufficient to parasitise a significant proportion but not all of the available hosts. Following wasp exposure, the larvae were placed in individual plastic pots (125 ml) and supplied with pre-weighed amounts of tomato leaf. The larvae were kept at 20°C, 70% r.h., L/D 16h/8h and weighed daily until the emergence of the wasp or until the caterpillar had pupated. Fresh leaves were supplied every second day and, to prevent wilting, the cut ends of the leaves were pushed into a water-filled 1.5 ml microcentrifuge tube through a hole that had been pieced in the lid. Uneaten leaves was removed, dried in an oven (90°C, 24 h) and weighed. The dry weight of food initially provided to each larva was calculated through drying leaf samples, the quantity of food consumed being the dry mass of food provided minus the dry weight of uneaten food. The effect of parasitism on the area of leaf consumed by *C. chalcites* larvae was also determined. Ten larvae of *C. chalcites* were parasitised as described above and provided with single tomato leaves at three, five and seven days after parasitism. Leaves were prevented from wilting by the method described above. Leaf area before feeding was calculated through scanning each leaf and analysing the image with Delta-T SCAN© software. After 24 h of feeding the leaves were rescanned and the total area of leaf consumed determined. Control larvae were of the same age as parasitised larvae but had been exposed to male wasps only. Ten replicates were completed for both control and parasitised larvae, at each time point.

Meteorus gyrator: a potential biocontrol agent against glasshouse noctuid pests?

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ABSTRACT

Glasshouse crops are subject to attack by a range of very damaging noctuid caterpillars. There is an urgent need to develop alternative, biological methods for their suppression. Here we present evidence that the endoparasitic braconid *Meteorus gyrator* will differentially parasitise a number of significant noctuid pests. The biology of *M. gyrator* on *Lacanobia oleracea* (an important noctuid pest of glasshouse tomatoes in the UK) has been presented elsewhere, but here we show that, under laboratory conditions, this wasp also shows considerable potential as a biocontrol agent against the Turkish moth, *Chrysodeixis chalcites*, a very serious pest of protected crops in mainland Europe: we record an 80% reduction in the weight of leaf-tissue eaten by parasitised *C. chalcites*, and a similar highly significant reduction in the area of leaf consumed. Small-scale glasshouse trials confirm that even low dose, inoculative releases of *M. gyrator* suppress the amount of crop damage caused by populations of *L. oleracea*, and rapidly reduce numbers of larvae feeding on tomato plants. However, the potential shown by *M. gyrator* against *C. chalcites* in the laboratory is not borne out under simulated field conditions. The reasons for the contrasting performance of this wasp against these two pests are discussed.

INTRODUCTION

The larvae of several species of noctuid moths occur as frequent pests in tomato glasshouses in Europe and the UK. In the British Isles the tomato moth, *Lacanobia oleracea*, is the most serious problem (Jacobson, 2000), whereas in continental Europe the Turkish moth, *Chrysodeixis chalcites* has become increasingly problematic in recent years (De Clerq *et al.*, 1998). Whilst both species can be controlled by chemical pesticides, increasing deployment of predatory or parasitic biocontrol agents against other horticultural pests has resulted in a general reduction in conventional insecticide usage. Although biocontrol agents are available for use against noctuid caterpillars (e.g. *Bacillus thuringiensis* formulations and *Trichogramma* egg parasitoids) these are not universally successful. As a result, there is now a pressing need to develop further alternative control measures for these pests, which are compatible with modern growing techniques and existing biological control practices.

Laboratory studies have revealed that the solitary endoparasitoid *Meteorus gyrator* (Hym.: Braconidae) has many traits that may allow it to perform well as a biocontrol agent against *L. oleracea* larvae. Important characteristics include its high fecundity, rapid rate of development, and a rapid and marked reduction in food consumption by parasitised hosts (Bell *et al.*, in press). However, little data is available as to its preference for, or performance against, other noctuid species. The first of three experiments described below was designed to record any oviposition preferences of *M. gyrator* towards a range of noctuid hosts. Secondly,

more time is needed to train people to carry out pest trapping correctly and to be able to identify the insects they have found. Software to receive these messages was available but the security of MTT's computer systems required further problem solving.

After two years, it has already been decided that the new pest and disease service will be continued by a private company while MTT will focus on the validation of the pest and disease models and the generation of new service systems. Close collaboration between the researchers and the service provider will be necessary for effective quality control of the system.

ACKNOWLEDGEMENTS

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20.06.2000 13:34:34:0400637***:F HEKÄÄAO HEKÄÄBO
20.06.2000 15:38:37:0405855***:F hekääA1#hekääB0
20.06.2000 21:09:22:0405676***:F hekääA0#hekääB0
20.06.2000 21:13:17:0407162***:F hekääA 0#hekääB0
21.06.2000 08:29:15:0400663***:F POKEMA0#POKEMB0POKEMC0
21.06.2000 08:31:09:0400663***:F POKÄRA0#POKÄRB0#POKÄRC0
21.06.2000 20:13:49:0400637***:F HEKÄÄA1 HEKÄÄBO
22.06.2000 15:06:02:0405855***:F hekääA0#hekääB0
22.06.2000 17:32:37:0400595***:F hekääA1OhekääB2O
22.06.2000 19:50:41:0405676***:F hekääA3#hekääB0
22.06.2000 23:31:36:0407162***:F hekääA3#hekääB1
23.06.2000 08:40:14:0405553***:F hekääA0 #hekääB0
23.06.200014:49:14:0400536***:FpokemA97#pokemB59#pokemC190#pokemD124#pokemE34#
23.06.2000 18:55:27:0400637***:F HEKÄÄA3 HEKÄÄB1
24.06.2000 16:17:28:0405855***:F hekääA1#hekääB0
24.06.2000 18:35:42:0407162***:F hekääA 0#hekääB1
24.06.2000 19:05:35:0405676***:F hekääA0#hekääB4
25.06.2000 07:09:09:0407305***:F pokemA1#pokemB2#pokemC2#pokemD1#pokemE2#
25.06.2000 12:40:46:0400595***:F hekääA10#hekääB20
25.06.2000 23:02:10:0405553***:F hekääA0 #hekääB0
26.06.2000 09:34:52:0400637***:F HEKÄÄA1 HEKÄÄB1
26.06.200009:34:53:0400536***:F okemA67#pokemB30#pokemC129#pokemD33#pokemE14#
26.06.2000 12:28:36:0400722***:F hekääA0#hekääB0
26.06.2000 13:04:35:0405855***:F hekääA0#hekääB0
26.06.2000 14:15:12:0407092***:F pokärA2#pokärB0#pokärC3#pokärD2#
26.06.2000 14:19:26:0407092***:F pokemA2#pokemB3#pokemC5#pokemD8#
26.06.2000 14:20:23:0407092***:F kakoiA5#kakoiB5#kakoiC3#kakoiD5#
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26.06.2000 14:59:08:0405155***:F pokemA8#pokemB4#pokemC9#pokemD12#pokemE8#

Potato late blight monitoring was arranged by sending a letter to selected farmers, including organic farmers. The letter included instructions on how to sample plants for late blight in the field which were later checked for the disease at MTT.

DISCUSSION

The reciprocal flow of pest and disease information was found to be very satisfactory for the farmers involved in the project according to a questionnaire sent out after the 1999 season. Many other farmers were also willing to send information as SMS text messages to MTT and most of them were ready to pay for the service in the future. The farmers main reason for using the service was the belief that correctly timed monitoring and treatment of pests and diseases could provide more ecologically and economically efficient control with optimized use of their time and other resources.

Automatic information flow from pest and disease models to farmers is therefore possible, but more time than expected was needed to reconcile the different software packages used before the system was reliable. The weather data used in the project is liable to charge and is therefore a big factor when fixing the price of such a service. However, it is possible in the future that weather data could be provided by farmers and companies themselves using their own weather stations. This would in many cases lead to the development of a more accurate and possibly field-specific forecasting service for farmers. Obtaining the necessary pest and disease monitoring information as SMS text messages from farmers was relatively easy to organize. After a short exercise they were able to send standard information. However, much

"The first flight of the carrot fly is starting. Check your sticky traps weekly" was a message sent to farmers growing carrots in the area where the first flight of carrot fly was forecasted (Figure 2).

Farmers growing cauliflower, cabbage or swede got the message "The second flight of the cabbage root fly is starting. Count eggs weekly" when the second flight of cabbage root fly was predicted (Figure 3).

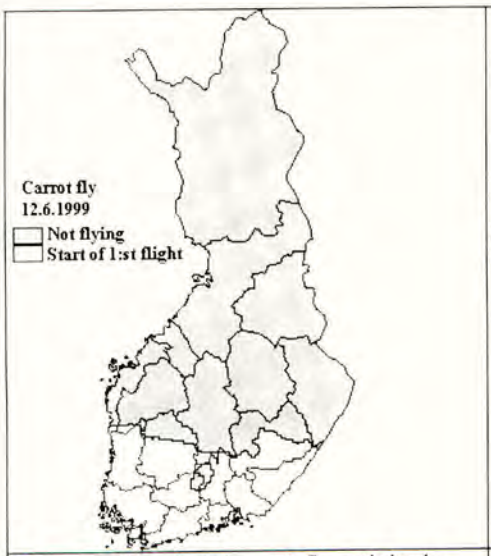

Figure 2: Forecasted carrot fly activity in June 6 1999.

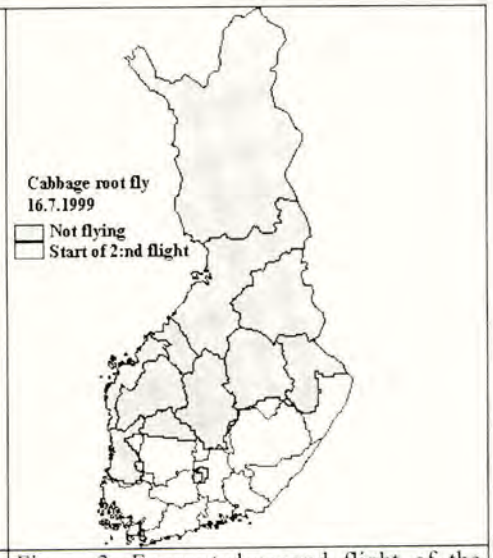

Figure 3: Forecasted second flight of the cabbage root fly was starting in southern Finland in July 16.

Potato farmers got the following message when potato late blight was forecasted: "There is a risk of potato late blight. Keep an eye on your crop. A first fungicide application may be required if wet weather continues and if susceptible cultivars are used."

Response messages

In 1999, 150 text messages were received mainly from pea growers concerning the appearance of pea moth. Messages were received by mobile phones in the year 1999 and then entered into the MTT database. In the year 2000 messages received were processed automatically and only validation of the incoming data was done manually. 110 messages had been received by the beginning of July.

An example of messages sent by farmers and advisers at the beginning of the growing season 2000 is shown below. This was the "raw data" as it was before any data management,(*** added for privacy protection). The order of the information per line is: date, time, phone number, data signal, pest code, trap code, number of the pest and # to separate trap specific information. Pest code HEKÄÄ means pea moth, POKEM is carrot psyllid, POKÄR carrot fly, KAKOI diamondback moth and KAKÄR cabbage root fly.

Commercial SONERA software was used for sending the SMS messages from the field to the MTT server where a special procedure of data management was developed for saving the data to a special database.

RESULTS

The number of farmers participating in the project was 272 of which 44 were growing potatoes, 128 peas, 57 carrots, 13 swede, 4 cabbage and 26 cauliflower. All the farms were in the south-western part of Finland (Figure 1).

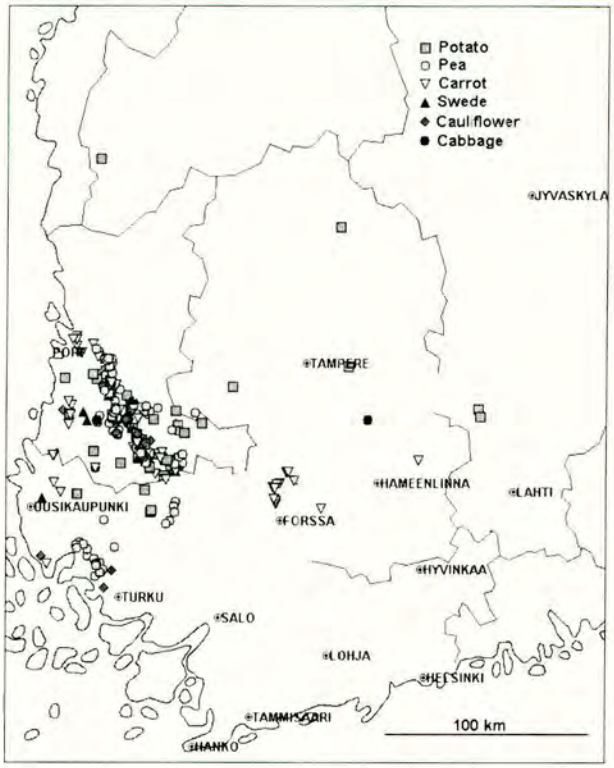

Figure 1: Farms participating in the project in south-western Finland.

Messages sent to farmers

During the 1999 growing season, 30 different text messages were sent from MTT to all farmers, (1000 SMS messages in all) and the capability to fully automate message delivery was achieved for the growing season in 2000.

METHODS AND MATERIALS

Forecasts and warnings

Forecasts were based on the models of MTT predicting carrot fly (*Psila rosae*) and cabbage root fly (*Delia brassicae*) activity (Markkula *et al.*, 1998.) Forecasts for bird-cherry aphid (*Rhopalosiphon padi*) (Kurppa, 1989) and pea moth (*Cydia nigricana*) activity were also used. Diamondback moth (*Plutella xylostella*) warnings were based on the use of entomological radar (Tiilikkala *et al.*, 1996) whenever the information was available on the World Wide Web home page of the University of Helsinki (www.helsinki.fi/~mleskine/kaiku/kaiut.html). The NegFry system was used to estimate the risk of primary attacks of potato late blight (*Phytophthora infestans*) (Hannukkala, 1998).

Meteorological data provided by the Finnish Meteorological Institute (FMI) were used as input information in all models. The data were received from 37 automatic meteorological stations once a day or every third hour for the late-blight forecast. All values were interpolated for every grid point of the mapping system by means of SAS and ARCInfo software. For the Agronet Services, results were visualized using ARCInfo TM software and the activity of pests displayed as thematic maps on the Agronet internet site.

Potato late blight forecasts were made using weather station data and the NegFry decision support system (Grønbech-Hansen *et al.*, 1995). NegFry is based on two models; one estimating the risk of primary attacks (Ullrich & Schrödter 1966), and the other timing subsequent fungicide applications (Fry *et al.*, 1983). For predictions, temperature, humidity and rain data were required and the disease forecasts were visualized using MapInfo software and displayed as a thematic map.

For the delivery of SMS messages, grid specific output values of the models were used as input values in the MTT's GIS system selecting target farmers from the project area. Basic information from the farms participating in the project were collected and stored in a special database which included: 1) cultivated plant, 2) location of fields, 3) date of sowing or planting time and 4) contact information of the farmer such as GSM phone number and E-mail address.

A critical part of the project was to find out how to combine the different software packages used for the interpolations, modelling, mapping, selection of target farms and the automatic sending of relevant messages to the right farmer at the correct time.

Response messages

A selected group of farmers and advisers monitored their fields daily and sent SMS text messages concerning the appearance of insect pests on their traps. Yellow sticky traps were used for monitoring the activity of the carrot fly and the carrot psyllids. A pheromone trap was used for monitoring migration of the pea moth. Activity of cabbage root fly and turnip root fly were estimated by counting eggs laid on cabbage plantings. All the monitoring methods were standard methods used in IP production of field vegetables in Finland.

Pest warnings and forecasts sent as SMS messages from models into "farmer's pocket"

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ABSTRACT

The Agricultural Research Centre of Finland (MTT) has developed a new pest and disease early warning system for Finnish farmers based on the use of mobile telephones and the internet. With this new information technology, farmers could receive warnings of approaching pest or disease problems as SMS (text) messages and read details about risks from MTT's electronic database and Internet service. Some farmers could also respond with text messages reporting the presence of any pests or diseases.

INTRODUCTION

New information technology was developed and tested for rapid pest and disease information delivery to farmers in a two year project. Farmers themselves took the initiative in the project which focussed on the use of mobile telephones rather than the internet. Although more than half of Finnish farmers have PCs and access to the internet, it was found that they were reluctant to spend time online to find out information about forecasted outbreaks of pests and diseases unless they knew it was relevant to their own decision making. However, specified text messages sent "into their pockets" while working in the field, was much preferred.

The aim of this project was therefore to develop a rapid communication system between researchers and farmers which would support farm-specific decision making during the growing season. In addition, another aim was to gather data for validation of pest and disease forecasting models by using real-life data sent by farmers and advisers to the MTT database.

The application of this new information technology was based on earlier modelling work at MTT and the information system by which pest and disease forecasts and warnings were delivered via agricultural newspapers and the Agronet Services. At the beginning of the 1999 growing season, SMS text messages (short message service for GSM technology) and Agronet were linked together to operate as a simultaneously functioning system. In the year 2000 the focus was on the management of the response data sent by farmers and regional advisers in the pilot scheme.

The target group of the project was chosen from IP-farmers (integrated production) producing field vegetables, peas and potatoes for the food manufacturing industry. Communication technology was based on the use of software designed to send and receive large numbers of SMS messages.

The finding that cyromazine and fipronil seed treatments applied at greatly reduced application rates were as or more effective against OM than granular in-furrow insecticide applications and that when chlorpyrifos and phosetbupirin+cyfluthrin granulars were included with fungicide(s), OS incidence was lower, suggests that placement may be a factor. Perhaps applying the insecticide as a seed treatment protects young onion bulbs from OM damage more efficiently than a granular in-furrow application. Alternatively, a granular in-furrow fungicide may help to control OS, because its spores are immobile and germinating hyphae must traverse the soil matrix to reach a germinating onion seedling (McDonald, 1994). A granular in-furrow insecticide may prove an additional barrier. More detailed research is required to understand the dynamics of pesticide placement in the control of OM and OS. It is also important to consider the dynamics of applying both insecticide and fungicide together as seed treatments.

ACKNOWLEDGEMENTS

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Table 3.Effectiveness of insecticide/fungicide combinations for onion smut control, 1999.

Treatment	Incidence of Onion Smut (%)				
	1st true leaf 7 June[1]	4 true leaf 25 June	6-7 true leaf 12 July	9-10 true leaf 17 August[1]	harvest 15-17 Sept[1]
Untreated	54.7a[2]	24.6b	26.4a	20.0a	20.5ab
CT	19.4cd	13.2c	4.17d-f	3.18d	4.03ef
MAN	28.2bc	14.6c	11.8bc	1.60d-h	7.11e
CHL	26.0bc	12.5cd	11.4bc	9.56c	12.4cd
CT + CHL	8.37ef	3.40ef	1.74ef	0.96e-i	1.59f-h
MAN + CHL	20.1cd	15.3c	4.85d-f	1.60d-g	4.67ef
PC	40.0ab	28.4b	15.6b	13.1bc	15.4bc
CT + PC	11.0d-f	2.52ef	2.42ef	0.66f-i	1.43f-h
MAN + PC	28.9bc	7.83c-f	9.22cd	2.49de	4.56ef
CYR	51.8a	32.5ab	27.2a	14.2ab	22.5a
CT + CYR	12.1de	3.51ef	4.06d-f	4.25de	3.06e-g
MAN + CYR	35.2b	13.3c	7.21c-e	2.14d-f	6.95de
FIP	34.5b	39.7a	22.7a	12.7bc	19.7ab
CT + FIP	20.8cd	11.2c-e	8.51cd	2.62de	3.32e-g
MAN + FIP	31.1bc	14.4c	9.35cd	2.68de	5.13e
CT + MAN	6.56e-g	3.40ef	0.92f	0.48hi	1.11g-i
CT + MAN + CHL	1.66g	1.49f	1.01f	0.33g-i	0.58hi
CT + MAN + PC	5.36e-g	4.09d-f	0.58f	0.26g-i	0.00i
CT + MAN + CYR	4.84fg	0.89f	0.82f	0.59g-i	0.13i
CT + MAN + FIP	4.46e-g	2.54ef	0.69f	0.00i	0.49hi

[1] Statistics performed on arcsin/x transformed data.
[2] Numbers in a column followed by the same letter are not significantly different at p=0.05, Fisher's Protected LSD test. Granular In-Furrow = CHL: chlorpyrifos (insecticide); MAN: mancozeb (fungicide); PC: phosetbupirin+ cyfluthrin (insecticide). Seed Treatments = CT: carbathiin+thiram (fungicide); CYR: cyromazine (insecticide); FIP: fipronil (insecticide).

DISCUSSION

Effective control of OM and OS was achieved with insecticide treatments in combination with carbathiin+thiram plus mancozeb. Cyromazine, fipronil, and phosetbupirin+cyfluthrin provided excellent OM control, while even at twice the recommended rate, chlorpyrifos was not as effective. Best OS control was achieved when chlorpyrifos was included in the treatment combination. The observation that the best OM control occurred with treatments that also controlled OS most effectively suggests that a biological interaction may be taking place. Onions are susceptible to OS for two-three weeks after germination, after which time they resist further infection. Thus, onions are infected by OS first. OS infection is often accompanied by secondary soft rot bacteria and the rotting produce is attractive to ovipositing OM (Hausmann & Miller, 1986). Therefore, it is conceivable that when OS is effectively controlled, OM is less as likely to thrive. Chlorpyrifos significantly reduced OS suggesting that it has fungistatic properties. It has fungistatic activity against *Sclerotium rolfsii*, the causal organism of southern stem rot, and has been recommended for control of this disease. Cyromazine, when used without a fungicide had the same incidence of OS as the untreated check suggesting that it does not cause an increase in OS.

combination with fipronil and cyromazine. OS incidence was as high in the treatments with carbathiin+thiram as it was in the treatments with mancozeb.

Chlorpyrifos alone significantly reduced OS in comparison to the untreated check in all assessments. Carbathiin+thiram plus chlorpyrifos or phosetbupirin+cyfluthrin significantly reduced incidence of smut in comparison to carbathiin+thiram.

Table 2. Effectiveness of insecticide/fungicide combinations for onion maggot control, 1999.

Treatment	Onion Maggot Damage (%)		
	1st gen (12 July)	1st & 2nd gen (17 Aug)	1st, 2nd & 3rd gen (22 Sept)
Untreated	21.2a[1]	17.7a	24.1a
CHL	4.51b-d	6.99bc	7.43bc
PC	2.89b-e	11.5ab	8.76b
CYR	2.07b-e	4.58cd	8.85b
FIP	2.86b-e	3.74cd	5.27b-d
CT	8.59bc	4.01cd	3.13c-g
CHL + CT	1.67c-e	0.33ef	4.81c-f
PC + CT	0.57de	0.00f	0.43gh
CYR + CT	0.26e	1.82c-e	1.33e-h
FIP + CT	0.00e	0.30ef	2.13d-h
MAN	13.4ab	2.55c-e	7.49bc
CHL + MAN	2.79b-e	0.60ef	2.88c-g
PC + MAN	0.74de	2.62c-e	5.13b-e
CYR + MAN	0.31e	0.00f	1.74d-h
FIP + MAN	4.82b-d	2.07de	4.75b-e
CT + MAN	2.67b-e	4.28c-e	3.46c-f
CHL + CT + MAN	0.00e	0.59ef	1.18f-h
PC + CT + MAN	0.81de	0.60ef	1.67e-h
CYR + CT + MAN	0.60c-e	0.40ef	0.44h
FIP + CT + MAN	0.24e	0.00f	0.61f-h

[1] Numbers in a column followed by the same letter are not significantly different at p=0.05, Fisher's Protected LSD test. **Granular In-Furrow** = **CHL**: chlorpyrifos (insecticide); **MAN**: mancozeb (fungicide); **PC**: phosetbupirin+ cyfluthrin (insecticide). **Seed Treatments** = **CT**: carbathiin+thiram (fungicide); **CYR**: cyromazine (insecticide); **FIP**: fipronil (insecticide).

RESULTS

1998 Experiment

Significant differences were found at all assessments except at the 3rd OS assessment (Table 1). No significant differences were found between the two cultivars in the untreated checks. Although not significantly different, fipronil was most effective against OM followed by cyromazine, phosetbupirin+cyfluthrin and chlorpyrifos. Although not significant, in-furrow treatments with chlorpyrifos and phosetbupirin+cyfluthrin had less OS than treatment with fungicide only; cyromazine and fipronil seed treatments had the same or a slightly higher incidence of OS.

Table 1. Effectiveness of insecticides in combination with carbathiin+thiram+mancozeb for the control of onion maggot and onion smut, 1998.

Treatment	Onion Maggot Damage (%)			Incidence of Onion Smut (%)	
	1st gen (9 July)	1st & 2nd gen (17 Aug)	1st, 2nd & 3rd gen (22 Sept)	1st true leaf (25 June)	3rd-4th true leaf (21 July)
Untreated (cv. Cortland)	29.3a[1]	28.4a	30.0ab	73.9ab	47.6a
Untreated (cv. Tribute)	23.3a	33.4a	53.1a	84.4a	60.0a
CT + MAN	25.7a	24.7ab	5.02c	56.5a-c	6.35b
CT + MAN + CHL	4.88b	12.1bc	3.29c	40.5c	2.53b
CT + MAN + PC	4.17b	5.33c	2.12c	46.2bc	4.93b
CT + MAN + CYR	1.65b	3.88c	1.83c	52.8bc	9.68b
CT + MAN + FIP	1.16b	1.53c	0.43c	64.8a-c	7.53b

[1] Numbers in a column followed by the same letter are not significantly different at p=0.05, Fisher's Protected LSD test. Granular In-Furrow = CHL: chlorpyrifos (insecticide); MAN: mancozeb (fungicide); PC: phosetbupirin+cyfluthrin (insecticide). Seed Treatments = CT: carbathiin+thiram (fungicide); CYR: cyromazine (insecticide); FIP: fipronil (insecticide).

1999 Experiment

Significant differences were found among OM treatments at all assessments (Table 2), but not for final yield. No significant interaction between insecticides and fungicides occurred. No consistent significant differences or trends among insecticides across assessments or with fungicide combinations were apparent. Insecticide combinations with carbathiin+thiram or carbathiin+thiram+mancozeb were more effective than those with no fungicide(s). Phosetbupirin+cyfluthrin was more effective when used in combination with carbathiin+thiram. When fipronil was used in combination with mancozeb, OM damage was significantly higher than when it was used in combination with carbathiin+thiram+mancozeb and carbathiin+thiram in the 1st assessment and with carbathiin+thiram+mancozeb in the last two assessments. Significant differences were found among OS treatments at all assessments (Table 3), but not for final yield. A significant interaction between insecticides and fungicides was found at the six-seven and nine-10 true leaf stages. Treatment combinations with carbathiin+ thiram +mancozeb had the least incidence of OS, followed by those with carbathiin+thiram and then those with mancozeb. An exception to this trend was observed when these fungicides were used in

MATERIALS AND METHODS

1998 Experiment

A preliminary study was conducted to evaluate the efficacy of chlorpyrifos, cyromazine, phosetbupirin+cyfluthrin and fipronil in combination with carbathiin+thiram and mancozeb. Formulations and application rates were: 1) Carbathiin+thiram - seed treatment, film-coated at 20 g a.i./kg of seed; 2) Cyromazine - seed treatment, film-coated at 50 g a.i./kg of seed; 3) Fipronil - seed treatment was film-coated at 25 g a.i./kg of seed; 4) Mancozeb - granular in-furrow applied at 6.6 kg a.i./ha at seeding; 5) Chlorpyrifos - granular in-furrow applied at 4.8 kg a.i./ha at seeding and 6) Phosetbupirin+cyfluthrin - granular in-furrow applied at 0.5 kg a.i./ha at seeding. Raw seed was included in untreated checks and the trial was arranged as a randomized complete block design with seven treatments and four replications. The trial was conducted at the University of Guelph - Muck Crops Research Station (MCRS) located at the Holland Marsh, Ontario. Each treatment plot consisted of 4x6m rows of onions spaced 40 cm apart seeded at 47 seeds/m on 15 May using a push V-belt seeder. Eight separate 2m sections were randomly selected for each of four OM assessments, three OS assessments and final yield. To determine initial stand, emergence counts were taken on 2, 9 and 16 June in each 2m section. OM damage was assessed at the end of 1st (9 July), 2nd (17 August) and 3rd (22 September) generations as determined by monitoring onion fly trap catches and degree days. In the two sections designated for the 1st OM assessment, plants were assessed twice weekly for damage from 16 June until 7 July and damaged plants were counted and removed. OS incidence was assessed at the 1st (25 June) and 4th (21 July) true leaf stages, and at harvest (22 September) by visual observation and destructive sampling. Harvest weight was taken from the remaining 2m section of onions on 6 October. Data were analyzed using the General Analysis of Variance function of the Linear Models section of Statistix, V.4.1

1999 Experiment

Four insecticide (chlorpyrifos, cyromazine, phosetbupirin+cyfluthrin, fipronil) and three fungicide combinations (carbathiin+thiram, mancozeb, carbathiin+thiram+mancozeb) and untreated checks were evaluated using a 4 x 5 factorial randomized complete block design with 20 treatments and four replications. Pesticides were applied at the rates used in 1998 and all seed treatments were commercially film-coated using the same cultivar (cv. Cortland). Onions were sown at MCRS on 4-6 May and emergence counts were taken on 21, 25, 28 May and 2 June. Dying onions, other than those in the plot designated for the yield assessment, were pulled and cause of death (OM, OS, OM + OS or other) was recorded. This was done twice weekly from 7 June to 12 August. OS incidence also was assessed in the sections designated for OM damage assessments and vice versa. These data were collected to account for the loss of each onion plant from the original stand to make the results more accurate and meaningful. This was done twice weekly from 7 June to 12 August. In 1999, only a 1x2m section was used for the 1st generation OM damage assessment. OM damage assessments were made on 12 July, 17 August and 22, 23 September after the 1st, 2nd and 3rd OM generations, respectively. OS assessments were made on 7 June (1st true leaf), 25 June (3rd and 4th true leaf), 12 July (6th and 7th leaf; 1st OM), 17 August (9th and 10th leaf; 2nd OM) and 15-17 September (harvest). On 21 September, harvest weight and bulb size were recorded. Interaction between insecticides and fungicides was analyzed using a 4 x 5 factorial analysis of variance.

Evaluation of insecticide and fungicide combinations for the control of onion maggot (*Delia antiqua*) and onion smut (*Urocystis cepulae*) in Ontario

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ABSTRACT

A study was conducted to determine the effectiveness of combinations of insecticides (chlorpyrifos, cyromazine, phosetbupirin+cyfluthrin and fipronil) and fungicides (carbathiin+thiram, mancozeb, and carbathiin+thiram+mancozeb) for control of onion maggot (OM) (*Delia antiqua*) and onion smut (OS) (*Urocystis cepulae*) in onions grown in Ontario. Effective OM and OS control was achieved with insecticide treatments in combination with carbathiin+thiram+mancozeb. Cyromazine and fipronil seed treatments provided best OM control. Best OS control was achieved with carbathiin+thiram seed treatment+granular in-furrow applications of mancozeb and chlorpyrifos.

INTRODUCTION

Onion maggot (OM) (*Delia antiqua*) and onion smut (OS) (*Urocystis cepulae*) are the most economically important pests of onions grown in Ontario, Canada. In the absence of control measures, stand losses from either pest can exceed 50% (Ritcey & Harris, 1995; McDonald *et al.*, 1996). Onions with OM damage or OS infection are unmarketable at harvest. To control OS, carbathiin+thiram ('ProGro 30/50D') is used as a seed treatment and mancozeb ('Dithane DG 75G') is applied in-furrow at the time of seeding. To control OM, chlorpyrifos ('Lorsban 15G') is applied as a granular in-furrow treatment at seeding. These pesticides have been used for years and are becoming less effective due to development of OM resistance to chlorpyrifos (Harris & Svec, 1976) and the occurrence of extremely high soil densities of OS. Recent studies have shown that some insecticides are effective against OM when applied as seed treatments (Ritcey and Harris, 1995). In 1997, cyromazine ('Governor 75WP') was registered as a seed treatment for OM. Although very effective some growers reported a higher incidence of OS. There also was concern a similar problem might occur with other promising insecticides including phosetbupirin+cyfluthrin ('Aztec 2/0.1G'), and fipronil ('Regent 80WG'). Since pesticides used for OM and OS control are applied at the same time and in the same place the opportunity exists for interactions to occur. Our objective was to evaluate the efficacy and interaction of insecticide and fungicide combinations applied for OM and OS control.

Applying prochloraz-manganese in 90 litres H_2O/100 m^2 rather than the standard 180 litres H_2O /100 m^2 resulted in a greater proportion of the active ingredient being retained in the top layer of casing, at least following the first spray. This may give better control of disease development initially but the subsequent falling levels of a.i. over time would reduce this benefit.

CONCLUSION

Total disease control by prochloraz-manganese after day 28 (i.e. third flush onwards) is unlikely to occur, as by this time much of the a.i. in the casing has disappeared, resulting in a fungicide concentration that fail to inhibit even the more sensitive isolates. Thus, observations of poor efficacy in later flushes are likely to be partly as a result of this loss of a.i. However this work demonstrates that prochloraz manganese still significantly controlled dry bubble disease, caused by two isolates with different sensitivities to this fungicide, up to the end of the second flush. There was a greater percentage reduction in symptoms for the more sensitive isolate but this advantage was cancelled out due to its more aggressive nature.

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Prochloraz -manganese levels in casing

Prochloraz -manganese was recovered from casing throughout the duration of the crop. The amount recovered however dropped significantly with time for both fungicide treatments, and following both sprays, to a point where < 25% of the total a.i. applied (110 g. a.i./100m^2) was recovered on day 21 or day 28 (Figure 3). This equates roughly to a concentration in the casing of < 9 ppm. Both isolates can tolerate this concentration so that disease establishment during the crop cycle should be possible, despite the use of fungicide.

A standard single spray of prochloraz-manganese (120 g a.i./100m^2) fungicide provides 55 g a.i./100 m^2, resulting in an approximate initial concentration in casing of 17.5 ppm per spray. When this was applied on day 3 in 180 litres H_2O/100 m^2, the amount recovered was much lower than expected at 34 g a.i./100 m^2 (10.8 ppm) whereas when it had been applied in only 90 litres H_2O /100 m^2, the amount recovered was only just higher than expected at 63 g a.i./100 m^2 (20 ppm). Some of this difference is likely to reflect difficulties in applying a set volume of liquid to a set area of mushroom bed so that a degree of over or under dosing is likely to occur. However, the greater the volume of water applied per unit area then the greater the likelihood that some of it will drip through to run-off, particularly if a wet casing-mix is used, resulting in a corresponding loss of some active ingredient. This appears to have been the case for the treatment on day 3 using 180 litres H_2O /100 m^2 but not for that using only 90 litres H_2O /100 m^2.

Good recovery of the expected 55 g a.i./100 m^2 was achieved following the application of the second spray on day 21when applied in either 180 or 90 litres H_2O /100 m^2 (Figure 3). At this stage, the casing is drier following the uptake of water by growing mushrooms so, providing the casing structure is still good, fungicide drenches are likely to be held within the casing layer with little or no run-off. The rate of disappearance appears to be faster after the second spray compared with the first and may suggest microbial degradation.

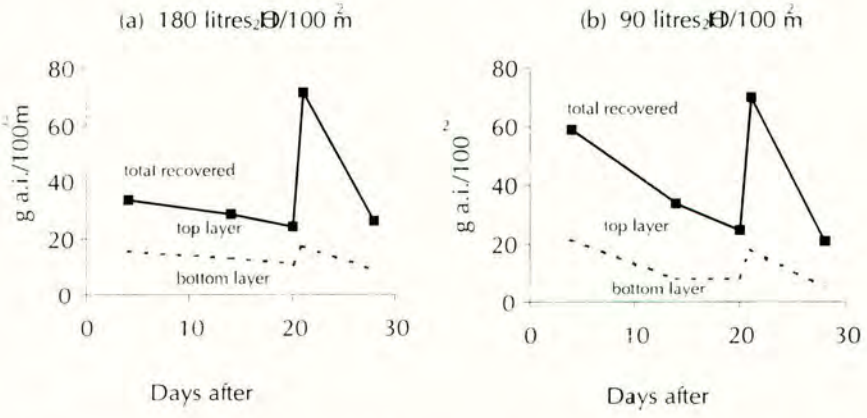

Figure 3. Amount of prochloraz-manganese recovered from casing following applications of two standard sprays (2 x 55 g a.i), applied on Days 3 and 21, in either (a) 180 litres H_2O/100 m^2 or (b) 90 litres H_2O/100 m^2. LSD values for log_e transformed data: comparisons within (a) and (b) LSD = 0.71; comparisons between (a) and (b) = 0.76.

higher for isolate 620, compared with isolate 182, due to the higher level of symptom development in the control treatment. There was no appreciable reduction in symptom expression when prochloraz-manganese had been applied in 90 litres rather than 180 litres $H_20/100\ m^2$, under the conditions of the experiment.

In the control treatment receiving no fungicide, there was a strong suggestion that isolate 620 was a more aggressive pathogen than isolate 182, with higher levels of dry bubble and spotting symptoms developing at the rate of inoculation used (Figure 2). This observation was confirmed in subsequent experiments (*Grogan,* unpublished), particularly for the dry bubble symptom. Therefore, although isolate 620 is more sensitive to prochloraz-manganese than isolate 182, it will nonetheless be difficult to control due to its tendency to produce more symptoms than isolate 182.

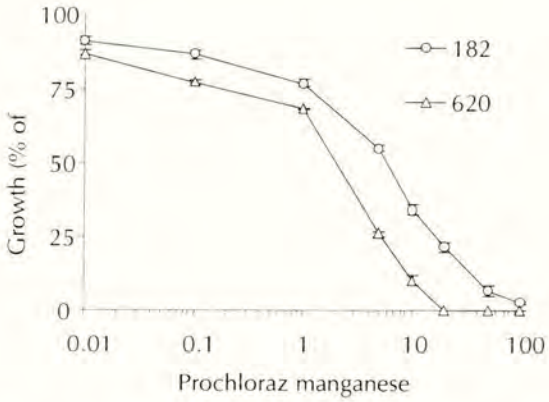

Figure 1. *In vitro* response of two *Verticillium* isolates to prochloraz -manganese over a series of experiments (mean ± SE).

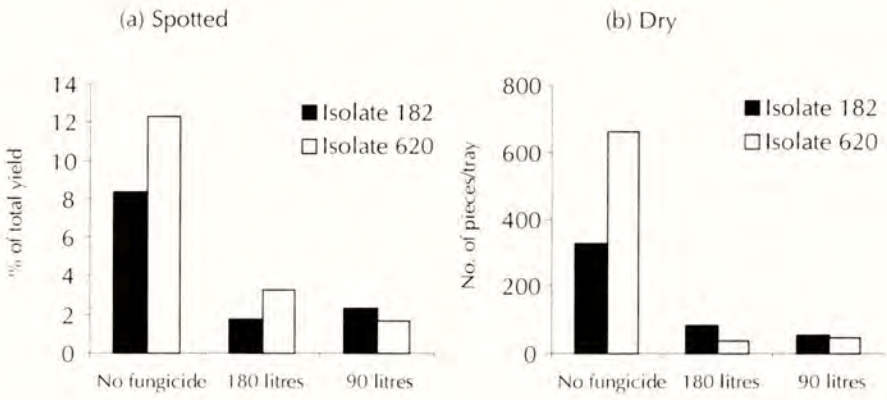

Figure 2. (a) Yield of spotted mushrooms over 2 flushes as % of total yield; LSD = 2.04. (b) Number of bubble pieces harvested over 2 flushes; LSD for square-root transformed data = 3.02.

of spawned compost (*Agaricus* strain Sylvan A12) and incubated (spawn-run) for 17 days at 25°C. Trays were cased with a 40-50 mm layer of black peat/sugar beet lime casing (Tunnel Tech Ltd., Stockbridge, Hants.) and further incubated (case-run) at 25°C. Fresh air was introduced into the cropping chamber on day 7-8 after casing, over a three-day period (airing), during which time the air temperature was reduced to 18°C. Harvested mushrooms were classified as either healthy or spotted. Pieces of dry bubble were also picked off at intervals during the crop. Control plots were picked before diseased plots to minimise cross contamination. The first "flush" of mushrooms was harvested from day 17 to 21; the second spray of prochloraz-manganese was applied to relevant trays when harvesting was completed on day 21 with water being applied to control trays; the whole crop was further watered on days 22 and 23 and the second flush was harvested from day 24 to 28. Two flushes were harvested in total.

Determination of prochloraz-manganese levels in casing

Samples of casing were removed on days 4, 14, 20, 21 and 28 after casing. Five cores of casing (26-mm diameter, to full depth at 50-mm approx.) were taken from each tray on each sampling day. The cores were split in half transversely to give 'top' and 'bottom' sub-samples, which were pooled together and then frozen (-15°C) until analysed. After defrosting the samples were weighed and mixed. Dry weights were determined by drying samples of casing to constant mass in a microwave oven. Residues were extracted from fresh casing (20 g) with methanol (60 ml, hplc grade) by tumbling end-over-end for 1 hour, then analysed by hplc, as described by Grogan & Jukes (2000).

Statistical analysis

Four replicate trays were prepared for each of nine treatments which consisted of three inoculation treatments (isolate 182, isolate 620, None), each receiving three fungicide treatments (standard rate in 180 litres H_2O/100 m^2, standard rate in 90 litres H_2O/100 m^2, None). Trays were positioned in stacks, in a growing room, according to a Trojan square design, which takes into account possible sources of variation within a three dimensional space. Data were analysed by ANOVA and LSD values were calculated for $P = 0.05$.

RESULTS AND DISCUSSION

***In vitro* characteristics**

Under *in vitro* conditions, isolate 182 was confirmed as being more tolerant to prochloraz - manganese than isolate 620, and was capable of some growth at concentrations of 20 and 50 ppm (Figure 1). Average EC_{50} values were 5.9, and 2.7, respectively.

Efficacy of prochloraz -manganese *in vivo*

Following the use of prochloraz-manganese to control dry bubble disease caused by two isolates with different fungicide sensitivities, a significant reduction in symptoms was observed (Figure 2). However, their was little difference between the two isolates in terms of the amount of dry bubble present following fungicide use, despite the fact that isolate 620 is more sensitive to prochloraz-manganese *in vitro*. Percentage reduction of symptoms was

In recent years growers have been reporting increases in the amount of *Verticillium* present on their farms, despite the use of prochloraz-manganese. This might be as a consequence of different sensitivities of *Verticillium* isolates to prochloraz-manganese, particularly as the mushroom industry in Britain, and elsewhere, relies heavily on this product to keep *Verticillium* under control. This project was commissioned to determine whether prochloraz - manganese effectively controlled the two different types of *Verticillium* identified in the survey, when grown under standard *in vivo* conditions. A modification of the standard drench application was included to determine if a reduced volume of water used per unit area would give better control.

MATERIALS AND METHODS

Verticillium isolates.

Two *Verticillium* isolates, isolate 182 and isolate 620, representing the two main types of isolate identified during the 1997 survey, were chosen for this study. The fungicide-resistance profiles of both isolates, in response to increasing concentrations of prochloraz - manganese, were established prior to the inoculation of the mushroom crop. Both isolates were grown on potato dextrose agar (Oxoid) amended with prochloraz-manganese at concentrations ranging from 0 to 100 ppm. Three replicate plates were prepared for each isolate at each concentration and all plates were incubated at 20°C. Colony radius was measured after 3 weeks and growth on the fungicide-amended medium was expressed as a percentage of the control. EC_{50} values were calculated by interpolation. Ten repeat experiments were carried out for isolate 182 and three for isolate 620.

Inoculum

Verticillium inoculum in the form of a spore suspension was prepared on the day when the mushroom crop was to be inoculated. Spores were washed from a pure culture of each isolate and the concentration of each spore suspension was determined using a haemacytometer. The spore suspensions were diluted so that a 100 ml volume applied to each cropping unit delivered approximately 1 million spores/m^2. Spore suspensions were sprayed onto the crop, using a separate sterilised wash bottle for each isolate, four days after the peat-based casing layer had been applied (no mushrooms are present at this stage in the crop cycle).

Prochloraz-manganese treatments

Two prochloraz-manganese treatments were tested in this study. These consisted of (a) a standard product application rate of 120 g in 180 litres H_2O/100 m^2 of mushroom bed area: first spray applied three days after casing and second spray after the first flush harvest and (b) the same standard product rate applied in half the standard volume of water as follows: 120 g in 90 litres H_2O/100 m^2 of mushroom bed area: first spray applied on three days after casing and second spray after the first flush harvest. Each 120 g spray contained 55 g a.i. and therefore all fungicide treatments received a total of 110 g a.i./100m^2.

Crop details

Compost produced by the HRI Mushroom Unit (compost batch 18/98) was used for this experiment. Wooden trays, measuring 91cm x 61cm and 17cm (deep) were filled with 50 kg

In vivo response of the mushroom pathogen *Verticillium fungicola* (dry bubble) to prochloraz-manganese

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ABSTRACT

Verticillium fungicola isolates from British mushroom farms differ in their sensitivity *in vitro* to the fungicide prochloraz-manganese. The two main types of isolate found are represented by isolate 182, which has an EC_{50} value of 5.9 ppm and grows at 50 ppm, and isolate 620, which has an EC_{50} value of 2.7 ppm and does not grow at 50 ppm. To test the efficacy of prochloraz-manganese against these two isolates, mushroom crops were inoculated with one or other isolate then treated with prochloraz-manganese at standard rates. Prochloraz-manganese gave good control of both isolates compared to untreated controls. Percentage control was better for the more sensitive isolate but it was also the more aggressive pathogen in the absence of any fungicide. Prochloraz-manganese levels in casing had dropped by day 28 after application to less than 25% of what had been applied. Loss of a.i. in the casing, coupled with either a more aggressive but fungicide-sensitive isolate or a less-aggressive but more fungicide-tolerant isolate, is considered to be responsible for the intractability of dry bubble disease of mushrooms.

INTRODUCTION

In Britain, 'Sporgon 50WP', containing 46% prochloraz-manganese (a.i.) is relied upon heavily by the mushroom industry to control the dry bubble pathogen *Verticillium fungicola*. This pathogen is resistant to the benzimidazole fungicides, which are used to control some of the other pathogens affecting mushrooms (Fletcher *et al.* 1989). It is a true parasite of *Agaricus bisporus*, deriving its nutrition from the host following intra-hyphal penetration of mushroom mycelium (Draght *et al.*, 1996). If infection occurs at an early stage in mushroom development, then undifferentiated masses of mushroom tissue will develop to give the characteristic "dry bubble" symptom. If maturing mushrooms are infected then spotting symptoms will develop making the mushrooms unmarketable. At present, the losses to the British industry due to *Verticillium* are estimated to be in the region of £2-3 million but if significant resistance to prochloraz-manganese was to emerge (as happened with benomyl in the 1980's) this loss would increase to an estimated £10 million.

A survey of *Verticillium* isolates from British mushroom farms, carried out in 1997 on behalf of the Horticultural Development Council (Grogan *et al.* 1998), indicated that a bimodal population of the pathogen existed with respect to *in vitro* sensitivity to prochloraz-manganese. Sixty-four percent of isolates tested were weakly resistant to prochloraz-manganese and had EC_{50} values (concentration required to reduce growth by 50%) in the region of 5 to 8 ppm while 30% of isolates were more sensitive, with EC_{50} values in the region of 1 to 4 ppm. Recent studies in the Netherlands and Belgium have also identified *Verticillium* isolates which are less sensitive to prochloraz-manganese *in vitro* (Geels, 1996; Desrumeaux *et al.*, 1998).

Stevenson, 1996). It is possible that this effect was due in part to preventing contact between basal leaves and a moist compost surface. It is unlikely, however, that such a treatment in primula or cyclamen would be economically viable. There is evidence that a fungicide treatment to young cyclamen plants, to protect the basal leaves, is effective at reducing subsequent grey mould development (O'Neill & McQuilken, 2000).

Adoption of sub-irrigation rather than overhead watering appears to be a useful component for integrated management of grey mould on calluna. It may also reduce the risk of grey mould on cyclamen plants close to crop maturity. However, sub-irrigation by itself is unlikely to provide sufficient control of the disease in any of the crops studied and this method will need to be integrated with fungicide treatment, or other disease management strategies, to provide a commercially acceptable degree of control.

ACKNOWLEDGEMENTS

We thank G Hilton and D Reid (ADAS) and Jim Thomson (SAC) for technical assistance. This work was funded by MAFF, the Horticultural Development Council, Campbell Scientific Ltd and S Coutts as part of a Horticulture LINK project (HortLINK 25), undertaken in collaboration with Horticulture Research International (Dr T Pettitt), Silsoe Research Institute (Dr B J Bailey) and the University of Reading (Dr M Shaw).

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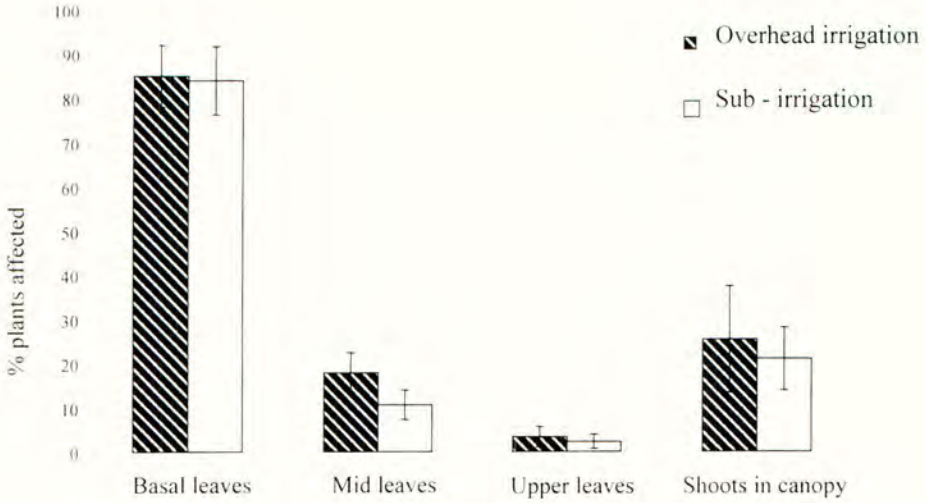

Figure 1. Occurence of grey mould on cyclamen grown under different irrigation regimes - 17 December (crop at first flower)

DISCUSSION

Irrigation was found to influence the development of grey mould on calluna cuttings and potted calluna plants, but had little effect on the disease on cyclamen and none on primula until past the normal marketing stage. Where an effect was detected, overhead irrigation increased botrytis compared with sub-irrigation. Assuming the greater disease severity was associated with increased leaf wetness, and the reduced disease on sub-irrigated plant with reduced leaf wetness, it is surprising that dripline irrigation did not reduce botrytis. It is possible that drip irrigation created a more humid microclimate in the plant canopy than sub-irrigation, especially at the plant base, sufficient to permit infection by *B. cinerea*. Subsequent testing of within crop humidity confirmed a consistently higher humidity with dripline than sub-irrigation.

On cyclamen, infection by *B. cinerea* occurred predominately on yellow and necrotic leaves in contact with the moist compost surface. Irrigation method had no significant effect on infection at this site, probably because sufficient moisture to permit infection was available from the compost surface and/or senescing leaves. Towards crop maturity, overhead irrigation increased the incidence of grey mould on leaves in the mid-canopy. These leaves often become chlorotic from lack of light and may be more susceptible to infection than other leaves.

Grey mould on primula occurred initially on basal leaves in contact with the compost surface and was unaffected by irrigation treatment. The disease progressed by contact spread between leaves and by growth along leaves into the stem base. On geranium, it was previously shown that use of a plastic mulch over the compost surface, combined with the use of forced heated air, significantly reduced grey mould (Pennypacker &

Table 2. Effects of irrigation methods on grey mould on potted calluna plants - 1998

Irrigation	% sporulating botrytis		% foliar browning	
	13 weeks	26 weeks	13 weeks	26 weeks
Overhead	21.2 (26.7)[a]	13.6 (16.2)	37.6 (37.6)	76.4 (61.7)
Sub-irrigation	15.4 (21.4)	4.4 (5.9)	32.6 (34.6)	68.0 (55.8)
Dripline (100 ml/plant)	28.2 (31.4)	18.4 (22.1)	48.2 (43.8)	78.0 (62.4)
Dripline (200 ml/plant)	24.4 (27.9)	15.0 (20.0)	43.0 (40.7)	80.4 (64.2)
LSD ($P = 0.05$)	(3.98)	(5.62)	(3.08)	(2.72)

[a] Figures in parenthesis are angular transformed values

Cyclamen

Grey mould was first observed 10 days after potting, affecting *c.* 5 % of plants, and increased to affect over 80 % by 17 October, as the crop was coming into first flower. Irrigation treatments had no significant effect on overall disease incidence or severity. Disease severity on 25 November , when plants were at full flower and ready for marketing, was almost identical on plants subjected to sub-irrigation (DI 39.0) or overhead irrigation (DI 40.5). Sporulating grey mould was only found on necrotic and yellowing leaves in contact with the compost surface until the plants were at first flower when the disease was
also recorded on leaves at mid-height in the plant canopy. Infection at this position was significantly less with sub-irrigation (10.7% of plants) than with overhead irrigation (18.0%) (Figure 1).

Primula

Irrigation treatment had no statistically significant effect on occurrence of grey mould. On 16 October, 3 weeks after potting, the disease was present on *c.* 5 % of plants in all treatments. This had increased to 20.9 - 23.3% of plants on 15 January, when the crop was in flower and ready for marketing. Infection occurred almost invariably on the basal whorl of leaves and generally on those in contact with the compost surface. Towards the end of the experiment, when plants were past the usual marketing stage, a second common infection site was on senescing flowers, and there was an increased occurrence of grey mould on leaves at points of contact with flowers using overhead irrigation (mean 28.3 sites/plot), than with sub-irrigation (12.5).

were compared at a probability of 5% and least significant difference (LSD) values calculated.

Disease assessment

Crops were examined at regular intervals to determine the incidence and severity of grey mould and the type and position of tissues affected. On calluna, foliar browning and degree of sporulating grey mould were recorded by estimating the proportion of each plant affected. On cyclamen, disease severity was assessed using a 0 - 5 scale (1 = up to 5 leaves affected, 5 = whole plant collapsed) and a disease index (DI) on a 0-100 scale calculated.

RESULTS

Calluna cuttings

Grey mould was first confirmed 2 weeks after the experiment was established and after 12 weeks, when cuttings were removed in preparation for potting, had progressed to affect approximately 50% of overhead-watered cuttings. The disease usually occurred on the tips of cuttings. Sub-irrigation significantly reduced both incidence of cuttings affected and the degree of foliar browning, compared with overhead irrigation (Table 1).

Table 1. Effects of irrigation methods on grey mould on calluna cuttings - 1999

Irrigation	% cuttings affected		% foliar browning	
	4 weeks	12 weeks	4 weeks	12 weeks
Overhead	18.0 (24.9)[a]	49.9 (45.5)	21.8 (27.9)	36.4 (37.2)
Sub-irrigation	7.8 (16.0)	23.7 (29.6)	11.9 (23.2)	26.3 (30.9)
LSD (P=0.05)	(3.85)	(5.68)	(4.05)	(4.66)

[a] Figures in parenthesis are angular transformed values

Calluna plants

Grey mould occurred in mid-May, 6 weeks after the start of the experiment, and affected most plants by September. Foliar browning and sporulating botrytis generally occurred within the crown and on foliage around the stem base. The disease was consistently less in plants grown by sub-irrigation compared with plants watered by dripline or from overhead (Table 2). There was no statistically significant difference between the low and high regimes of drip irrigation.

fungicides, the current work aimed to establish the influence of overhead, drip and sub-irrigation of crops of three important ornamental species grown under commercial conditions.

MATERIALS AND METHODS

Crop production and irrigation treatments

Experiments were carried out from Autumn 1997 to Spring 2000 at The Scottish Agricultural College (SAC) Auchincruive on calluna cuttings and mature plants, and at ADAS Arthur Rickwood on cyclamen and primula. No fungicides were applied against grey mould in any of the crops. Calluna plants severely affected by grey mould were placed evenly between plots at establishment of the calluna trials. Treatments were applied from potting of plug plants through to the usual point of sale, or from striking of cuttings (calluna) to potting.

Calluna cuttings cv. Sun Rise were grown in module trays (140 cells of $5cm^3$) of propagation compost on capillary sandbeds, under low polythene tunnels within a glasshouse. Irrigation treatments were by hand-watering from overhead, or sub-irrigation using the sandbed, and were applied as required for 12 weeks (February - May).

Calluna plants cv. Flamingo were grown in 1 litre pots in an unheated, net-sided polythene tunnel. Irrigation treatments were: 1) overhead watering by sprinkler at 1.6 l/plot (100 ml/plant); 2) sub-irrigation using capillary sandbeds, 3) dripline irrigation at 100 ml/plant, 4) drip line irrigation at 200 ml/plant. Plants were watered approximately every 3 days from late April to early November.

Cyclamen plants cv. Sierra White were grown in 13 cm half-pots within plastic carry-trays on the concrete floor of a heated greenhouse (minimum 12°C). Irrigation treatments were: 1) overhead watering by hand, 2) sub-irrigation by filling a 1 cm deep reservoir in the plastic tray. Plants were watered as required from July to December; shade screens were used during hot weather.

Primula plants cv. Danova were grown in 9 cm pots on the floor of a heated glasshouse (minimum 7°C). Irrigation treatments were: 1) overhead sprayline irrigation with pots stood on capillary matting, 2) sub-irrigation using seep-hoses laid over capillary matting. Plants were watered as required from September to March.

Trial design and statistical analysis

All trials were fully randomised complete block designs with four replicates. Plot size was $0.25m^2$ (2 trays) for calluna cuttings, 1 m^2 (16 plants) for potted calluna, $6m^2$ (96 plants) for cyclamen and $2.25m^2$ (121 plants) for primula. Results were subjected to analysis of variance after transformation of % data where required. Treatment means

Influence of irrigation method on development of grey mould (*Botrytis cinerea*) in greenhouse crops of calluna, cyclamen and primula

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ABSTRACT

The influence of irrigation method on the development of grey mould on protected crops of calluna, cyclamen and primula was investigated as a potential component of an integrated disease management strategy. On calluna, foliar botrytis was significantly reduced when plants were watered by sub-irrigation compared with overhead or dripline irrigation. On cyclamen, the incidence of leaves affected by botrytis was generally not affected by watering method although there was a significant reduction associated with sub-irrigation on leaves at mid-canopy level towards crop maturity. Irrigation method had no effect on the disease on primula. It is suggested that the differing effects of irrigation on development of grey mould on calluna, cyclamen and primula relates to the primary infection routes by *B. cinerea* in these crops. Sub-irrigation reduced disease risk in crops where infection occurs in the upper or mid-canopy leaves, but not where the disease originates on leaves at compost level.

INTRODUCTION

Grey mould, caused by *Botrytis cinerea*, is a common disease on UK greenhouse crops of calluna, cyclamen and primula and chemical control using the fungicides currently available is often inadequate (O'Neill, 1999; O'Neill & McQuilken, 2000). The number of fungicide treatments applied against grey mould is frequently at least three per crop and can exceed eight in long-season crops of cyclamen (Coutts, per. comm.) or calluna (McQuilken, pers. comm.). There is increasing interest in reducing fungicide usage on ornamental crops, to satisfy consumer demand, to reduce the risk of selecting fungicide-resistant pathotypes and to reduce the occurrence of fungicide deposits on the marketed product.

Prolonged high humidity and/or leaf wetness is widely reported to favour infection by *B. cinerea* (Carre & Coirer, 1984; Cole *et al.*, 1996; Sirjusingh & Sutton, 1996), indicating that irrigation methods which result in different crop wetness characteristics could affect development of the disease. Crops grown with reduced leaf wetness should be less at risk from grey mould. As part of a project seeking to manage the disease by integration of non-chemical treatments with occasional use of crop-safe and effective

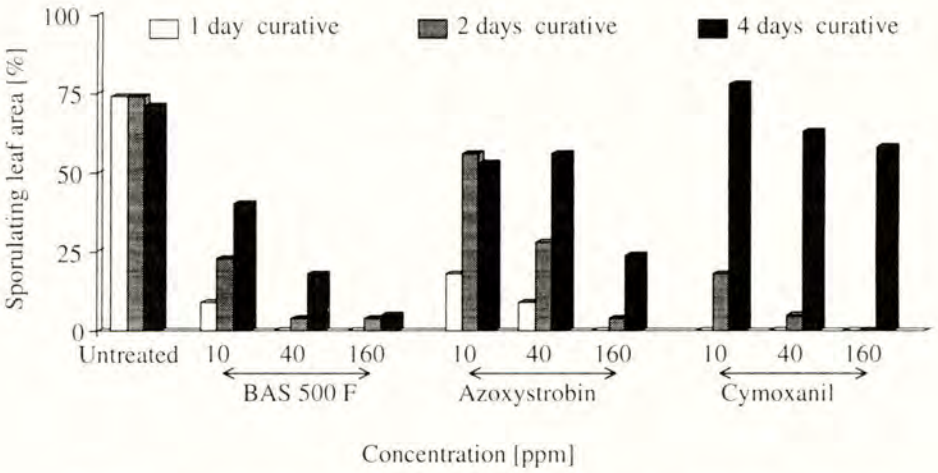

Figure 4. The curative activity of BAS 500 F and other fungicides against *P.viticola* from applications made 1, 2 or 4 days post inoculation.

DISCUSSION

BAS 500 F is a new, broad-spectrum strobilurin fungicide from BASF. In field testing, BAS 500 F has demonstrated greater efficacy compared to the current market standard products. Controlled studies in the laboratory and glasshouse have demonstrated that good disease control in the field can be attributed to activity preventing zoospore release, mobility and germination as well as suppression of post-infection stages of *P. viticola*. However, strobilurins have demonstrated the capacity to select resistant strains of pathogenic fungi under field conditions (Anon. 2000). Therefore, the use of BAS 500 F will be recommended preventatively, in mixtures or in alternation programmes with effective fungicides from different cross-resistance groups and with a restricted number of applications per season.

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Table 1. Effect of BAS 500 F and other fungicides on the mobility, integrity and germination of zoospores of *P. viticola* (in vitro).

Compound	Concentration [ppm]	Inhibition of mobility after 2 minutes	Lysis after 6 minutes	Germinated spores after 4 hours [%]
Untreated		-	-	100
BAS 500 F	0.01	+	-	2.0
	0.1	+++	+++	0.5
	1	+++	+++	0.3
	10	+++	+++	0
Azoxystrobin	0.01	+	+	70
	0.1	++	+	4.3
	1	+++	++	3.4
	10	+++	+++	3.4
Cymoxanil	0.01	-	-	59
	0.1	-	-	52
	1	-	-	44
	10	-	-	50

- = < 5%; + = 5 – 25 %; ++ = 26 – 60 %; +++ = 61 – 100 %

Curative activity

To investigate the curative activity of BAS 500 F, applications were made at various intervals post-inoculation to glasshouse grown plants. The curative effect of BAS 500 F depended on the time and concentration of application (Figure 4). At 1-day post inoculation (1-dpi), 40 ppm BAS 500 F was sufficient to prevent the development of downy mildew symptoms. The activity of the other tested fungicides was similar when application was made at 1-dpi although a higher rate of azoxystrobin was required for the same level of efficacy. When the fungicides were applied at later times post inoculation (2 or 4-dpi), BAS 500 F at 160 ppm was less effective than at 1-dpi but still reduced formation of lesions and sporulation to a maximum of 5% and was, especially at the 4-dpi application more active than both the other fungicides. Cymoxanil showed activity similar to BAS 500 F at 2 dpi, but was less effective at 4-dpi.

Microscopic evaluation of curative treated plants revealed that BAS 500 F strongly inhibited the development of *P. viticola* within the grape leaf. The development of most of the colonies was suppressed at the developmental stage present at the time of treatment. The inhibited fungal structures were usually encapsulated by a layer of callose and necrotic leaf cells. When, due to low application concentrations or late treatment, some sporulation occurred, it arose from only a few isolated colonies.