POSTER SESSION 4B

PEST AND DISEASE MANAGEMENT IN HORTICULTURAL CROPS

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Aspects of the epidemiology of *Botrytis cinerea* on covered pot-grown ornamentals

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ABSTRACT

The half-distance for conidial dispersal of *Botrytis cinerea* was approximately 0.7 m in a glasshouse grown Fuschia crop. There was clearly more dispersal and an increased half-distance of 1 m in a sprayed portion of the crop. In a commercial *Primula* crop, which developed disease progressively as it matured, there was no evidence for association between the positions of infections on any scale except between individual pots in direct contact. Variety differences in disease incidence were clear, but there was no association of disease with other factors. This is consistent with secondary spread being a relatively unimportant cause of disease in the primula crop. Alternative sources of infection are seed and external inoculum, combined with a long and variable latency; management should therefore focus on these factors.

INTRODUCTION

B. cinerea conidia are frequently isolated as part of the air-spora, so that otherwise healthy crops will almost always be at risk of infection from distant sources. In some cases, primary infection may be via seed (Barnes, 2002) or overwintering or otherwise latent mycelium. Secondary spread within crops is due to direct contact or to dispersal of conidia by splash, air currents, or insect vectors. *B. cinerea* conidia are not particularly abundant in the air, comprising in one study only 0.42% of the total air-spora (Gregory and Hirst, 1957). Furthermore, there is evidence to suggest that the conidia themselves are not genetically uniform, but originate from many different sources; it is an open question to what extent conidia from different sources may differ in ecological specialisation (Kerssies *et al.*, 1997).

Removal of senescent onion leaves has been found to reduce the number of *Botrytis* sp. spores in the air above a crop by up to 50% (Kohl *et al.*, 1995) but there is an increase in *Botrytis* sp. in the air-spora during leaf removal in raspberries (Jarvis, 1980). The use of drip as opposed to overhead irrigation, decreases splash dispersal (Legard *et al.*, 2000). Conidial concentrations have been found to increase after the application of fungicides (Hausbeck and Pennypacker, 1991), indicating that the spraying mechanism itself may be responsible for the spread of conidia. Finally, increasing plant spacing reduces contact spread (Legard *et al.*, 2000).

Inferences about secondary dispersal patterns and the factors influencing them can come from observations of infection gradients surrounding a source of inoculum. Splash-borne spread leads to the number of spores spreading a given distance falling off exponentially with

distance from the source, as does transport by insect vectors. Movement in air currents leads to transport being inversely proportional to some power of distance from the source (Fitt *et al.*, 1987).

Two studies are discussed here. First, the dispersal of conidia around a focal point was studied in a fuschia crop to determine the effect of crop spraying on dispersal and the likely dispersal distance of conidia. Second, disease within a primula crop was mapped intensively over a growing season. The alternative hypotheses for the origin of disease were new infections due to infection by conidia originating at a few foci and latent disease already present within the crop at establishment. Associations of disease with wind speed due to fan turbulence, light, temperature and host variety were examined.

MATERIALS AND METHODS

Dispersal distances

One thousand young fuchsia plants established from cuttings were placed in two long columns, on two parallel benches separated by 95 cm. Each bench had 64 rows of 8 plants. The plants were placed far enough apart not to be touching, 3.5cm x 3.5cm. In the test column 3 plants were removed from the centre row and replaced with 3 fuchsias, at the same growth stage as the other plants, with visibly sporulating *B. cinerea*. The temperature was set at 18°C with a relative humidity of 85-95 %. A plastic curtain, 2.5 m high, was placed along the centre of the glasshouse between the benches to divide them. It did not reach the roof, and had a 10-cm high gap at the bottom. The plants on one bench were then sprayed with water using a hand held pump sprayer. The following day the curtain was removed. Seven weeks after spraying, no visible symptoms were present. One leaf was removed from each plant and placed on a PDA plate. One week later the presence or absence of *B. cinerea* mycelium and spores was noted. The proportion of infected plants within 20-cm wide intervals (bins) at successive distances from the focal point (0-20 cm, 20-40 cm etc) was calculated. The disease incidence in each interval was calculated by dividing the number of diseased plants by the total number of plants in that interval.

Spatial pattern in a commercial *Primula* crop

Visits were made monthly to a commercial grower in Buckinghamshire. Disease was scored in each glasshouse compartment in which primula was being grown. Any leaves showing necrotic lesions were collected and isolations made, in order to determine if *B. cinerea* was present. On 7 January 2000 disease was mapped plant by plant. On 21 January the plants, which had not yet been removed for sale or due to disease were re-mapped and light, temperature and windspeed measurements made.

RESULTS

Dispersal distance

The pattern of isolations of *B. cinerea* was centred on the infector plants, confirming that they were a major contributor to the infections detected. There is clearly less dispersal to corresponding distances in the unsprayed half, suggesting a definite increase in dispersal due to the disturbance caused by spraying. The relationship between number of isolations and distance appears to conform better to an exponential distribution than a power law distribution (Figures 1, 2). (However, if the analysis is done separately for the two halves of the experiment dispersal in the unsprayed half seems to conform better to a power-law than an exponential: c.f. Figures 1 and 2). Under the exponential model the half distance of dispersal for the sprayed bench was 1 m and for the unsprayed bench was 70 cm.

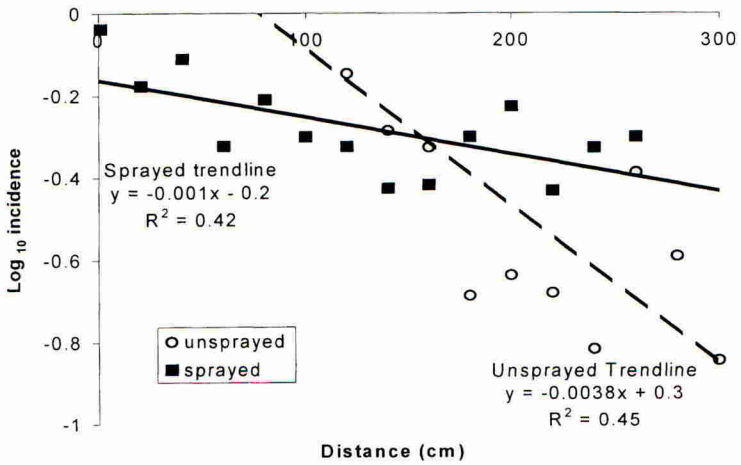

Figure 1. Relation between log $_{10}$ proportion of leaves from which *B. cinerea* was isolated and distance from inoculum source.

Spatial pattern in a commercial *Primula* crop

On the first visit to the commercial nursery, 3700 primulas were present of which 2700 of these were examined covering the whole cropped area. Only one lower leaf with sporulating *B. cinerea* was found. 2.5% of lower leaves had spots or patches of necrosis, but infection could not be confirmed by isolations or an antibody test.

In subsequent visits no disease was detected until November 1999, after which two further assessments were made. Necrotic leaf lesions mostly proved not to be associated with *B. cinerea*, and sporulating infection was scarce in two blocks until January, when the crops flowered; few further infections were seen in compartment B in which disease first became common (Table 1).

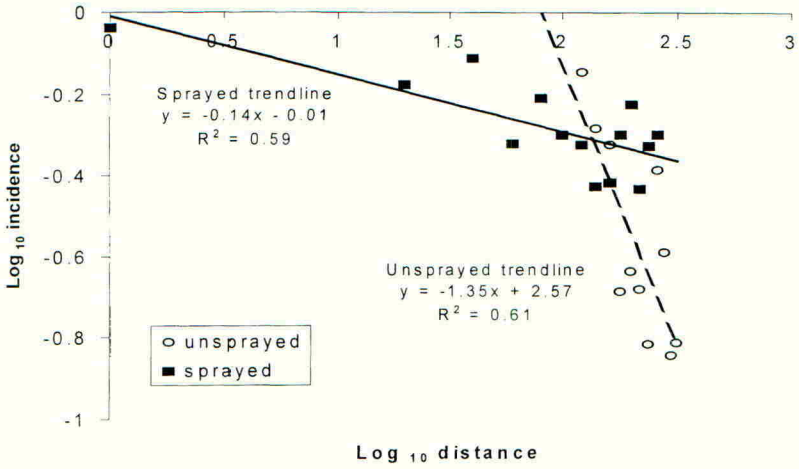

Figure 2. Relationship between log10 proportion of leaves from which B. cinerea could be isolated and log10 distance from inoculum source.

Table 1. Symptoms observed and isolations of *B. cinerea* made in a crop of *Primula* over three months. Plants were sown in September 1999 and flowered in December/ January. 900 plants were scored in each compartment. All non-specific necrotic lesions were placed on PDA to determine whether they contained *B. cinerea*.

Glasshouse compartment	Category	November	December	January
A	Necrosis, not *B. cinerea*	20	28	62
	B. cinerea infected plants[a]	1	0	236
B	Necrosis, not *B. cinerea*	55	34	42
	B. cinerea infected plants	24	3	2
C	Necrosis, not *B. cinerea*	14	56	57
	B. cinerea infected plants	3	7	69

[a] Either visibly sporulating or sporulating within 7 days on agar.

Compartments contained distinct blocks of different cultivars, but not in a balanced or intermingled arrangement. For example, in block A, there were blocks of Royal Suis (3.2% of the plants), Forza (13%), Select (29%) and Gemini Cream (61%). Much the worst affected plants in January were cv. Forza. There were approximately 20% as many infected plants in the much larger block of cv. Select. Cvs. Gemini Cream or Royal Suis were completely free of disease, even close to the boundaries with Forza and Select.

The wind, light and temperature measurements at different positions had no correlation with disease. A map of the position of diseased plants in a subsequent crop, in October 2000, was subdivided into quadrats of various sizes. The proportions of quadrats containing given

numbers of infected plants were very close to those expected from a fully random distribution, except at the smallest possible scale (4 plants/quadrat) where local spread by leaf contact was obvious.

DISCUSSION

In the dispersal experiment, spread on the control side was consistent with turbulent dispersal over longer distances from the infected plant, with a power-law exponent of 1.4. *B. cinerea* is known to be dispersed both as dry spores on turbulent currents and splash dispersed as dry conidia coating water droplets (Johnson and Powelson, 1983).

Mapping of the disease in primulas at commercial growers showed little sign of spread from initial foci. With a half-distance spread by conidia of 1 m we would predict clustering on this scale if spread was from local sources. All plants of different varieties and from 2 different suppliers were grown in groups in the same greenhouse. Varieties had clearly different disease incidences. This could be related to intrinsic susceptibility, or to the prevalence of disease in the stock plants from which seed was derived, since we have shown that *B. cinerea* on primula can be seedborne and give rise to systemic asymptomatic infection appearing when plants mature (Barnes, 2002; Barnes and Shaw, 2002).

These greenhouses were ventilated by a number of large fans suspended from the ceiling, The air movement created by the fans was clearly detectable at plant level but did not have any detectable relationship to the disease distribution.. This is consistent with conidia being relatively unimportant in spreading disease. However, the fuschia results clearly show that spraying operations increase the distance over which conidia are distributed.

Because the fuschia plants never became symptomatic it is not possible to say in what form *B. cinerea* survived on the leaves tested. It may have been in the form of ungerminated spores, or a latent infection. It is clear that background levels of infection in the fuschia must have been low enough to allow us to detect the conidial gradients seen; but no real inferences about epidemiology on fuschia can be made from our observations, which were designed to study dispersal using a convenient plant with low background infection. The essential point is that clustering on a 1 m scale due to conidial dispersal is implied by the fuschia results but is absent from the primula data.

The pattern of disease in the primulas is consistent with findings reported elsewhere: latent infection of primula plants can be present from very early in the plant's life, becoming symptomatic only when the environmental conditions and the physiological status of the host are correct (Barnes and Shaw, 2002). Table 1 suggests that very few of the non-sporulating necrotic symptoms appearing in an immature crop can be attributed to *B. cinerea* infection. The latent *B. cinerea* may come from infected seed (Barnes, 2002) or from conidial infection at any stage. An important practical implication of these results is that in some ornamental species, such as primula, conidial dispersal may be only of secondary consequence and control should be aimed more at finding the source of primary disease and reducing it.

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Integration of different fungicide groups in spray programs for the control of powdery mildew in grapevines

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ABSTRACT

Applications of either wettable sulphur, penconazole, azoxystrobin or trifloxystrobin at various grapevine growth stages were evaluated in five field experiments in 2001/2002. In 4 of the 5 unsprayed plots, all bunches were more than 50% covered with mildew and unmarketable. Most programs (except those with only wettable sulphur and penconazole) with 4 to 5 sprays between the growth stages of 5 leaves separated and just after fruit set provided good control. The most effective programs were those where early applications of either wettable sulphur or penconazole were followed by trifloxystrobin applied in 2 or 3 consecutive sprays around flowering.

INTRODUCTION

Powdery mildew (*Uncinula necator)* is the major fungal disease of grapes costing the Australian grape industry over 30 million dollars annually.

Fungicide programs based on wettable sulphur alone or in combination with DMI's (demethylation inhibiting fungicides) are widely used to control the disease in Australia. While these programs have provided good control in the past there are concerns with phytotoxicity when sulphur is applied at temperatures >35°C and the development of DMI resistant strains of powdery mildew that have recently been detected in Australia (Savocchia *et al.* 1998).

New fungicides with activity against powdery mildew have been developed (Margal *et al.* 1998, Dacol *et al.* 1998), but few critical studies have been done in Australia to determine where they best fit in spray programs used on grapes. This paper reports on studies where the efficacy of strobilurin fungicides was evaluated when applied in programs with other types of fungicides.

MATERIALS AND METHODS

Products and formulations

Fungicides and rates per litre used were 0.5 g Amistar WG (500 g/kg azoxystrobin), 0.15 g Flint (500 g/kg trifloxystrobin), 6 g Thiovit (800 g/kg sulphur) and 0.125 ml Topas (100 ml/L penconazole).

Sites

Experiments were conducted at the Lenswood and Nuriootpa experimental stations, situated approximately 30 km east and 100 km north east of Adelaide respectively. At Lenswood, treatments were applied to Chardonnay vines planted 1.5 m apart with 3 m row spacings. At Nuriootpa, experiments were carried out on Chardonnay vines planted 2.25 m apart with 3.5 m row spacings, Crouchen vines planted 1.2 m apart with 3.7 m row spacings and Verdelho vines planted 2 m apart with 3.5 m row spacings. Plots varied from 3 to 8 vines each, replicated 5 times and arranged in a random block design.

Spray application and timing

Fungicides were applied with a "Solo" motorised back pack sprayer utilising up to 500 L/ha in early spring to 1000 L/ha when vines were in full canopy in mid summer. The combinations of different spray timings is shown in Tables 1a-e.

Assessment and analysis

At harvest, fifty bunches were selected at random from the middle 1 or 2 vines of each plot and assessed for disease incidence and severity. A 0 to 10 rating scale (Emmett, unpublished) was used to rate each bunch where 1=<1%, 2=2%, 3=5%, 4=10%, 5=20%, 6=40%, 7=60%, 8=80%, 9=90% and 10=100% of the bunch infected with powdery mildew. Data was analysed with the analytical software package "STATISTIX for Windows V2" using general analysis of variance to generate values for least significant differences (LSD, $P < 0.05$).

RESULTS

Powdery mildew developed extensively in all plantings, and by harvest most bunches in the unsprayed plots were severely diseased (Tables 1a-e).

In experiment 1, the lowest level of bunch infection was found in treatments where trifloxystrobin was applied in December. On the other hand the highest levels of disease in the sprayed treatments developed when penconazole was applied in December (Table 1a). In programs where penconazole and trifloxystrobin were applied, additional applications of wettable sulphur in early November and January did not improve the control of bunch infection.

In experiment 2, all programs controlled powdery mildew and although there was no significant difference between the spray programs, most disease developed in the wettable sulphur/penconazole program (Table 1b).

Table 1(a-e). Incidence and severity of powdery mildew on bunches treated with various fungicide regimes applied at different vine growth stages, 2001/2002.

Treatment, application date and vine growth stage[1]						Bunches diseased (%)[2]		Bunch area diseased (%)[2]	
(a) Experiment 1 – Chardonnay – Lenswood									
Nov 1 GS 14	Nov 15 GS 15	Nov 28 GS 18	Dec 12 GS 23	Dec 27 GS 27	Jan 1 GS 29				
-	-	-	-		-	100	a	98.1	a
-	F	F	T	T	-	98.5	a	10	d
-	T	T	F	F	-	47.5	b	0.8	d
-	S	S	F	F	-	44.1	b	0.7	d
-	F	F	S	S	-	96.7	a	7.2	d
-	S	T	T	S	-	100	a	49.3	b
S	T	T	F	F	S	45.2	b	1.0	d
S	S	T	T	S	S	99.6	a	28.5	c
(b) Experiment 2 – Chardonnay – Lenswood									
Nov 1 GS 14	Nov 8 GS 15	Nov 22 GS 18	Dec 8 GS 23						
-	-	-	-			99.5	a	55.4	a
S	F	F	F			50.6	b	1.0	b
S	T	T	T			97.5	a	14.9	b
(c) Experiment 3 – Verdelho – Nuriootpa									
Nov 1 GS 15	Nov 14 GS 17	Nov 28 GS 23	Dec 12 GS 26						
-	-	-	-			100	a	83.3	a
S	S	S	S			92.5	ab	4.6	bc
S	T	T	S			95.4	a	14	b
T	T	F	F			41.8	c	0.6	c
F	F	T	T			78	b	6.6	bc
(d) Experiment 4 – Chardonnay – Nuriootpa									
Oct 16 GS 17	Nov 1 GS 18	Nov 14 GS 21	Nov 28 GS 27						
-	-	-	-			95.7	a	20.5	a
S	S	S	S			58.5	b	1.0	b
S	T	T	S			64.9	b	1.7	b
T	T	F	F			9.6	c	0.2	b
F	F	T	T			23.2	c	0.3	b
(e) Experiment 5 – Crouchen – Nuriootpa									
Oct 16 GS 12	Nov 1 GS 15	Nov 14 GS 17	Nov 28 GS 26	Dec 12 GS 29					
-	-	-	-			100	a	97.6	a
S	S	T	T	T		100	a	33.8	b
S	S	A	A	A		100	a	21.9	b
S	S	F	F	F		45	b	0.6	c

[1] GS= Growth stage (Coombe 1995),

[2] Treatments with the same letter are not significantly different from one another

Treatments: S = Wettable sulphur 6g/L, T = penconazole 0.125 ml/L, F = trifloxystrobin 0.15g/L, A = azoxystrobin 0.5g/L, - = no treatment.

At Nuriootpa, various programs of 4 sprays were applied to both the Verdelho (Experiment 3) and Chardonnay (Experiment 4) vines. In both plantings the lowest incidence of bunch infection developed in the vines where the initial two applications of penconazole were followed by trifloxystrobin (Table 1c and 1d). Similarly the highest level of bunch infection in the sprayed plots developed in the wettable sulphur/penconazole programs. In the Crouchen vines (Experiment 5) the lowest levels of disease developed in vines where trifloxystrobin was applied on three occasions following two applications of wettable sulphur (Table 1e). This treatment was significantly better than similar programs of either azoxystrobin or penconazole.

DISCUSSION

These results show that powdery mildew infection of grape bunches can be controlled with as few as four applications of fungicides, even in conditions of severe disease pressure. In these experiments the application of fungicides between the growth stages of 5 leaves separated and just after fruit set provided good control with most programs. Strobilurins were generally more effective when applied during or just after flowering compared to before flowering. In programs where the efficacy of different strobilurins was compared, trifloxystrobin was more effective than azoxystrobin.

These results confirm other studies showing that grape berries become resistant to powdery mildew 4 weeks or more after fruit set (Gadoury *et al.*, 1999). In our experiments, only low levels of disease were detected in bunches that remained unsprayed for 12 weeks or more after fruit set. During that time they were subjected to high levels of inoculum from adjacent unsprayed vines, and new shoot growth that developed after fruit set became heavily infected with powdery mildew. In commercial vineyards further applications of wettable sulphur would normally be used to protect this growth.

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Comparison of strategies for timing protective and curative fungicides for control of onion downy mildew (*Peronospora destructor*) in New Zealand

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ABSTRACT

Different strategies for timing protective and curative fungicides against downy mildew in South Auckland, New Zealand, were compared over two seasons. Fungicide application strategies were based on calendar timing, presence of disease and weather patterns (using a modified version of DOWNCAST). Success of treatments was measured as percentage leaf necrosis, and mean bulb yield. Weekly applications of mancozeb with metalaxyl applied after disease alerts provided the highest bulb yield. In the second season, use of the disease forecaster enabled a 40% reduction in number of fungicide applications with no statistically significant loss in yield.

INTRODUCTION

Epidemics of downy mildew (*Peronospora destructor* (Berk.) Casp. Ex Berk.) can cause substantial production losses in North Island onion-growing areas of New Zealand. Losses arise when damage to foliage leads to small size and poor storage quality of onion bulbs (Chupp & Sherf 1960). Downy mildew tends to appear suddenly (Jesperson & Sutton 1987, Whiteman & Beresford 1998) during cool, moist seasons (Fullerton *et al.* 1986). Downy mildew severity varies from year to year, depending on weather conditions, fungicide applications and locality (Wright 1992).

Management of onion downy mildew in New Zealand involves 7-10 day applications of protective fungicides during the growing season with additional curative (systemic) fungicides when disease risk is perceived to be high (Wright 1992). Protective fungicides, such as mancozeb, kill sporangia on contact (Gunn 1991), but are only effective if applied before infection has occurred (Viranyi 1981). They do not protect new leaf tissue that develops between sprays (Gunn 1991). Curative fungicides, such as metalaxyl, penetrate the plant surface, killing *P. destructor* mycelium within plant tissues (Gunn 1991). However, only a limited number of systemic fungicide applications (usually three) is recommended each growing season to reduce the risk of the fungus developing resistance to the chemical.

The relationship between weather and downy mildew is well-documented (Howard *et al.* 1994; Mukerji 1975; Schwartz 1995). A Canadian forecasting system DOWNCAST (Jesperson & Sutton 1987), which uses five meteorological criteria, was developed to identify

conditions suitable for *P. destructor* sporulation and subsequent infection of leaves by deposited spores. Disease management procedures using a modified version of this system have been proposed (de Visser 1998), one of which is used here.

While onion growers need to minimise the risk of economic losses from downy mildew, they also need to minimise fungicide applications to reduce both production costs and other adverse effects of agrochemical use. More precise systems for timing fungicide sprays need to be developed. This paper compares different strategies for timing protective and curative fungicides against downy mildew in the Pukekohe district of South Auckland over two seasons.

MATERIALS AND METHODS

Trials were carried out in the same field over two seasons at the Crop & Food Research Centre at Pukekohe. The soil type was a Patumahoe mottled clay loam. Seed of the bulb onion cultivar 'Pukekohe Long Keeper' were direct-seeded on 15 July 2000 and 6 June 2001 using a Stanhay seed planter. In 2000, coated seed was used (Sumisclex 2%, Benlate 5%, Thiram 1%), and in 2001 non-coated seed (dusted with 1% Thiram) was used. Plant density 1 month after sowing was 55-65 plants/m of bed. A basal fertiliser application of 15% potassic superphosphate (1 tonne/hectare) was applied to the experimental sites six weeks prior to sowing. Nitrogen was applied at 130 kilograms/hectare (as urea) each year, with half applied at early crop emergence and the remainder eight weeks later. Three months after sowing, onion plants were thinned to c. 5-7 cm spacing within rows. Irrigation and the control of weeds and insect pests during the growing seasons were managed as in local commercial practice.

The treatment plots were randomised in each of four replicate five-row beds, twelve plots per bed. Each datum bed was flanked on both sides by a non-sprayed guard bed. Four non-sprayed control plots were located randomly within the three centre guard beds. Within each plot 50 plants were marked out for downy mildew assessments.

The fungicides used were mancozeb as Dithane ® M45-WDG, Dow AgroSciences (750g/kg mancozeb as water dispersible granule); and metalaxyl as Ridomil® Gold MZ WG, Syngenta (40g/kg metalaxyl-M plus 640g/kg mancozeb, as water dispersible granule). The rate of Dithane ® was 200g/100 litres water; that of Ridomil ® was 300g/100 litres water. The surfactant Contact (wetter, spreader, 25ml/100 litres) was added to the fungicide solution, which was applied at the rate of 700 litres/hectare.

Twelve treatments consisting of combinations of three mancozeb regimes and four metalaxyl regimes were tested in both years.

The mancozeb regimes were applied:

1. Weekly, starting at 2-3 leaf stage (MAN-weekly);
2. Fortnightly, starting at 2-3 leaf stage (MAN-fortnight);
3. Weekly, starting when infection was first found in the trial (MAN-weekly/ONSET).

The metalaxyl regimes were:

1. None (NORID);
2. Three applications 28 days apart, with the first applied 14 days after the first mancozeb application (RID28);
3. Three applications, applied 1-2 days after 'Infection Event' - beginning at the 2 leaf stage (RID-ALERT);
4. Three applications - first applied when downy mildew was first found in the trial (c. 1% plants infected) then two more applied 1-2 days after 'Infection Event' (RID-ALERT/ONSET).

Meteorological data was collected using Campbell Scientific Instruments CR10 data loggers with Model 107 thermistor probes and Model 237 surface wetness sensors on a weather station 150m from the trial site. Wet and dry bulb air temperatures were measured in stacked plate temperature screens 1.5m above ground and used to calculate relative humidity.

Requirements for sporulation and infection in this study were based on modified DOWNCAST criteria, defined as follows:

Sporulation (production of sporangia):

(1) Mean temperature between 0800 and 2000 hrs during the previous day < 24°C; and
(2) Mean hourly temperature at night (2000-0500 hrs) between 4 and 24°C; and
(3) < 0.2mm rain between 0100 and 0500; and
(4) Relative humidity (RH) >95% continuously between 0100 and 0500.

Infection by sporangia when either:

(1) Leaf surface wetness between 0500 and 0800 immediately following the sporulation event;
(2) Leaf surface remains wet for 3 hours between 1900 and 2400 hrs on the evening following the sporulation event; or
(3) Leaf surface remains wet for 3 hours between 1900 and 2400 hrs the second evening following the sporulation event.

Sporulation-infection events were identified within the meteorological records using a Microsoft Excel 6.0 spreadsheet. When three sporulation-infection periods occurred in a 5-day period, an infection alert was deemed to have occurred. Timing of metalaxyl applications was based around such alerts. The incidence of downy mildew symptoms on leaves was assessed on the 50 marked onion plants in each plot on December 14 and 28 in 2000 (first season) and on December 10 and 21 in 2001 (second season). For each plant, the number of fully emerged leaves was counted, disregarding dead or shrivelled leaves. Three months after harvest, onion bulbs were graded and weighed.

RESULTS

Number of applications

Total number of fungicide applications for each treatment over two seasons is shown in Table 1. In the first season, the weekly and fortnightly mancozeb applications commenced on the 3 October 2000. The metalaxyl regimes commenced 17 October (RID28) and 14 November (RID-ALERT/ONSET) after disease detection in the crop. Infection alerts occurred three

times: on 25 October, 29 November, and 12 December (RID-ALERT and RID-ALERT/ONSET). The maximum number of metalaxyl applications (3) was not reached for RID28/MAN-weekly/ONSET and RID-ALERT/MAN-weekly/ONSET treatments.

Table 1. Total number of fungicide applications each treatment received in each season.

	Metalaxyl treatment							
	NORID		RID28		RID-ALERT		RID-ALERT/ONSET	
Mancozeb treatment	00/01	01/02	00/01	01/02	00/01	01/02	00/01	01/02
MAN-weekly	13	17	16	20	16	20	16	20
MAN-fortnight	6	9	9	12	9	12	9	12
MAN-weekly/ONSET	8	10	10	12	10	13	11	13

In the second season, the weekly and fortnightly mancozeb applications commenced 27 August 2001. The metalaxyl regimes commenced 10 September (RID28) and 15 October (RID-ALERT/ONSET) after disease detection in the crop. Infection alerts occurred five times: on 11 September, 1 October, 15 October, 11 December, and 19 December (RID-ALERT and RID-ALERT/ONSET). The maximum number of metalaxyl applications (3) was not reached for the RID28/MAN-weekly/ONSET treatment (Table 1).

Foliage Disease

Percentage foliar necrosis was significantly greater in the control plots than the fungicide treated plots in both seasons (Table 2), although significant treatment differences only occurred in 2000/01 for mancozeb regimes (P = 0.042). In the 2001/02 season mancozeb treatment differences were significant at the 10% level, but not the 5% level.

Table 2. Factorial analysis of mean percent of foliage necrosis for main effects of mancozeb and metalaxyl regimes applied to onions in South Auckland, New Zealand in 2000/01 and 2001/02.

Regime	2000/01 mean % necrosis	2001/02 mean % necrosis
MAN-weekly	6.5[a]	25.5
MAN-fortnight	8.3[b]	32.0
MAN-weekly/ONSET	7.4[ab]	26.9
P =	0.042	0.056
NORID	8.3	29.7
RID28	7.2	26.4
RID-ALERT	6.8	27.3
RID-ALERT/ONSET	7.2	29.1
P =	0.495	0.282
P (interaction mancozeb/metalaxyl) =	0.359	0.082
Control	19.0	41.2

Numbers with the same letters (within each regime) are not significantly different from each other.

Metalaxyl treatments were not shown to have a significant effect on percentage leaf necrosis in either season.

Bulb Weight

In the 2000/01 season there was no clear response of onion bulb weight to either spray regime compared to the control (one way ANOVA over all treatments, P=0.895). In 2001/02 the non-sprayed control had significantly lower (P< 0.001) bulb weight than any of the fungicide treated plots. There were significant effects for both mancozeb and metalaxyl regimes (Table 3). There was no significant interaction between mancozeb and metalaxyl regimes in either season.

Table 3. Factorial analysis of mean bulb weight (g) for main effects of mancozeb and metalaxyl regimes applied to onions in South Auckland, New Zealand in 2000/01 and 2001/02.

Regime	2000/01 mean bulb weight (g)	2001/02 mean bulb weight (g)
MAN-weekly	125	109[a]
MAN-fortnight	123	96[b]
MAN-weekly/ONSET	121	98[b]
P =	0.727	0.001
NORID	123	94[a]
RID28	123	103[ab]
RID-ALERT	123	108[b]
RID-ALERT/ONSET	122	99[ab]
P =	0.999	0.005
P (interaction mancozeb/metalaxyl) =	0.808	0.457
Control	111	66

Numbers with the same letters (within each regime) are not significantly different from each other.

DISCUSSION

The low incidence of downy mildew in the 2000/01 season was associated with a low number of sporulation-infection events (three) and the late appearance of disease in the crop. Treatment plots had significantly less percentage leaf necrosis than the non-sprayed control (one way ANOVA over all treatments, P< 0.001). Only mancozeb regimes showed any significant treatment effect. There was no significant difference in bulb weights between treatments and controls (one way ANOVA over all treatments, P=0.895). The number of fungicide applications did not affect any control achieved, i.e. the treatment with the smallest number of fungicide applications gave as good disease control and bulb weights as the treatment with the greatest number (6 compared to 16, Table 1). Due to the low incidence of disease, results from the first season are not discussed further.

The 2001/02 season was much more conducive to downy mildew, with 41 percent leaf necrosis in untreated plots (Table 2). Weather conditions were much more favourable (an earlier, wetter spring with more infection alerts), although other factors (residual inocula from previous season, later planting, earlier germination) also could have contributed. Percentage foliar necrosis in treated plots were all significantly lower than in control plots, but no conclusion about strategies could be based on this measure. Crop yield (mean bulb weight)

proved the best measure of regime effectiveness, with both fungicide regimes providing significant increases in mean bulb weight.

Weekly applications of mancozeb provided the best control of downy mildew (highest mean bulb weights, Table 3). Waiting until the disease was first detected in the crop before beginning mancozeb applications saved 7 spray applications but resulted in significantly lower bulb yield.

All metalaxyl treatments enhanced disease control compared with mancozeb only. Significant increase in yield was only achieved when metalaxyl was applied following weather alerts.

In summary, combining fortnightly mancozeb sprays with a weather based metalaxyl programme reduced the total number of fungicide applications in the 2001/02 season by 40%, whilst maintaining effective control of disease (no significant difference in bulb weight between mancozeb regimes cf. other metalaxyl treatments).

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Integrated approaches to control of grey mould (*Botrytis cinerea*) in greenhouse crops of container-grown ornamentals

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ABSTRACT

A simple method based on monitoring and control of high humidity periods was developed to aid management of grey mould (*Botrytis cinerea*) in greenhouse crops of pot, bedding and nursery stock plants. A nightly heat-boost and ventilation treatment during the final 12 weeks of cyclamen production reduced disease severity by 29-45% and increased the proportion of marketable plants by 17%. In trials on commercial nurseries, excellent control of cyclamen botrytis was obtained when control of the glasshouse environment was combined with a fungicide spray programme. In crops of calluna, cyclamen and helianthemum, various fungicide programmes resulted in significant reductions of botrytis, with pyrimethanil and tolylfluanid effective on all three species. Crop management practices such as regular picking-off of dead leaves and increased air-movement at plant canopy level were shown to reduce botrytis.

INTRODUCTION

Grey mould caused by *Botrytis cinerea* is a common disease of many pot, bedding and nursery stocks plants, causing production losses in the UK of c. £7 M annually. Control of the disease is primarily by fungicides (O'Neill & McQuilken, 2000a) although in recent years, with reduced fungicide availability and occurrence of resistant pathotypes, these have not always provided commercially acceptable control (Yourman & Jeffers, 1999). Introduction of the anilinopyrimidine fungicides has brought prospects for improved control (Forster & Staub, 1996). High humidity is a major factor favouring leaf infection by *B. cinerea* conidia (Jarvis, 1980). There is evidence that modifying irrigation practice to reduce leaf wetness and high humidity can reduce the disease (O'Neill & McQuilken, 2000b). Spread of the disease by direct contact is also important in some crops, often arising from infested plant debris. The aim of the work described here was to evaluate non-chemical methods of control, particularly humidity reduction and debris removal, alone and integrated with timely use of fungicides.

MATERIALS AND METHODS

Trial details

Trials were conducted in greenhouse compartments at research sites (HRI Efford, Lymington, Hants; SAC Ayr, Auchincruive Estate, Ayr) and in commercial greenhouses in Argyll (calluna), Cambridgeshire (helianthemum) and Herefordshire, Lincolnshire and Norfolk (cyclamen). Except where indicated, all trials were of a randomised block design with four-fold replication, with extra replication of the untreated and a minimum of 10 plants per plot. Crops were grown to good commercial practice from the plug plant stage until ready for marketing. Disease incidence (% plants affected) and severity (0-5 index for cyclamen and helianthemum; % browning for calluna) were assessed when plants were ready for marketing (cyclamen index: 0 - healthy; 1 - one dead leaf affected; 2 - more than one dead leaf affected; 3 - one green leaf affected; 4 - more than one green leaf affected; 5 - plant collapsed).

Experiments

The efficacy of heat-boost and increased ventilation in controlling botrytis on cyclamen, cv. Sierra, was evaluated in four 20 m^2 compartments (c. 440 plants in each) in a linear array at HRI Efford in autumn 1999 (experiment 1) and 2000 (experiment 2). No fungicides were applied to the crops and leaf debris was not removed. In 2001, similar experiments were carried out in Herefordshire (experiment 3) and Lincolnshire (experiment 4) in paired glasshouses with additional heat and ventilation applied to one house in each pair. Humidity reduction was achieved by increasing the heating pipe temperature and ventilation. This was applied nightly from 02:00 to 04:00 h, and in some experiments was set to trigger only when within-crop humidity, measured with a Hycal monolithic humidity sensor (model IH-3602) exceeded 90% for more than 3 h.

Debris removal and increased air movement (experiment 5) were evaluated in a crop of cyclamen, grown on a commercial nursery in Norfolk in autumn 2000, and compared with a 5-spray fungicide programme of pyrimethanil alternating with tolylfluanid. Debris removal on cyclamen was done by hand, either once at plant-spacing or every 2-3 weeks in place of fungicide treatment. Increased air-movement around plants was achieved by use of a 60 mm diameter perforated polythene tube laid along the centre of benches (3 rows of cyclamen either side) fitted to a small fan to inflate the tube and blow air horizontally through the leaf canopy sufficient to gently move leaves.

Programmes of alternating sprays of two fungicides were evaluated in full-season trials on calluna, cyclamen and helianthemum on commercial nurseries (experiments 6-8). Fungicides were applied from immediately after potting until marketing, usually at 2 week intervals, using a pressurised sprayer operating a 200 –300 kPa, spraying to the point of run-off. For cyclamen, a single nozzle was used to apply fungicide into the crown of individual plants, as practised commercially. Fungicides used were: azoxystrobin (Amistar, 25% SC) at 0.25 g/l; chlorothalonil (Bravo 500, 50% SC) at 1.1 g/ml; dichlofluanid (Elvaron, 50% WG) at 0.85 g/l; iprodione (Rovral, 50% WP) at 0.5 g/l; prochloraz Mn (Octave, 50% WP) at 0.5 g/l; pyrimethanil (Scala, 40% WP) at 0.8 g/l; tebuconazole (Folicur, 25% SC) at 0.26 g/l and tolylfluanid (Elvaron Multi, 50% WP) at 0.85 g/l.

RESULTS

Reducing long-duration high humidity periods

In 1999 (experiment 1), although the severity of botrytis was low there were clear differences between treatments. Disease severity at marketing (0-5 scale) was reduced from 1.6 (grower standard treatment) to 0.9 where a routine nightly heat boost was used. The sensor-driven predictive system was almost as effective (Table 1). An increase of just 1% in marketable plants compensated for the additional heating cost. In 2000 (experiment 2), a severe attack of botrytis occurred and both the routine and sensor-triggered treatments again reduced disease. Results in the paired-glasshouse crops of cyclamen in Herefordshire and Lincolnshire, in which a humidity sensor-driven heat boost and ventilation treatment was applied in only one glasshouse in each pair, were consistent with control of botrytis by humidity reduction. The high level of disease at Herefordshire was associated with high humidity in the plant canopy (Table 2). Although the heat boost/vent treatment decreased relative humidity, the reduction was insufficient to avoid the plants from being affected by grey mould. Even in the heat boost/vent treatments there were times when the relative humidity exceeded 90% for periods longer than 3 h due to insufficient supply of heat and the external vapour pressure being greater than inside the greenhouse.

Table 1. Effect of glasshouse heat boost and increased ventilation treatments on incidence and severity of cyclamen grey mould at marketing (unreplicated comparisons).

Treatments		% plants affected	Disease severity (0-5)
1999 (HRI Efford) – Exp. 1			
1.	Grower standard	67.2	1.6
2.	Constant 2°C above ambient	53.4	1.3
3.	Routine heat boost/vent	45.9	0.9
4.	Sensor-driven heat boost/vent	44.5	1.1
2000 (HRI Efford) – Exp. 2			
1.	Grower standard	75.7	3.5
2.	Routine heat boost/vent	57.7	2.5
3.	Sensor driven heat boost/vent	64.5	2.3
2001 (Herefordshire) – Exp. 3			
1.	Grower standard	100.0	4.4
2.	Sensor-driven heat boost/vent	85.0	3.1
2001 (Lincolnshire) – Exp. 4			
1.	Grower standard	81.3	3.4
2.	Sensor-driven heat boost/vent	52.1	2.2

Table 2. The mean night-time relative humidity (%) of grower standard and sensor-driven heat boost/vent treatments measured in the aspirated screen and in the plant canopy (mean of three sensors) in a cyclamen trial in Herefordshire (experiment 3).

Week	Grower standard		Sensor-driven heat boost/vent	
	Aspirated	In-plant	Aspirated	In-plant
40	83	83	81	84
41	92	91	87	87
42	91	91	87	87
43	92	92	88	90
44	92	92	83	86

Debris removal and increased air-movement

Disease severity at marketing was greatest on untreated plants and least with regular picking-over to remove dead leaf debris, which was similar to the fungicide programme (Table 3). Increased air-movement around plants had a small but significant effect on disease control. Removing only leaves that were visibly affected by botrytis was not as effective as removal of all dead leaves.

Table 3. Effect of picking-over, increased ventilation and a fungicide programme on grey mould of cyclamen at marketing - autumn 2000 (experiment 5)

Treatment		% plants affected	Mean disease severity	% plants unmarketable
1.	Untreated	64.5	2.9	39
2.	Dead leaves removed (x5)	27.1	1.0	14
3.	Botrytis-affected leaves removed (x5)	41.7	1.9	29
4.	Extra ventilation via polythene tube	58.3	2.2	24
5.	Ventilated and dead leaves removed	37.5	1.7	27
6.	Fungicide programme (5 sprays)	41.7	1.3	19
	Significance (19 df)	<0.001	<0.001	<0.05
	SED between treatments	5.2	0.34	7.2
	vs untreated	4.5	0.30	6.2

Fungicide programmes and integrated control treatments

Many of the fungicide treatments (experiments 6-8) resulted in significant reductions in disease, with programmes of pyrimethanil and tolylfluanid giving good control in all three trials (Table 4). In the paired glasshouse trials (experiments 3-4), disease severity in cyclamen was least where a 6-spray fungicide programme was combined with a sensor-driven heat-boost and ventilation treatment. Picking-over once had little effect. Fungicide treatment was noticeably less effective in the glasshouses where no environmental control was applied (Table 5).

DISCUSSION

These results indicate that short duration heat-boost and ventilation treatments aimed at preventing periods of high humidity (>90%) for greater than 3 h within the plant canopy of can reduce the severity of grey mould in geeenhouse crops of cyclamen. Possibly this relates to effects on germination of *B. cinerea* conidia on the leaves. Laboratory studies have shown that, at 10-20°C, rapid germination of *B. cinerea* conidia on cyclamen leaves is strongly favoured by high humidity (>95%) for at least 3 h; moreover, germination can be prevented by reducing humidity to 80%, providing conidia have imbibed moisture for less than 3 h (Pettitt & O'Neill, unpublished). Previous studies with late-planted glasshouse tomatoes have shown that continuous increased temperature and ventilation between dusk and dawn can reduce botrytis (Morgan, 1984), although routine use of this approach was prohibitively expensive. Application of extra heat and ventilation only when conditions are conducive to infection by *B. cinerea* conidia is economically more attractive and was demonstrated to be effective in the cyclamen trials reported here.

Table 4. Effect of selected fungicide programmes on *B. cinerea* on container-grown ornamentals at marketing

Treatment (alternating sprays)	Mean % plants affected		Mean disease severity	
Calluna (5 sprays) (Experiment 6)			% browning	
Untreated	100	(90)	22.9	(28.4)
Azoxystrobin/Pyrimethanil	70	(58)	8.0	(16.3)
Tolylfluanid/Pyrimethanil	71	(58)	8.4	(16.7)
LSD (P=0.05)		(12.1)		(3.1)
Cyclamen (5 sprays) (Experiment 7)			0-5 index	
Untreated	65		1.9	
Pyrimethanil/Tolylfluanid	25		0.5	
Azoxystrobin/Tolylfluanid	72		1.6	
Iprodione/Tolylfluanid	69		1.8	
Significance (34 df)	<0.05		<0.05	
SED	1.5		0.3	
Helianthemum (8 sprays) (Experiment 8)			0-5 index	
Untreated	18		1.5	
Pyrimethanil/Tolylfluanid	4		0.3	
Pyrimethanil/Prochloraz Mn	13		0.9	
Pyrimethanil/Chlorothalonil	5		0.5	
Pyrimethanil/Azoxystrobin	11		0.8	
Significance (31 df)	<0.001		<0.001	
SED	3.6		0.37	

Angular transformed values are shown in parentheses.

Table 5. Effect of humidity reduction, fungicide treatment and picking over on severity of botrytis on cyclamen at marketing

Treatment		Mean disease severity at marketing			
Fungicide	Picking over	Lincolnshire trials (Experiment 3)		Herefordshire trials (Experiment 4)	
		+ H/V	- H/V	+ H/V	- H/V
1. Untreated	No	2.2	3.4	3.1	4.4
2. Pyrimethanil (x1)	No	1.7	4.1	2.8	4.2
3. Programme[a]	No	0.2	1.9	0.7	3.1
4. Untreated	Yes	2.1	3.4	2.0	4.0
5. Pyrimethanil	Yes	1.3	4.8	2.4	4.2
6. Programme[a]	Yes	0.2	1.9	0.5	3.3
Significance (15 df)		<0.05	<0.05	<0.05	<0.05
SED		0.70	0.71	0.50	0.93

[a] Pyrimethanil/dichlofluanid/prochloraz/dichlofluanid/pyrimethanil/dichlofluanid.

+ H/V - with heat boost/vent; - H/V without heat boost/vent.

Although increased gentle air movement around cyclamen plants had only a slight effect on botrytis occurrence, the potential for greater use of air movement as a disease management tool warrants further investigation. Forced heated air from 22:00 to 06:00 h, combined with a plastic mulch on pot tops, which could reduce humidity around plants, significantly decreased the incidence of sporulating botrytis on lower necrotic leaves of geranium stock plants (Hausbeck *et al.*, 1996).

When it is not possible to reduce humidity within a greenhouse by increased heat and ventilation (e.g. due to high outside humidity), fungicide treatment and picking-over to remove leaf debris provide alternative control methods for cyclamen. It is interesting that the fungicide programme on cyclamen (Table 5) was considerably more effective at the lower disease pressures found in the two glasshouses where heat-boost and ventilation was implemented, supporting an integrated approach to management of botrytis.

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The effect of rain splash on the development of rose blackspot and implications for a disease control strategy

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ABSTRACT

The development of rose blackspot lesions was monitored within a rose bush throughout 2001. Temperature, humidity, leaf wetness, height of rain splash and intensity of rainfall were simultaneously recorded within the bush. The greatest, earliest development of the disease was in the bottom 30 cm of the bush, coinciding with the highest concentration of rain splashes early in the growing season. Lesion development increased more rapidly in the wetter months of September and October 2001 when there was increased durations of both relative humidity and leaf wetness. Temperature during the monitoring period had little effect on lesion growth. The implications for disease control are discussed.

INTRODUCTION

Roses are an important horticultural crop in the UK, with exports alone worth £669,000 in 1997. Blackspot is one of the most severe diseases of field grown roses, caused by the host specific facultative fungal parasite *Diplocarpon rosae*, a disease that is confined only to the genus *Rosa*. The pathogen is now found world-wide and is endemic wherever roses are cultivated in gardens and is often a major problem to growers (Horst, 1983). The symptoms are dark brown-black lesions of 2-12mm in diameter which appear on the upper surface of the leaf. Leaf tissue surrounding the spots turns yellow and chlorosis extends throughout the leaflet until defoliation occurs (Horst, 1983). Acervuli which form within the lesions produce conidia which are dispersed throughout the rose bush by water run-off or splash. (Saunders, 1966). The fungus overwinters as acervuli on infected dead leaves, stems and thorns (Cook, 1981) and hence the main inoculum source for the disease has been reported to be from diseased leaves at the bottom of the bush (Cook, 1981) with initial disease spread by upward splash by rain. The current recommended control is therefore to gather and burn infected leaves from under the bushes and to spray fungicides regularly. Commercial growers spray fortnightly whilst amateur gardeners may spray nearly as frequently.

The aim of the work reported here was to monitor disease development and spread of the pathogen within a rose bush in relation to environmental conditions. The relationship between temperature, rain intensity, rain splash, leaf surface wetness and disease development throughout a rose bush was investigated.

MATERIALS AND METHODS

Environmental monitoring

Air temperature (°C) and relative humidity (%) were recorded hourly (Gemini Tinytag Plus) throughout 2001 in and around rose bushes. Rainfall was recorded weekly. Leaf surface wetness was recorded every hour using the Gemini Tinytag leaf wetness recorder.

Upward splash was measured using a splashmeter, adapted from Shaw (1987). The device was placed within a rose bush in May 2001 and evaluated at intervals. Raindrops were allowed to fall into small troughs containing glass fibre, saturated with a cellulose binding dye, UVITEX NFW (Ciba-Geigy) diluted at a rate of 1:100 with distilled water. 3 ml of the dye was placed in each trough. The splash drops were caught on a cylinder of chromatography paper placed in the centre of the troughs. The frequency and height of raindrops and the percentage of rain-splash were measured.

D. rosae lesion and disease development

D. rosae lesion development was monitored each week from April to October 2001 by assessing the occurrence and growth of lesions on individual leaflets. At each assessment date, the area covered by lesions on each leaflet was determined. A total of 80 leaflets was examined in this study. Rose blackspot disease incidence was also assessed at intervals as the percentage leaves infected on the bush.

RESULTS

Environmental conditions and lesion development

Rainfall was most frequent from August to December 2001 and was correlated with leaf wetness and high relative humidity. Disease development on leaflets appeared to be related to increased leaf wetness and relative humidity (Figure 1). This was confirmed when total hourly durations of leaf wetness (>80%) and relative humidity (>80%) were calculated for corresponding lesion development assessments on leaflets. As leaf wetness and relative humidity durations increased, so did the rate of lesion development (Table 1). The most rapid lesion development was therefore in the wetter months of September and October 2001, whereas the slowest disease development was in the drier months of June and July. This was despite the fact that mean temperatures were higher in these latter time periods (Table 1).

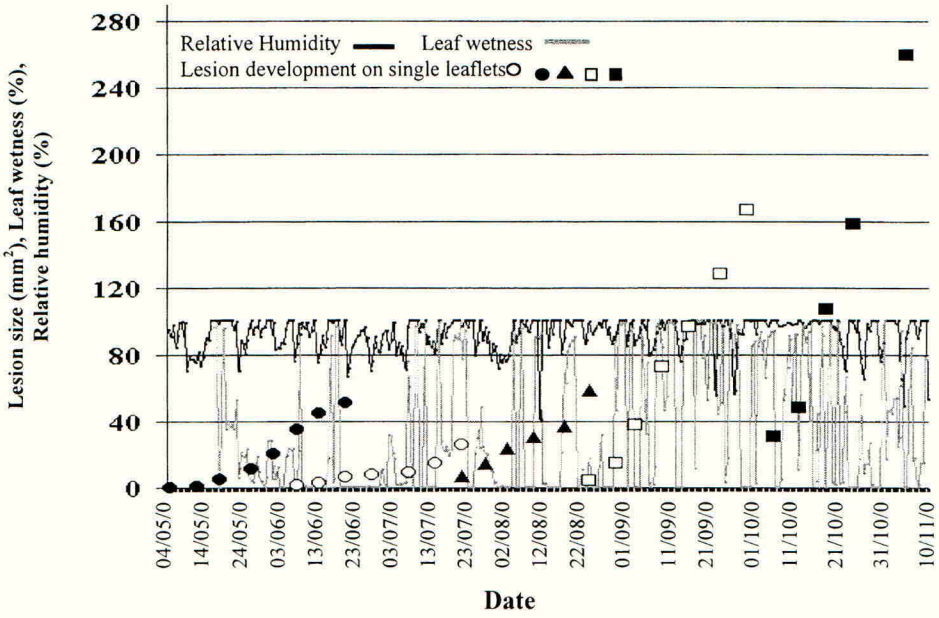

Figure 1. Humidity, leaf wetness and *D. rosae* lesion development for May to November 2001.

Table 1: Effect of temperature, relative humidity (>80%) and leaf wetness (>80%) duration on the rate of *D. rosae* lesion development in 2001.

Time period	Hours relative humidity > 80%	Hours leaf wetness >80%	Mean temperature (°C)	Rate of lesion development (mm^2 / day)
11/5/01-19/06/01	396	108	17	1.23
06/06/01-20/07/01	492	144	18	0.48
20/07/01-23/08/01	379	135	19	1.39
23/08/01-03/10/01	625	378	14	4.06
10/10/01-14/11/01	658	236	10	6.82

Rain-splash and disease transport

The splashmeter allowed the height and density of rain splashes within the bush to be quantified. The greatest concentration of rain splashes was at the base of the bush with less frequent rain splashes being recorded high on the splashmeter (Figure 2). Rose blackspot symptoms occurred first at the bottom of the bush (0-30cms) which coincided with the greatest frequency of rain splash on the splashmeter (5-15cms) from 80-100%. Symptoms slowly progressed to the middle of the bush (30-60cms) where the frequency of rain-splash ranged from 30-40% and then to the top of the bush where the frequency of rain-splash ranged from 0-5% (Figures 2 and 3). At this height there were few rain splashes to initiate infection. At the end of the season (October 2001), rose blackspot was evenly distributed throughout the bush, as lesions were initiated high in the bush by upward splashes, which allowed a trickle down effect to occur. A period of heavy rainfall between 2nd and 10th August 2001 resulted in rain-splashes reaching 55cms up the bush (Figure 2).

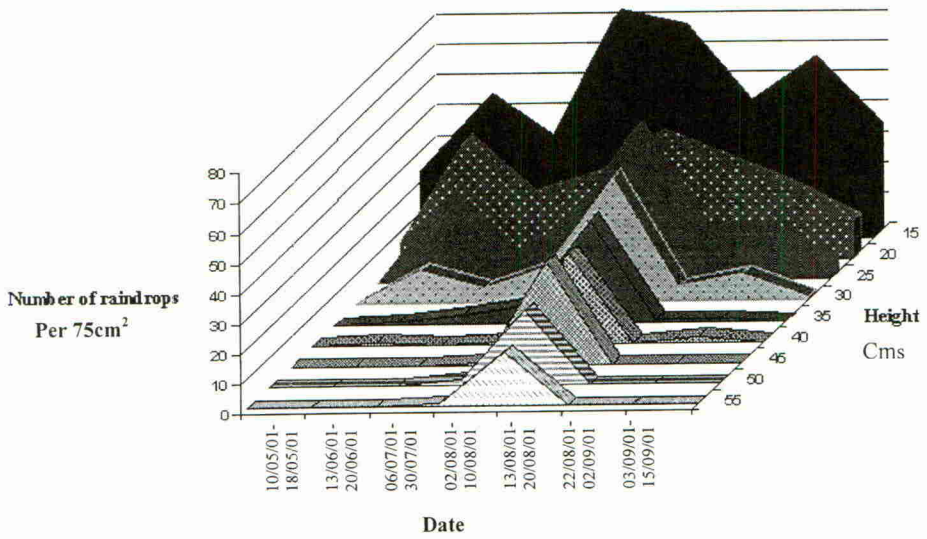

Figure 2: Number of raindrops splashed at different heights within a rose bush from May to September 2001.

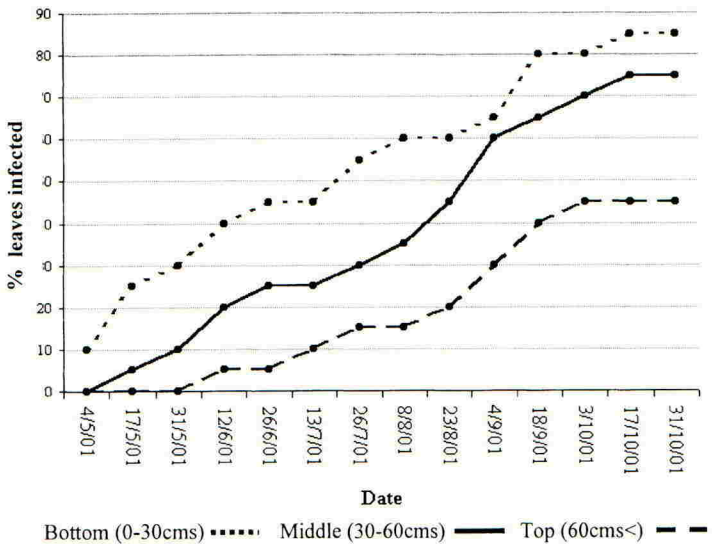

Figure 3: Progression of rose blackspot disease (% leaves infected) within a rose bush in 2001.

DISCUSSION

The period of heavy rain between the 2nd and 10th August followed by further rainfall, high humidity and leaf surface wetness resulted in rapid lesion development from 22 August. For the period of the experiment, temperature was not a limiting factor in the development of rose blackspot disease as the fastest rate of lesion development occurred in the period with the lowest mean temperature.

High rates of lesion development coincided increased durations of leaf wetness and high humidity. It would appear however that high humidity alone, even combined with increased mean temperature does not result in faster lesion development (Table 1). Further investigation in determining the role of surface wetness and the relationship to temperature is required. The greatest, earliest development of rose blackspot was in the bottom 30cms of the bush, which coincided with the highest concentration of rain splashes in the growing season. The results suggest that the advice to pick up and burn dead leaves would be insufficient to give good blackspot disease control and that water splash is clearly vital in spreading infection throughout the bush.

Implications for a disease control strategy

The findings in this study lead us to suggest the following modifications to the traditional disease control strategy of this disease:

- Most roses in the field have acervuli on the stems and thorns (Cook 1981). Work reported here clearly shows how water splash spreads the disease throughout a bush. Commercial

growers should therefore reconsider their practice of trickling water over uprooted rose bushes prior to root pruning and potting on as this has the effect of spreading infective conidia throughout several hundred rose bushes.

- The old advice to clear up dead, infected leaves from under bushes continues to be beneficial but additionally, commercial and amateur growers should pay particular attention to obtaining good spray coverage of the bottom 30cms of the bushes early in the growing season. This would help to prevent upward spread of the disease.

- A fungicide wash of the canes in the winter may also help to reduce disease inoculum.

ACKNOWLEDGEMENTS

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Effect of leaf wetness duration and temperature on the development of leaf spot (*Septoria apiicola*) on celery

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ABSTRACT

Septoria leaf spot (*S. apiicola*) of celery is the most destructive disease of field-grown crops and work is ongoing to develop an integrated strategy for improved disease management. Controlled environment experiments were carried out to quantify the effect of environmental conditions (temperature and leaf wetness duration) on disease, as a preliminary step towards the development of a spray-timing decision tool. There was a significant trend for disease severity to increase with both temperature (5 to 25°C) and leaf wetness duration (1 to 96 h). Disease development occurred at lower temperatures and shorter leaf wetness durations than has been previously reported, although resulting disease severity was low (<5 %). Under optimum conditions for infection (20°C, ≥24 h leaf wetness), Septoria leaf lesions with pycnidia occurred on young celery plants, 10 d after inoculation.

INTRODUCTION

Septoria leaf spot (*S. apiicola*) of celery, also known as 'late blight', is the most destructive disease of field-grown crops. Initially seen as small brown spots on leaves, the disease can progress rapidly to cause extensive defoliation and render the whole crop unmarketable, if left unchecked. Following recent specific off-label approvals for the use of azoxystrobin (Amistar) and difenoconazole (Plover) on celery, as well as chlorothalonil (Bravo 500) and copper fungicides, Septoria leaf spot can be effectively controlled by fungicide applications. However, sprays may need to be applied at 10-day intervals for the duration of the growing season to ensure a marketable crop. Current research aims to develop integrated management strategies for celery leaf spot, including the use of a range of fungicides representative of different chemical groups, applied only when environmental conditions are conducive for disease development.

Leaf wetness duration and temperature strongly influence the development of celery leaf spot (Mathieu & Kushalappa, 1993) and are obvious parameters that could aid spray decisions. For example, Lacy (1994) identified 12 h leaf wetness as a useful threshold above which there is significantly increased likelihood of Septoria spore germination and leaf infection. In three successive seasons, spray timing based on this threshold reduced the number of sprays required to maintain disease control from seven to five in an inoculated

crop in the USA. The controlled environment experiment described in this paper was conducted to determine the effect of temperature and leaf wetness duration on disease, as a preliminary step towards the development of a spray-timing decision tool for celery crops in the UK.

MATERIALS AND METHODS

Celery plants artificially inoculated with *S. apiicola* were incubated at six temperatures (5, 10, 15, 20, 25 and 30°C) in a controlled environment (CE) cabinet (SGC 297 Growth chamber; Sanyo Gallenkamp plc). At each temperature, six leaf wetness periods were tested (1, 6, 24, 48, 72 and 96 h), with ten plants per temperature-wetness combination. The experiment was conducted over time with one inoculation per temperature. Leaf wetness treatments were in a randomised block design within the CE cabinet and the order of temperature treatments in sequential experiments was randomised.

As temperatures were tested over time, their effect could have been confounded with that of inoculum. To minimise confounding, percentage spore germination was determined to ensure that spore viability remained uniform for all temperatures (>90 % germination). In addition, the experiment was repeated at a single temperature (20°C) to provide data to test for concordance or otherwise of the experimental technique.

For inoculum preparation, 10-30 g dried celery leaves previously infected with *S. apiicola* were immersed into 150-400 ml distilled water, left for 30 min and agitated. The suspension was strained through muslin and adjusted to 10^6 conidia/ml using a haemocytometer. To check spore viability, a sample of spore suspension (20 µl) prepared for each temperature treatment was pipetted and spread on to each of three plates of potato dextrose agar amended with streptomycin. Percentage spore germination, based on 100 spores per plate, was determined after incubation for 24 h at 20°C.

Young celery plants cv. Celebrity used for the experiment had 3-4 true leaves when the trial commenced. Inoculated plants were sprayed to run-off with the spore suspension using a spray bottle with atomiser and were then placed in a misting chamber in the CE cabinet. For each experiment, six uninoculated control plants were also placed in the misting chamber but separated from the inoculated plants. In order to maintain leaf wetness, intermittent mist was provided by a timer-operated cold mister. Temperature and relative humidity within the mist chamber were monitored with a data logger. The plants received a 12 h day/12 h night light regime.

At the end of each wetness period, ten plants and the uninoculated control plants were removed and dried for approximately 30 min with an electric fan. Plants for the 1 h leaf wetness treatments were dried immediately after inoculation. Initially, the 2nd and 3rd true leaves were marked to allow subsequent disease assessments on these leaves. In later experiments using older plants, the 3rd and 4th true leaves or 4th and 5th true leaves were marked, as appropriate, to ensure that assessments were made on leaves of a common age. After drying, plants were potted into 9 cm diameter pots and placed on capillary matting in a glasshouse. Temperatures and relative humidity in the glasshouse were monitored with a data logger. Plants were grown on for 4 weeks to allow symptom development, during

which time they were watered around the base to avoid leaf wetting and were spaced to prevent contact between plants.
Twice weekly from the time of inoculation, the incidence of Septoria lesions was assessed on each plant. At 14 and 28 days after inoculation, percentage leaflet area affected by Septoria lesions was estimated for each of the 3 leaflets of the two previously marked leaves.

Statistical analyses was conducted using response surface methodology available in Statistica (Anon, 2000). The two factors of interest were incubation temperature and the duration of wetness. These were not linear in their effect on the percentage of area covered by lesions and therefore a response surface design was employed for analysis. The regression surfaces, using distance weighted least squares, were prepared for disease severity data at 14 days (data not presented) and 28 days after inoculation.

RESULTS

Spore germination exceeded 90 % for each set of inoculum used in the experiment. There was no disease development on the untreated control plants.

The shortest time to symptom development (10 days) occurred when leaves were incubated at 20°C with 24 h, or more, leaf wetness. For temperatures >20°C and leaf wetness durations ≥24 h, the appearance of symptoms was also generally rapid with 100 % incidence being reached 15 days after inoculation (Tables 1 and 2). Although lesions were seen on plants for the majority of other leaf wetness-temperature combinations, first symptoms were not frequently observed before 15 days post-inoculation but disease continued to develop on individual plants until the end of the experiment (28 days after inoculation). The final disease incidence was also less than 100 %. For example, when plants were incubated at 20°C with only 6 h leaf wetness duration after inoculation, it was 18-28 days before symptoms first occurred, and the final disease incidence of 20 %.

Table 1. Effect of temperature and leaf wetness duration on the number of days between inoculation with *Septoria apiicola* and appearance of the first lesions on celery.

	Wetness duration (h)					
Temp (°C)	1	6	24	48	72	96
5	15-28[a]	15-28	18-28	11-28	15-28	15-28
10	-	25-28	-	18-28	**15**	**15**
15	11-28	11-28	15-28	11-28	11-28	15-28
20	25	-	22-28	11-18	**11-15**	**11**
20[b]	15-28	18-28	**10-15**	**10-15**	**10**	**10**
25	22-28	15-28	**15**	**11-15**	**11-15**	**11-15**
30	18-28	18-28	15-28	**15**	**11-15**	**15**

[a] The range represents the period during which first lesions appeared on the 10 plants sampled.
[b] Duplicate test at 20°C.
Combinations where all plants developed symptoms within 15 days in bold.

The effects of temperature and leaf wetness on disease severity at 28 days (Table 3) were modelled by regression analyses. Quadratic coefficients for temperature and leaf wetness

duration, and their interactions, were statistically significant at $P<0.001$. Inspection of the residual versus predicted plot, and the probability plot for the residuals, indicated that the data and model were satisfactory. There was no global function for the 3-dimensional response surface plot (Figure 1), since local least squares smoothing was used to take account of changes in disease severity at higher temperatures and leaf wetness durations. The response surface plot (Figure 1) shows an optimal ridge for wetness duration and temperature.

From the above analyses, there was a clear relationship between disease severity assessed 28 days after inoculation and increased temperature and leaf wetness. The percentage area of leaflets affected by lesions reached a maximum at approximately 25°C and 72 h leaf wetness duration. After 72 h duration and temperatures >25°C, disease severity tended to decline, such that at 30°C, disease severity after 28 d was broadly similar to that at 15°C. Infection occurred at temperatures as low as 5°C, but at <10°C considerable periods of continuous leaf wetness (>72 h) were required before disease severity of >5 % were recorded. At lower temperatures (5-15°C), wetness continued to have an increasing effect on lesion development beyond 72 h.

Table 2. Effect of temperature and leaf wetness duration on incidence of Septoria lesions, 25 days after inoculation of celery plants with *Septoria apiicola*.

	% Disease incidence[a] for different leaf wetness durations					
Temp (°C)	1 h	6 h	24 h	48 h	72 h	96 h
5	50	80	70	80	80	90
10	0	10	0	90	**100**	**100**
15	90	70	60	40	80	70
20	10	0	30	**100**	**100**	**100**
20[b]	50	20	**100**	**100**	**100**	**100**
25	10	20	**100**	**100**	**100**	**100**
30	20	10	60	**100**	**100**	**100**

[a] % disease incidence for ten plants per treatment; [b]duplicate test at 20°C.
Combinations where all plants developed symptoms within 25 days in bold.

Table 3. Effect of temperature and leaf wetness duration on % leaf spot severity 28 days after inoculation of celery plants with *Septoria apiicola*.

	% Disease severity[a] for different leaf wetness durations					
Temp (°C)	1 h	6 h	24 h	48 h	72 h	96 h
5	0.7	3.5	2.9	2.2	1.5	**19.0**
10	0.0	0.0	0.2	3.8	**32.8**	**50.1**
15	0.1	0.2	0.3	**10.5**	**24.2**	**25.8**
20	0.0	3.3	0.3	**20.2**	**34.7**	**45.2**
20[b]	0.2	0.1	**8.4**	**35.5**	**52.6**	**31.4**
25	0.3	0.5	**20.9**	**43.2**	**55.7**	**50.9**
30	0.0	0.1	0.4	**10.7**	**31.1**	**26.5**

[a] Mean % disease severity for six leaflets per plant, ten plants per treatment; [b]duplicate test at 20°C.
Disease severity >5% in bold.

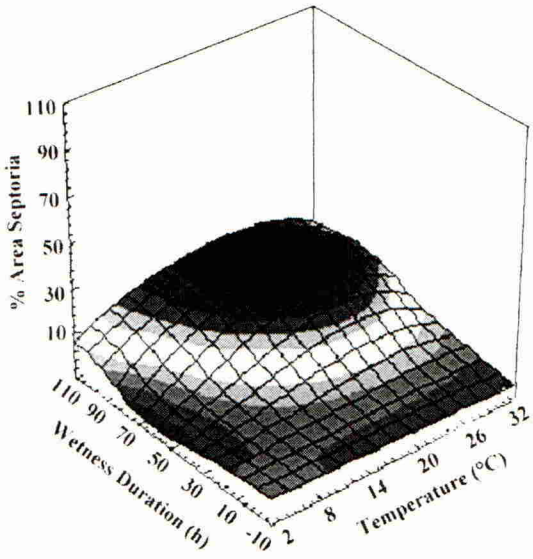

Figure 1. Response surface plot of percent area of Septoria lesions per leaflet versus temperature and leaf wetness duration, 28 days after inoculation, using distance weighted least squares.

DISCUSSION

The controlled environment experiment provided detailed information on the effects of leaf wetness duration and temperature on the development of celery leaf spot (*S. apiicola*) on young plants. Characteristics of disease development, such as the number of days to symptom appearance, optimal temperature/leaf wetness combinations and the decline in disease severity at temperatures >25°C, correspond with results from previous research (Mathieu & Kushalappa, 1993; Lacy, 1994).

Symptoms were, however, observed at lower temperatures and after shorter periods of leaf wetness than has been previously reported. For example, infection occurred at temperatures as low as 5°C, although it required 96 h of leaf wetness at this temperature before the disease severity 28 days later exceeded 5 % leaf area affected. Similarly, when leaf wetness duration was restricted to just 1 hour, there was a low incidence of infection at most temperatures, although this resulted in a disease severity of only 0.1 - 0.7% leaf area affected, 28 days later. The ability for infection to occur under these less than optimal conditions should be taken into account during development of a risk prediction scheme.

Further experiments are planned to test leaf wetness durations between 6 and 24 h at different temperatures, to determine more precisely the minimum leaf wetness duration which allows severe disease (>5% leaf area after 28 days). In addition, the effects of fluctuating temperatures on disease development will be studied, to more closely reflect

day/night temperature variations under a field situation. For example, Krauthausen *et al.*, studying parsley Septoria (*S. petroselini*) reported that with fluctuating incubation temperatures (24°C day/18°C night) resulting disease severity was greater than with constant incubation temperatures.

Based on the findings of this work together with information from in-crop data loggers on typical conditions occurring during the growing season, low, moderate and high-risk thresholds for temperatures and leaf wetness durations that are conducive for disease development will be determined. These could form the basis of a spray-timing decision tool for growers.

ACKNOWLEDGEMENTS

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Assessment of the impact of water treatments on potential indicators of microbial suppression of root disease in hydroponic tomatoes

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ABSTRACT

'Active', UV irradiation, and 'passive', slow sand filtration (SSF) treatments, known to control disease spread in recirculating irrigation systems, were tested against *Phytophthora cryptogea* root rot on tomato in recirculating hydroponic systems. The impact of these two treatments on the populations of two possible indicators of natural disease suppression, total aerobic bacteria and fluorescent Pseudomonads, was compared with non-treated controls. Both treatments successfully controlled the secondary spread of *P. cryptogea*, although neither showed any signs of curative effects on plants already infected. There were no significant effects of the nutrient solution treatments on the populations of aerobic bacteria or fluorescent Pseudomonads, which seemed most affected by development of the tomato plants. However, no measurable differences in disease suppression were seen in any of the treatments and the possibility of subtle population shifts not measurable by cfu counts cannot be ruled out.

INTRODUCTION

Interest across Europe in the use of closed growing systems, where excess water and nutrient solution is collected and re-used is increasing. However, a potential problem with the use of such closed systems is the increased risk of widespread dissemination of pathogen spores (*e.g. Phytophthora* spp.) from small point-sources. Such potential for rapidly spreading disease in closed systems has been demonstrated in a wide range of crops (Stanghellini & Rasmussen, 1994). Detailed studies of tomato systems were carried out at HRI Stockbridge House (1991-2000), which demonstrated the potential of a range of water cleaning systems for removing this risk of disease spread. These experiments also showed the occasional development of disease suppression, which was considered to be microbial in origin and possibly linked to changes in populations of fluorescent Pseudomonads. Whilst diverse water treatment systems appear efficient at limiting the spread of secondary inoculum of both *Pythium* and *Phytophthora* spp. (Mc Pherson *et al.*, 1995), anecdotal observations (McPherson & Pettitt, unpublished) indicated that some systems, especially slow sand filtration (SSF) had a

'curative' influence on disease downstream of water treatment. McPherson *et al.* (1995) postulated that using 'active' or 'passive' disinfection methods may be crucial for the establishment of a beneficial microflora in closed soilless cropping systems. 'Active' disinfection methods like heating, ozonation or UV-irradiation may completely eliminate all micro-organisms from treated nutrient solutions and reduce the populations in the rhizosphere (Zhang & Tu, 2000), whereas the effluent from a slow filtration unit can contain a relatively high concentration of micro-organisms (Waechter-Kristensen *et al.*, 1997). The work considered here formed part of a wider EU-funded project ('MIOPRODIS') aimed at preventing disease in closed soilless growing systems by optimising microbial disease suppression. Detailed assessments were made of the microbiota in tomato nutrient feed solutions of which counts of total bacteria and fluorescent Pseudomonad colony forming units (cfu) are considered here in relation to pathogen dispersal, disease spread and the impact of UV irradiation ('active') and SSF ('passive') water treatments.

MATERIALS AND METHODS

Experimental design and set-up

Experiments were run for two seasons (2000 at Stockbridge Technology Centre and 2001 at HRI Efford) using an identical experimental set-up. Four treatments were compared: uninoculated controls, inoculated controls, UV-treated systems, where recirculating nutrient solution was irradiated with UV using Hanovia Ltd treatment chambers, each with a WS200 control unit, and SSF-treated systems (Wohanka, 1992). There were 4 replicate independent recirculating hydroponic systems per treatment. Replicate systems were in 4 blocks arranged using a constrained randomisation to allow adjustment for positional effects within the greenhouse. Each independent hydroponic system consisted of a white polyethylene-lined galvanised steel trough containing 3 rockwool slabs (Grodan Talent) on each of which 5 tomato plants were grown, giving 15 plants per system. The slabs were numbered 1 to 3, with slab 1 being at the top of each trough and slabs 2 and 3 downstream. When area losses for the gaps between each independent system were allowed for, each system was estimated to have a cropping area of 8 m^2. This gave an initial plant density of 1.8 plants m^{-2}. One side-shoot was allowed to grow on each plant at the first truss and this took the notional plant spacing up to a commercially more realistic spacing of 3.75 plants m^{-2}.

Systems drained separately into 160 litre black polyethylene tanks. Nutrient solution collected in these tanks was topped up with fresh solution and the pH and conductivity (EC) were adjusted to maintain them in the range (pH 5.2-5.4 and EC 2.4-2.8 dSm^{-1}). By adding ballast to each tank, the optimum tank volume for the control of EC and pH, whilst giving the maximum volume for operational convenience, was found to be 140 litres. In the UV and SSF treatments, water was treated before draining into the collection tank. Nutrient solution was applied to each plant via the top of its block using drip feed lines. There were 23 drippers per system. These were arranged, 6 per slab (ie 1 per plant for the first 4 plants and 2 for the end plant), 1 to a test jug (to check applied volumes) and 4 spares (blocked off) that could be used as bleeds if needed.

Tomato seeds (cv. Espero) were sown in Kiem rockwool propagation blocks and supplied with bottom heat using Hotbox heatwave panels. Seedlings were blocked-on 7-9 days later and transferred to capillary benches in the tomato propagation block. Slab contact was

achieved 1 month after sowing and subsequently crops were maintained according to good agronomic practice.

Inoculations

A mixture of zoospores of three isolates of *Phytophthora cryptogea* (CBS 113.19; IMI 324217 & A987) of known high pathogenicity to tomatoes was used in inoculations. Two approaches to inoculation were used. In the first year uniform infection was attempted by inoculating all of the systems, except non-inoculated controls, simultaneously from a common tank inoculation, and recirculating the nutrient solution to these systems. Once infection was established, each system was separated from the others and run independently. In the second year, systems were maintained as independent units right from the start, with inoculations applied to individual plants in each inoculated system. To imitate the main source of inoculum in a recirculating system, inoculum was applied to each plant where the irrigation drippers applied nutrient solution. To determine whether it was possible for infection to be transferred within irrigation troughs, only plants on slabs 2 and 3 were inoculated.

Assessments

Simple assessments of the microbial populations were carried out at regular intervals during the development of the crops by plating samples of hydroponic solution, collected from the rockwool slabs onto selective agar media and counting cfu. Total aerobic bacteria were estimated on R2A (DIFCO), fluorescent pseudomonads on King's B medium (King *et al.*, 1954) and *P. cryptogea* propagules were first captured on membrane filters (Pettitt *et al.*, 2002), and then resuspended and counted on plates of a modified selective agar containing antibiotics (BNPRA, Pettitt & Pegg, 1991). Other microbial populations were assessed but are not considered here.

Disease was assessed at the end the experiments using a visual index (0-10) of the severity of root browning, and at intermediate times using plating of randomly sampled root segments onto BNPRA.

RESULTS

In both seasons establishment of initial infections was slow. This effect was more marked with the direct plant inoculations in 2001, where infection of inoculated plants was not established until week 45, despite 9 repeat inoculations. However, once infection was established, the production and dispersal of secondary inoculum was rapid, with *P. cryptogea* zoospores readily detectable in rockwool slabs and in nutrient solutions in collection tanks (Table 1 and 2). Treatment of the recirculating nutrient solution did not strongly affect the production of inoculum in inoculated plants (Tables 1 and 2), and also did not appear to have any curative effects on disease development as indicated by the severity of root browning (Figures 1a & b). Nevertheless, both UV and SSF treatments did successfully eliminate pathogen propagules from the recirculating nutrient solution between the collection tanks and the uninoculated slabs (Table 2). Also when, in 2001, use was made of 'infector' plants to assess the distribution of disease within systems, both water treatments prevented the spread of disease from inoculated to uninoculated plants in the same systems (Figure 1b).

Table 1. Numbers of Phytophthora cryptogea cfu per litre of hydroponic solution in samples collected from tomatoes in recirculation systems in the 2000 season

Treatment	Sampling date					
	20/04/00	15/05/00	02/06/00	03/07/00	21/07/00	18/08/00
Uninoculated control	0	-	0	0	0	0
Inoculated control	0	+	43	72	53	47
UV – treated	0	+	54	81	269	134
SSF – treated	0	+	48	116	266	47

Table 2. Numbers of *Phytophthora cryptogea* cfu per litre of hydroponic solution in samples collected from slabs and from collection tanks in the 2001 tomato crop (collected 20/11/01)

Treatment	Sample		
	Slab 1 (uninoculated)	Slabs 2 & 3 (inoculated)	Collection tanks (pre-treatment)
Uninoculated control	0	0	0
Inoculated control	625	700	29
UV – treated	0	130	15
SSF – treated	0	600	9

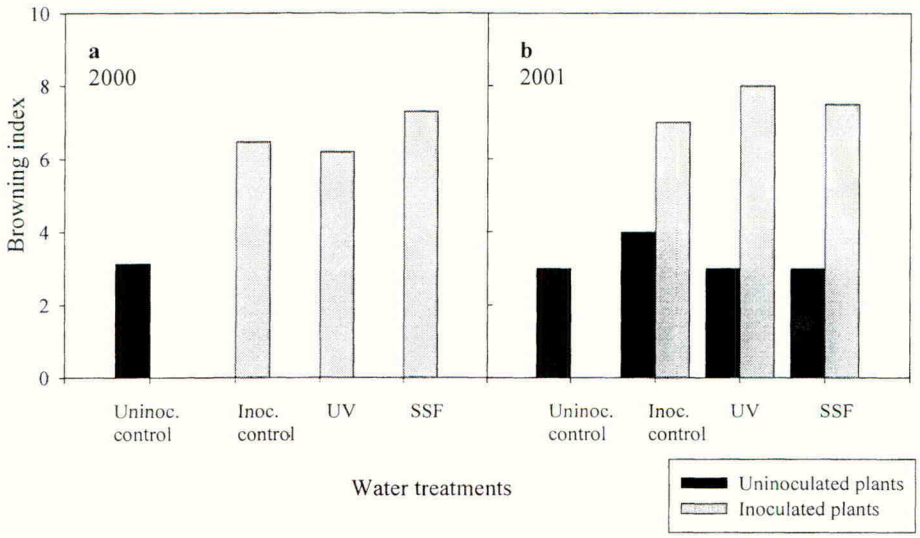

Figure 1. Comparisons of disease spread as indicated by the levels of root browning seen in inoculated and uninoculated plants at the termination of the 2000 and 2001 experiments

These observations of browning symptoms were supported by isolations from randomly sampled root segments, which showed widespread infection of uninoculated plants in inoculated controls but not in UV- and SSF-treated systems. In all systems assessed there were no measurable differences in disease suppression between the different treatments. The populations of culturable aerobic bacteria remained remarkably stable throughout both experiments between 5.1 and 6.25 $\log_{10}$ cfu ml^{-1} with no significant differences irrespective of treatment or sampling time. In contrast the fluorescent Pseudomonads generally declined over the cropping period in both 2000 and 2001 (Table 3). As a result of the late disease establishment, the 2001 crop was continued into November, when the fluorescent Pseudomonad population showed a sharp increase (Table 3). Despite these changes over time, however, there were still no significant differences between the treatments on individual sampling dates in the numbers of cfu recovered.

Table 3. Changes in fluorescent Pseudomonad populations in tomato nutrient feed solution samples as indicated by $\log_{10}$ cfu counts ml^{-1} on King's B agar

Treatment	Sample date									
	2000					2001				
	25/04	09/05	25/05	20/06	01/08	13/06	18/07	14/08	17/09	20/01
Uninoc. control	4.46	3.56	-	1.00	1.45	1.97	1.45	0.81	0.63	2.52
Inoc. control	4.15	3.78	2.52	1.99	0.92	3.02	1.77	1.19	1.60	2.99
UV – treated	3.53	3.90	2.18	1.12	-	2.07	1.81	0.94	0.90	2.71
SSF – treated	3.77	3.71	2.48	1.26	-	2.53	1.78	2.03	1.04	2.71

DISCUSSION

The spread of *P. cryptogea* root rot disease was successfully controlled in tomatoes by the application of UV or SSF treatments to the recirculating nutrient solution, a result in keeping with previous studies on other plant species (Wohanka, 1992; Postma *et al.*, 2001) and on tomatoes (McPherson *et al.*, 1995). There was little evidence of disease suppression in either the 2000 or the 2001 experiment. Disease suppression on a large scale in hydroponic crops can be elusive, only occurring in about 30% of crops, and is best highlighted when closed systems are compared with run-to-waste systems (McPherson, unpublished). Postma *et al.* (2000) observed microbial disease suppression in small-scale cucumber systems infected with *Pythium aphanidermatum* over 35 days, in which total aerobic bacteria populations increased with time in new rockwool slabs. However, in the current study, once established, total aerobic bacteria populations remained stable over the longer term. Measuring the total bacterial populations is a very crude assessment of effects on the microbiota, as large shifts in the species composition of these populations may occur as indicated by changes in fluorescent pseudomonad populations as well as other groups not presented here. These changing populations appeared largely influenced by the stage of crop development, relating possibly to the availability of carbohydrates, and not to differences in nutrient solution treatments. No apparent differences in the populations of the two possible indicators of disease suppressiveness were seen between treated and untreated recirculated nutrient solutions. In the absence of any major shifts in suppression it is impossible to determine whether this is

linked with large-scale or more subtle microbial changes. However, if large shifts are responsible, the results of this study indicate that the mode of treatment for cleaning recirculating feed solutions would have little impact on natural disease suppression.

ACKNOWLEDGEMENTS

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Spinosad: A natural insecticide with novel mode of action for control of pests in UK field vegetable crops

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ABSTRACT

Field trials results from 2001 showed that foliar applied spinosad can provide thrips control in leeks and salad onions and control of caterpillar pests in head and flowering cabbage and brussels sprouts. Spinosad exhibits a favourable environmental and mammalian toxicity profile and excellent safety to beneficial insects. It has a novel mode of action and is effective on target pests by contact and ingestion. It is selectively active on insects in the Orders: Lepidoptera, Diptera, Thysanoptera, and some Coleoptera and Hymenoptera. Our data highlight examples of how spinosad could be used to help UK vegetable growers achieve high levels of pest control resulting in high quality produce. In the future, UK vegetable growers will have fewer insecticides available to them as many currently approved products will not be defended during re-registration at EU or country level due to mounting costs of meeting regulatory hurdles.

INTRODUCTION

Spinosad is the first active ingredient proposed for a new class of insect control products, the Naturalytes®. Spinosad is derived from the metabolites of the naturally occurring bacterium, *Saccharopolyspora spinosa*. (Thompson G D *et al,* 1997). The bacterium was discovered from a soil sample taken from a rum still in the Caribbean in 1982. The organism was identified as a new species of actinomycete bacteria in 1986 and within one year scientists had identified the most highly active metabolites of *S. spinosa*. The metabolites (spinosyns) are produced in a state-of-the-art fermentation facility. The name spinosad is derived by combining the species name, "spinosa," with the two most active metabolites, spinosyns A and D. A highly effective formulation was identified and developed through five years of extensive testing around the world and contains a mixture of spinosyn A (85%) and spinosyn D (15%).

In 1997, the first spinosad products were approved and launched in the USA, under EPA reduced risk classification, for use on cotton, turf and ornamentals. Today it is registered in over 150 crops and in 76 countries. In the UK, it is currently registered for control of thrips in ornamental nursery plants and it is hoped that it will be available for use by UK vegetable and fruit growers by 2004.

Spinosad provides UK vegetable growers with a unique package of very desirable features:

- Highly effective on many pest species.
- Low application rates resulting in low environmental loading.
- Safe for use with most beneficial insects.
- Unique mode of action with no known cross-resistance to existing products.
- Low mammalian toxicity
- Low avian and fish toxicity.

Spectrum of Activity

Spinosad is highly active on many insects including species from the orders Lepidoptera, Diptera, Hymenoptera, Thysanoptera, and a few Coleoptera and may be used to control pests in both agricultural and horticultural environments, including glasshouses, amenity turf, gardens, and around homes. Spinosad has been developed to provide rapid control of Lepidoptera and other pests with minimum disruption of beneficial insects and other non-target organisms. It has a novel mode of action and is effective on target pests by contact and ingestion. Spinosad has contact activity on all life stages of a pest including egg, larva and adult; eggs must be sprayed directly but larvae and adults can be effectively dosed through contact with treated surfaces (Thompson *et al* 2000). However, it is most effective when ingested. Spinosad is generally not active on sucking insects such as aphids. Spinosad demonstrates large margins of safety to predaceous insects such as lady beetles (Coccinellidae), lace wings (Neuroptera), bigeyed bugs (Geocoris spp.), and minute pirate bugs (Orius spp.). Hymenopteran parasitoids and pollinators are sensitive if treated directly but most are able to tolerate dried residues on foliage. Extensive trials and commercial use confirm that spinosad has no issues of phytotoxicity (Anon 2001).

Symptomatology

Sensitive insects exposed to spinosad exhibit unique symptomatology typified by a general paralysis accompanied by loss of body fluids resulting in flaccid paralysis. Due to the combination of contact and ingestion activity, the onset of insect control occurs quickly and is irreversible. Symptoms appear almost immediately and mortality occurs within hours. Spinosad's speed of kill is comparable to most synthetic insecticides. However, it acts significantly faster than slow-acting products like *Bt*, *Beauveria* fungi, and other traditional biologicals (Table 1). This is in largely due to contact mode of entry, rather than a strict reliance on ingestion as with most traditional biologicals.

Table 1. Relative speed of activity of various classes of insect control products

Category	Speed of Activity
Pyrethroids	Minutes to Hours
Spinosad	Hours
Carbamates/Organophosphates	Hours
Biologicals (Bacterials/Fungals,etc)	Days
Insect Growth Regulators	Days to Weeks

Intoxicated insects remain on the plant for one to two days without feeding, whereas insects treated by excitatory compounds, such as pyrethroids or organophosphates, tend to fall off the plants more rapidly.

Mode of Action

In insects, the mode of action of spinosad is associated with excitation of the insect nervous system (Salgado 1998). Spinosad uniquely alters the function of nicotinic receptors and GABA-gated ion channels (Salgado 1998, Dow Agrosciences unpublished data), in a manner consistent with the observed neuronal excitation. However, spinosad does not interact with known binding sites for other nicotinic or GABAergic insecticides such as neonicotinoids, fiproles, avermectins and cyclodienes (Orr, Dow Agrosciences unpublished data). These data indicate that spinosad acts through a unique insecticidal mechanism.

Resistance

Spinosad has a novel mode of action that makes it ideal for resistance management programs. It has shown no cross-resistance with existing chemistries and can be rotated with all other classes of existing and experimental products (Sparks *et al,*1995). Spinosad has excellent activity on many insects with historic resistance problems. Therefore, Dow AgroSciences (DAS) is taking a proactive stance in regard to resistance management. Specific resistance management strategies have been identified for key pests; these strategies may vary depending on crop, pest, and geography.

MATERIALS AND METHODS

This paper reviews field trials results in which spinosad has been tested for foliar control of caterpillars in brassicas and thrips in onions and leeks. Data were obtained from replicated block field trials carried out in the UK and in Belgium on commonly grown varieties. In the foliar caterpillar trials the target was *Pieris rapae* in head cabbage, flowering cabbage and Brussels sprout. Spinosad is most effective when ingested and was applied to larvae at egg hatch as the best activity is obtained when treatments target small larvae. Spinosad @ 200 ml/ ha (96 g a.i./ha) was applied in 400 litres/ha water using a small plot sprayer and medium nozzles. The reference treatments included deltamethrin and lambda cyhalothrin applied at label rates. In field trials which involved foliar applications to control *Thrips tabaci* in alliums (leeks, onions and salad onions), spinosad @ 200 ml/ha (96 g a.i./ha) was applied in 400 litres/ha water using a small plot sprayer and medium nozzles. The reference treatments were deltamethrin, which is recommended off-label in the UK, and lambda cyhalothrin, which is recommended in Belgium. Applications were made when plants were young and first signs of thrips were evident.

RESULTS AND DISCUSSION

Brassicas

There are 36,000 ha of brassicas grown in the UK (MAFF Basic Horticultural Statistics Census, 1999). They are attacked by several species of caterpillar and if left uncontrolled, these pests will produce substantial crop losses either by causing direct damage, by feeding or presence of frass on the marketable parts of the crop. In the UK, chemical control of foliar caterpillars in these crops is dominated by a series of applications of synthetic pyrethroids. Trials data (Table 2) clearly showed that spinosad has both knockdown activity and

persistence equivalent to newer generation pyrethroids. In other parts of the world, spinosad has shown good activity on other brassica pests, *Pieris brassicae*, *Mamestra brassicae* and *Plutella xylostella* at label rates of 96 g a.i./ha (200 ml/ha) as tested in the current trials for control of *Pieris rapae*. Other work has shown that activity in some crops may be enhanced through the addition of a penetrating surfactant and field trials are ongoing in the UK to further test this effect. As well as providing good caterpillar control, at harvest, it was found spinosad treatments had a positive impact on marketability (Table 3) and quality was at least comparable to that achieved by synthetic pyrethroids. Spinosad achieves high levels of pest control which offers UK vegetable growers opportunities to generate high quality produce. It also allows them another class of product apart from pyrethroids and this must aid, in the long term, the management of resistance. In addition, spinosad will also enable growers opportunities to develop IPM strategies.

Table 2. Foliar control of *Pieris rapae* in various brassica crops

% control of caterpillars (25plants/plot)					
Treatment	Brussels sprout (n=3)		Head Cabbage (n=3)		Cauliflower (n=2)
	3DAT	9DAT	3DAT	9DAT	7DAT
Untreated[1]	7.5	6.9	7.0	6.2	1.75
Spinosad 200 ml/ha	83.3	94.0	95.3	92.3	87.5
Deltamethrin 250 ml/ha	88.0	94.0	92.7	90.0	100
Lambda-cyhalothrin 50 ml/ha	92.7	92.3	91.3	90.3	87.5

[1] Number of caterpillars on 25 plants

Table 3. Components of yield (quality): marketable yield in brassica crops

Percentage of yield in each class			
Treatment	Brussels sprout (n=2)		
	Marketable	Class 2	Unmarketable
Untreated	89.1	6.5	4.3
Spinosad 200 ml/ha	92.3	6.0	1.7
Deltamethrin 250 ml/ha	93.3	5.0	1.5
Lambda cyhalothrin 50 ml/ha	93.6	4.3	2.0

Percentage of yield in each class			
Treatment	Head Cabbage (n=3)		
	Marketable	Class 2	Unmarketable
Untreated	64.2	16.8	18.6
Spinosad 200 ml/ha	84.2	11.7	2.9
Deltamethrin 250 ml/ha	85.1	8.9	6.1
Lambda cyhalothrin 50 ml/ha	77.4	15.2	6.5

Percentage of yield in each class			
Treatment	Cauliflower (n=2)		
	Marketable	Class 2	Unmarketable
Untreated	29.7	21.2	79.0
Spinosad 200 ml/ha	84.5	9.1	13.0
Deltamethrin 250 ml/ha	76.9	15.4	15.0
Lambda cyhalothrin 50 ml/ha	65.2	21.9	24.0

Alliums

The effective control of onion thrips (*Thrips tabaci*) is a high priority for producers of leeks, salad onions and also bulb onions. The characteristic silvering or flecking on the leaves reduces crop quality and if severe can render crops unmarketable. Thrips larvae and adults are usually found inside the inner leaf sheaf. Within this sheltered environment they feed on the young plant tissue with the feeding damage causing the flecking on the leaves as they elongate and develop. The crops are especially vulnerable to thrips attack in hot summers, often during August and early September, and usually six foliar applications of insecticides are made in a season. There are over 2800 hectares of leeks, 2298 hectares of salad onions and 9522 hectares of bulb onions grown in England (MAFF Horticultural Statistics 1999). Leeks and salad onions are particularly at risk from thrips damage as the only insecticide currently available for their control is deltamethrin (SOLA 1273/99). Malathion, dimethoate and chlorpyrifos have recently lost approvals for use on these crops. Experimental seed treatments have shown evidence of some early season control but are unlikely to provide sufficient protection to the crop on their own and field foliar insecticide applications will still be required. Spinosad may offer growers another addition to their armoury of thrips control agents, especially as deltamethrin is currently only offering low levels of control.

From the limited number of trials it is apparent that spinosad has activity against thrips in these crops (Table 4). Spinosad is already approved in other countries for control of *Thrips tabaci* and *Franklinella occidentalis* in other crops and so inherent activity against these pests is well proven. Insect control in leeks is always a challenge due to the leaf structure of the crop, which enables thrips larvae and adults to take refuge inside the inner leaf sheaf, well protected from insecticide sprays. Spinosad has no systemic activity and successful control relies on applying an early first application before flecking damage is seen. In these trials (Table 4) spinosad offered control of thrips that was clearly superior to that achieved by deltamethrin. Spinosad also markedly increased crop marketability of leeks and salad onions compared to deltamethrin treated crops (Table 5) producing a greater quantity of Class 1 produce. However, it is likely that the trial sprays were applied later than ideal and additional work is now being undertaken to define application timing and improve spray coverage by increasing water volumes and the addition of adjuvants. Dow Agrosciences will develop resistance management strategies for spinosad which include regular monitoring of thrip populations, limiting numbers of sprays and recommending spray rotations with other chemistries. However, with a limited choice of products available to the grower, there is great risk of abuse and overuse.

Table 4. Control of *Thrips tabaci* in leeks and salad and bulb onions

% control of Thrips (20plants/plot)			
Treatment	Leeks (n=4) 9 DAT	Salad onion (n=2) 5DAT	Bulb onion (n=2) 5DAT
Untreated	25.5[2]	7.1[1]	5.9[1]
Spinosad 200 ml/ha	58	60	54
Deltamethrin 300 ml/ha	31	48	-
Lambda Cyhalothrin 50 ml/ha	0	-	52

[1] Number of thrips per plant. [2] Percentage plant damage as a result of thrips feeding

Table 5. Components of yield (quality): marketable yield in leeks and salad onion crops

Percentage of yield in each class						
Treatment	Leeks (n=2)			Salad onions (n=2)		
	Class 1	Class 2	Unmarketable	Class 1	Class 2	Unmarketable
Untreated	24.5	65	10.5	40.5	45.5	14
Spinosad 200 ml/ha	58	40	2	68	32	0
Deltamethrin 300 ml/ha	37.5	56	6.5	35	53	12

In the future, UK vegetable growers will have fewer insecticides available to them as many currently approved products will not be defended during re-registration at EU or country level due to mounting costs of meeting regulatory hurdles. However, growers will still need to produce high quality produce to meet the demands of consumers and supermarkets. The efficacy of spinosad compares favorably with the best synthetic standards such as pyrethroids. However, spinosad also offers a unique product with a novel mode of action, low impact on many beneficial insects and the environment and low mammalian, avian and fish toxicity. Dow Agrosciences is progressing studies to enable spinosad to expedite registrations for use in UK vegetable production.

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A pheromone monitoring system for pea midge *(Contarinia pisi)* in vining peas

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ABSTRACT

Lures containing synthetic forms of the three components of the female pea midge sex attractant 2-acetoxytridecane, (2*S*,11*S*)-diacetoxytridecane and (2*S*,12*S*)-diacetoxytridecane were shown to be effective in attracting newly emerged male pea midge *(Contarinia pisi)* to sticky based delta traps. Trapping in fields, previously cropped with vining peas was carried out in 1999-2001 in the UK. Recordings of catches showed peak times of emergence at a range of sites. A monitoring system was shown to be effective in providing early warning for protection of nearby susceptible vining pea crops. A commercial version of the system was made available for the 2002 season.

INTRODUCTION

Pea midge is a localised pest in northern Europe, particularly in UK and France. Larvae from eggs laid in the shoots of peas, feed within the developing bud and this results in sterile flowers and significant yield losses of up to 75%. Large populations of midge can develop in areas of intensive pea production. During June and July adults emerge from the soil of the previous year's infested pea crop and after mating, females fly to nearby pea crops where oviposition occurs. Vining peas are susceptible to high levels of damage due to the characteristic of determinacy, which exposes a greater proportion of flower buds to damage.

To control the pest, oviposition is prevented, by applying insecticide to susceptible crops as soon as adult midge can be found within the leaves of the growing point. This entails regular crop inspection, and often treatment is applied too late to prevent egg laying. Some crops may be treated prophylactically. Attempts at predicting emergence have been made by examining pupal development following extraction from soil. However such a prediction is affected by weather conditions, soil type and local climatic and soil conditions and is largely inaccurate.

A monitoring system based on the sex attractant pheromone was considered to be an ideal tool by Wall, *et al.*, (1985) who showed that female midge attracted males by means of a sex pheromone. This showed the potential for monitoring emergence (Wall, *et al.*, 1994). The identification of the pheromone as 2-acetoxytridecane, (2*S*, 11*S*)-diacetoxytridecane and (2*S*,12*S*)-diacetoxytridecane (Hillbur, *et al.*, 1999, 2000, 2001) allowed the production of synthetic components and their use in a trapping system to provide a prediction of midge infestation.

MATERIALS AND METHODS

Monitoring sites were identified each year by soil sampling and extraction of overwintering cocoons by wet sieving and flotation in magnesium sulphate. In 1999, two fields with a high and low population i.e. more than 2 x 10^6 cocoons and less than 0.5 x 10^6 cocoons per ha were selected. In 2000 and 2001, three fields were selected with high overwintering populations.

The pheromone components were synthesised at the Institute of Organic Chemistry, Hamburg University and used singly or in combination in the field tests. The components were dispensed at various dose rates onto 1 cm length dental cotton rolls (Celluron No. 2) at the Swedish University of Agricultural Science. The lures were suspended by a plastic covered wire inside the body of an Oecos pea moth delta trap, with a sticky insert placed inside the trap base. In addition, racemic isomers of the three components were also prepared and used in the trials in 1999.

During June of each year, traps containing one of each lure were placed at 10 m distances along tramlines of each field, all of which were currently in winter wheat. The dosed traps were replicated 4 times in randomised block experiments and the sticky bases inspected regularly and changed each time. Numbers of insects and their identity were regularly recorded. Capture data were analysed by analysis of variance for each capture date.

RESULTS

In 1999 pea midge were first caught between 11th and 14th June. Highest numbers were recorded on 17th June. There were highly significant differences in catches between the three-component lure compared with the single (2-acetoxytridecane) or two-component (2*S*, 11*S*)-diacetoxytridecane and (2*S*, 12*S*)-diacetoxytridecane lures at the low population site (Figure 1), although there were no significant differences between the catches recorded on the three-component and the two-component lures at the high population site (Figure 2). The racemic compounds (Triple R) were not as effective. All midge were male pea midge.

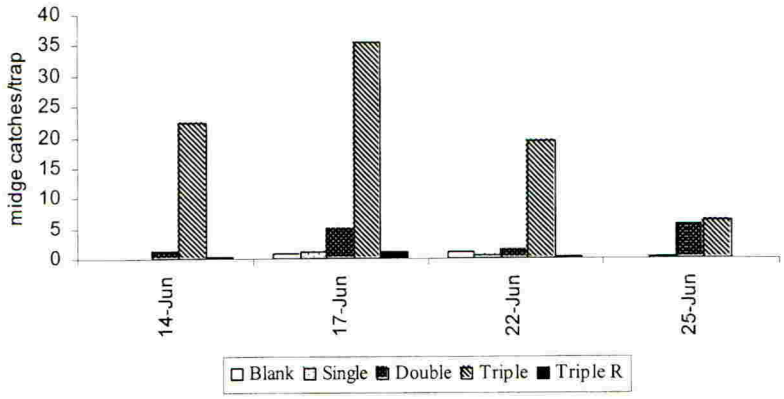

Figure 1. Midge trap catches Walkington 1999

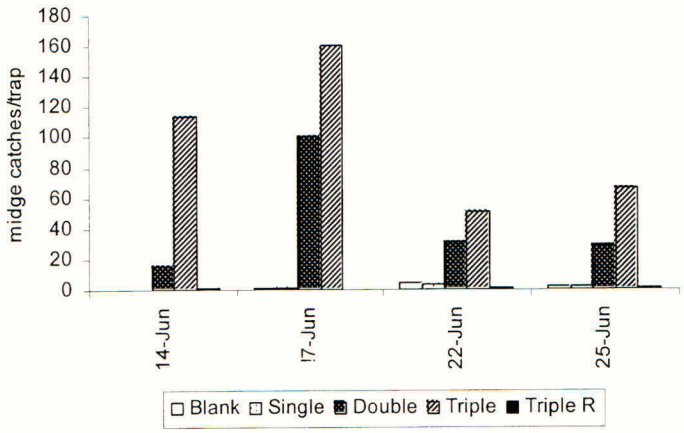

Figure 2. Midge trap catches Market Weighton 1999

In 2000 the three component lures were used; midge were first recorded on 11th June. At all sites the 1µg, 10µg and 100µg dose rates were all effective in attracting male midge, but overall doses of 1µg and 10µg trapped consistently higher numbers of midges at all three sites. The data are shown in Figures 3, 4 and 5.

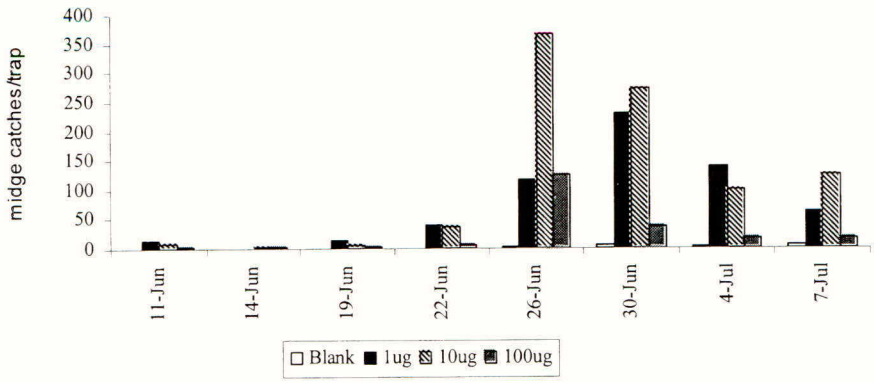

Figure 3. Mean trap catches Arras Hill 2000

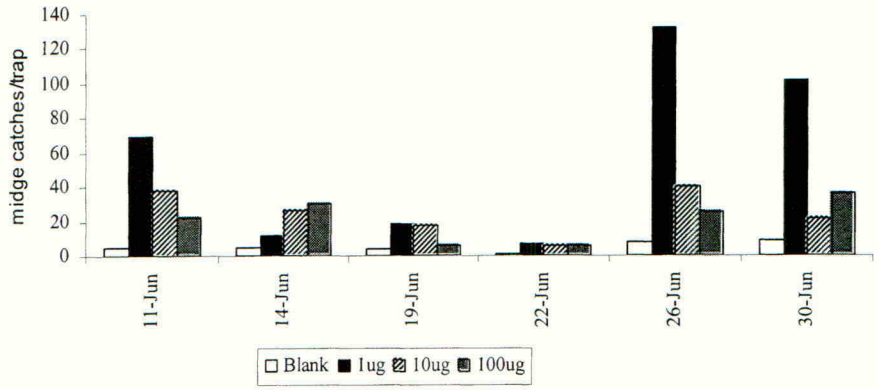

Figure 4. Mean trap catches Kilham 2000

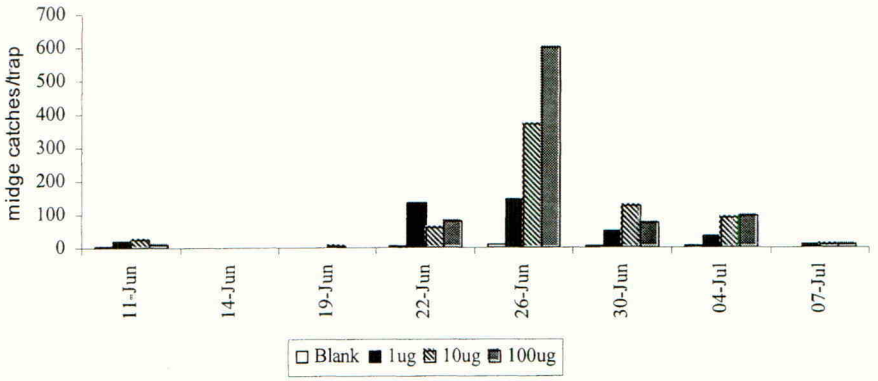

Figure 5. Mean trap catches Warter Estates 2000

In 2001 catches were first recorded on 6th June at all three sites, although there were large differences in overall numbers between sites. The recording period was extended to examine the persistency and release rate of the lures. At all sites, 10µg of the three-component lure gave consistently high catches on average over the season (Figures 6, 7 and 8). Trapping indicated at least two emergence periods at one of the sites (Tibthorpe) although the times of the peak emergence varied by several days between the three sites.

In each of the three years, local pea crops were examined for the presence of pea midge on each day that trap catches were recorded. In no case were midge found in the peas prior to the first recorded trapped midge. However, it was not possible to determine a delay period between peak emergence and crop infestation in this series of experiments.

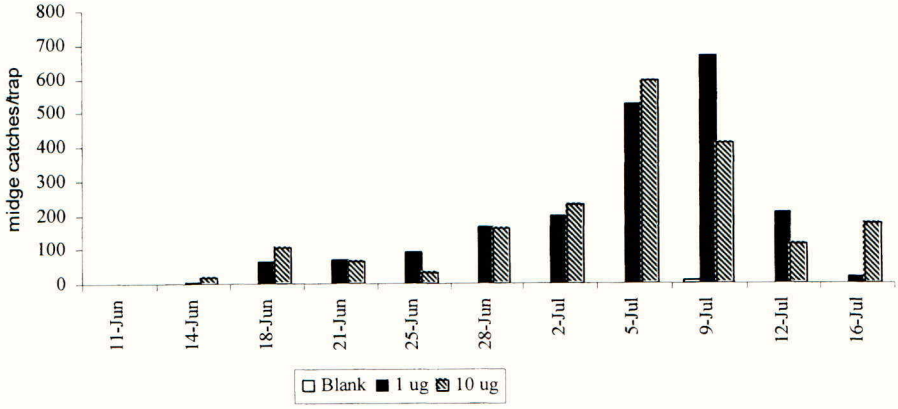

Figure 6. Mean trap catches Walkington 2001

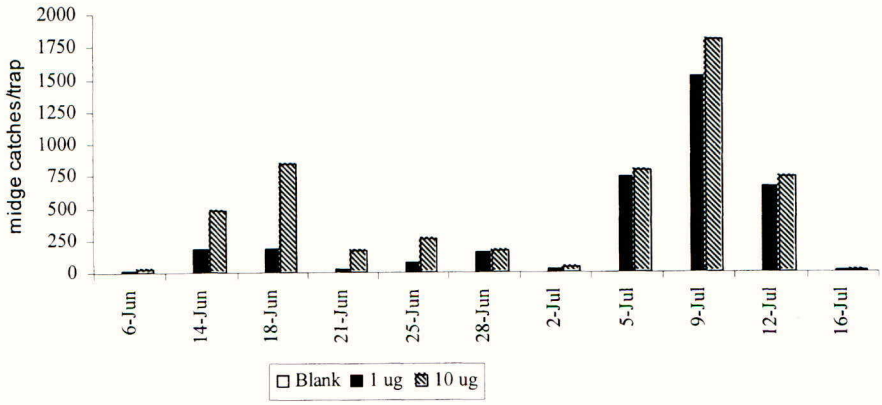

Figure 7. Mean trap catches Market Weighton 2001

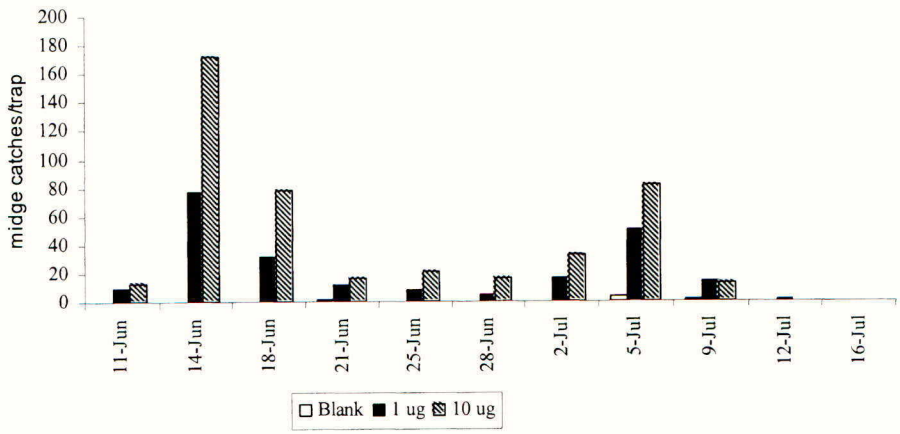

Figure 8. Mean trap catches Tibthorpe 2001

DISCUSSION

The results showed a clear response to the three-component pheromone compound in attracting male pea midge to sticky traps. The 10µg dose rate was consistently successful in catching midge at all the trapping sites and the use of delta sticky traps was a satisfactory method of using the lures and recording insects. In this work, the delay period between recording midge catches in the emergence site and crop infestation was not determined, but the advance notice of emergence allows sufficient time to organise pea crop inspection and subsequent spray action if necessary.

In 2001, an indication of midge emergence and numbers over time was useful in assessing peak emergence and population size. At high overwintering population sites, the peak numbers appeared to exceed 500 per trap on any one occasion and this figure can provide a guide in the use of traps as a monitoring and warning system in the field.

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Efficacy of spinosad in controlling some pests from the family Tortricidae

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ABSTRACT

The modern biological insecticide spinosad was tested against codling moth, plum fruit moth and leaf rollers in a field study lasting two to three years conducted in various parts of Poland. In the conditions of the experiments this insecticide was found to be highly effective in reducing fruit damage due to attacks by the above pests. The efficacy of this compound was influenced by the rate per hectare, and the timing and number of treatments. These factors were different in various years and orchards. Due to its selectivity, spinosad seems to be a very useful tool for integrated fruit production.

INTRODUCTION

Among various pests occurring in top fruit orchards in Poland, codling moth (*Laspeyresia pomonella*) and the complex of leaf rollers (Tortricidae) are most important on apple trees, whereas plum fruit moth (*L. funebrana*) is important on plum trees. For many years organophosphate insecticides were used against these pests, with a shift during the last decades towards insect growth regulators (IGRs) such as the chitin inhibitors and juvenoids (e.g. fenoxycarb). Due to an increasing acceptance of the concept of integrated fruit production (IFP) by growers and consumers all over the world, new groups of selective insecticides are still needed. An example of such a compound is spinosad which comprises two active components, spinosyn A and D produced by the soil actinomycete *Saccharopolyspora spinosa* (Thompson *et al.*, 1997).

METHODS AND MATERIALS

Investigations into the usefulness of Spinosad 480 SC in controlling codling moth, plum fruit moth and the complex of leaf rollers (mainly *Pandemis heparana*, *Archips rosanus* and in some regions *Spilonota ocellana* or *Adoxophyes orana*) were conducted in some commercial orchards in various regions of Poland. Five to six experiments with each pest were done. Depending on the orchard and region, each experimental plot area comprised 12 to 200 trees. In the case of codling moth and plum fruit moth, pheromone traps were used for determining the beginning and dynamics of the flight of the moths. Timing of the treatments was based on trap counts according to the procedure of Koslinska *et al.* (1987) and Kozlowski (1994) as well as on the development stage of eggs. Depending on the area, 1-2 traps were used. They were inspected every 2 or 3 days and specimens counted and removed on each occasion. In the case of tortricids, the timing of treatment was based on visual evaluation of larval development and species composition. Fenitrothion (Owadofos 540 EC) was used as the industry standard, and there was an untreated control.

Treatment effectiveness was assessed during harvest by inspecting 800 or 1000 fruits collected from trees growing within the central part of each plot, and recording the level of damage. In 2001, treatment effectiveness against leaf rollers in experiments 4 and 5 was assessed by counting the number of living larvae found in 200 leaf clusters.

The effectiveness of the treatments was calculated in two ways - a) by Abbott's formula (Abbott, 1925), b) statistically (ANOVA) on data transformed according to Freeman-Tukey's formula. Mean differences were evaluated with Duncan's range 't' test at 5% probability level.

RESULTS

Codling moth control

Table 1. Efficacy of spinosad in controlling codling moth (*Laspeyresia pomonella*)

Treatment	Rate in l per ha	Number of treatments	Time of treatment[2]	Picked crop		Fallen crop	
				% wormy fruit	% effecti-veness[1]	% wormy fruit	% effecti-veness[1]
Experiment 1 (1999)							
untreated	-	-	-	6.8 b[4]	-	66.0 b	-
spinosad	0.42	2	A, B	1.5 a	77.8	18.0 a	72.7
fenitrothion	2.25	2	A, B	1.1 a	83.3	15.0 a	77.3
Experiment 2 (2000)							
untreated	-	-	-	4.0 b	-	17.0 b	-
spinosad	0.42	1	A	0.6 a	84.4	4.0 a	76.5
fenitrothion	2.25	1	A	0.8 a	81.3	4.5 a	73.5
Experiment 3 (2000)							
untreated	-	-	-	5.0 b	-	16.0 b	-
spinosad	0.42	1	A	1.0 a	80.0	2.0 a	87.5
fenitrothion	2.25	1	A	0.9 a	82.5	4.5 a	71.9
Experiment 4 (2001)							
untreated	-	-	-	3.1 b	-	28.5 c	-
spinosad	0.3	4	A[3], AII[3]	0.3 a	92.0	3.5 b	87.7
spinosad	0.42	4	A[3], AII[3]	0.0 a	100.0	1.5 a	94.7
spinosad	0.42	2	A, AII	0.1 a	96.0	3.0 b	89.5
fenitrothion	2.25	2	A, AII	0.4 a	88.0	3.0 b	89.5
Experiment 5 (2001)							
untreated	-	-	-	7.9 b	-	12.5 c	-
spinosad	0.3	2	A[3]	0.9 a	88.9	4.0 b	68.0
spinosad	0.42	2	A[3]	0.5 a	93.7	0.0 a	100.0
fenitrothion	2.25	1	A	0.4 a	95.2	0.0 a	100.0

[1] Calculated according to Abbott's formula
[2] Beginning of hatching of the 1st (A) and 2nd (B) generation caterpillars, AII - second spray against the first generation
[3] Application was done twice in the 7-10 days interval
[4] Means followed by the same letter are not significantly different (Duncan's MRT, P=0.05)

Depending on the time of application, number of treatments, dose rate and initial level of infestation the efficacy of spinosad varied from 77.8% to 100% whereas that of the standard (fenitrothion) ranged from 81.3% to 95.2% (Table 1). The best result (100% efficacy) was obtained with spinosad applied four times at a rate of 0.42 l/ha (Table 1). The efficacy decreased by only 4% when the same dose rate was applied twice.

Plum fruit moth control

Against this pest spinosad was tested in five experiments in which the infestation level on untreated plots varied from 1.8% to 19.8% of wormy fruit. The effectiveness of the compound was influenced by factors mentioned above, and varied from 71.4% to 100%. At the same time the effectiveness of the standard preparation varied from 80% to 100% (Table 2).

Table 2. Efficacy of spinosad in controlling plum fruit moth (*Laspeyresia funebrana*)

Treatment	Rate in l per ha	Number of treatments	Time of treatment[2]	Picked crop		Fallen crop	
				% wormy fruit	% effecti-veness[1]	% wormy fruit	% effecti-veness[1]
Experiment 1 (1999)							
untreated	-	-	-	19.8 b[4]	-	48.5 b	-
spinosad	0.42	2	A, B	1.5 a	92.4	1.0 a	97.9
fenitrothion	2.25	2	A, B	2.0 a	89.9	2.5 a	94.8
Experiment 2 (2000)							
untreated	-	-	-	10.5 b	-	27.0 b	-
spinosad	0.42	2	A, B	0.5 a	95.2	3.5 a	87.0
fenitrothion	2.25	2	A, B	0.0 a	100.0	2.0 a	92.6
Experiment 3 (2000)							
untreated	-	-	-	2.5 b	-	11.5 c	-
spinosad	0.42	2	A[3]	0.5 a	80.0	0.5 a	95.7
fenitrothion	2.25	2	A[3]	0.5 a	80.0	2.0 b	82.6
Experiment 4 (2001)							
untreated	-	-	-	4.3 b	-	9.0 c	-
spinosad	0.2	4	A[3], B[3]	0.0 a	100.0	3.0 b	66.7
spinosad	0.3	4	A[3], B[3]	0.0 a	100.0	2.0 ab	77.8
spinosad	0.42	4	A[3], B[3]	0.0 a	100.0	0.5 a	94.4
fenitrothion	2.25	2	A, B	0.3 a	94.1	1.0 a	88.9
Experiment 5 (2001)							
untreated	-	-	-	1.8 b	-	5.5 b	-
spinosad	0.2	2	A[3]	0.5 a	71.4	0.5 a	90.9
spinosad	0.3	2	A[3]	0.3 a	85.7	0.0 a	100.0
spinosad	0.42	2	A[3]	0.0 a	100.0	0.0 a	100.0
fenitrothion	2.25	1	A	0.0 a	100.0	0.5 a	90.9

[1] Calculated according to Abbott's formula
[2] Beginning of hatching of the 1st (A) and 2nd (B) generation caterpillars
[3] Application was done twice in the 10-14 days interval
[4] For explanations see Table 1

The worst result with spinosad was obtained when it was applied twice at a rate of 0.2 l/ha (Table 2 - experiment 5), but the same rate of preparation applied four times, was very effective (Table 2 - experiment 4).

Leaf roller control

Table 3. Efficacy of spinosad in controlling leaf rollers (Tortricidae) in apple orchards

Treatment	Rate in l per ha	Number of treatments	Time of treatment[2]	% damaged fruit	% effecti-veness[1]	Number of caterpillars per 200 leaf clusters[5]
Experiment 1 (2000)						
untreated	-	-	-	6.3 c[4]	-	
spinosad	0.42	2	A, B	0.7 a	88.9	
fenitrothion	2.25	2	A, B	1.2 b	81.0	
Experiment 2 (2000)						
untreated	-	-	-	3.4 b	-	
spinosad	0.42	2	A, B	0.4 a	88.2	
fenitrothion	2.25	2	A, B	0.8 a	76.5	
Experiment 3 (2000)						
untreated	-	-	-	12.4 c	-	
spinosad	0.42	1	B	2.4 a	80.6	
fenitrothion	2.25	1	B	4.7 b	62.1	
Experiment 4 (2001)						
untreated	-	-	-	6.2 c	-	13 b
spinosad	0.2	1	A	2.5 b	59.7	3 a
spinosad	0.3	1	A	2.1 b	66.1	1 a
spinosad	0.42	1	A	1.4 a	77.4	0 a
fenitrothion	2.25	1	A	1.1 a	82.3	1 a
Experiment 5 (2001)						
untreated	-	-	-	6.7 b	-	17 c
spinosad	0.2	4	A[3], B[3]	1.8 a	73.1	8 b
spinosad	0.3	4	A[3], B[3]	1.5 a	77.6	4 ab
spinosad	0.42	4	A[3], B[3]	0.9 a	86.6	2 a
fenitrothion	2.25	2	A, B	1.1 a	83.6	5 b
Experiment 6 (2001)						
untreated	-	-	-	5.8 c	-	
spinosad	0.2	3	A, B[3]	2.3 b	60.3	
spinosad	0.3	3	A, B[3]	1.5 b	74.1	
spinosad	0.42	3	A, B[3]	0.6 a	89.7	
fenitrothion	2.25	2	A, B	2.1 b	63.8	

[1] Calculated according to Abbott's formula

[2] A - before flowering (beginning of pink bud); B - beginning of larvae hatching from eggs laid by the first generation of leaf rollers

[3] Application was done twice in the 7-18 days interval

[4] For explanations see Table 1

[5] Counted 2 weeks after the spray at timing A

In the case of these pests 6 experiments were done. As in the experiments with codling moth and plum fruit moth, the efficacy of spinosad depended on the time of application, number of treatments and level of infestation and varied from 60.3% to 89.7% (Table 3).

In most cases the efficacy of spinosad applied at the rate of 0.42 l/ha, measured by the number of damaged fruits, was similar or even better than that of fenitrothion. In experiments 4 and 5 (Table 3) the effect of spinosad was observed not only on fruits but also on leaf clusters. It was found that the number of living caterpillars of leaf rollers on leaves was significantly reduced on all plots treated with spinosad, but the best results were obtained with a rate of 0.42 l/ha.

DISCUSSION

A number of field experiments performed in commercial orchards indicated that Spinosad 480 SC is a useful tool for the control of such important pests of fruit trees as codling moth, plum fruit moth and leaf rollers. In Polish apple orchards this last group of pests very often plays a more important role than codling moth.

Analysing all the data we can state that for codling moth control this natural compound could be recommended at rates ranging from 0.3 l/ha to 0.42 l/ha. However when the lower rate is used, the application should be repeated whereas when the higher rate is used, a single treatment at the beginning of hatching of the 1st generation of caterpillars guarantees results comparable or even better than those obtained with fenitrothion.

Our three years work showed that spinosad has also a high intrinsic activity against plum fruit moth. In the case of this pest a single application with a dose of 0.42 l/ha against first and second generation caterpillars should be made. This compound applied at 0.3 l/ha also significantly reduced the number of wormy fruits, but where there are high populations of the pest, two treatments against each generation of plum moth should be made.

Spinosad also provided very good control of the tortricid complex, within which usually *Pandemis heparana* and *Archips rosanus* and in some orchards *Adoxophyes orana* and *Spilonota ocellana* were the most numerous species. Taking into consideration all the collected data one can say that spinosad applied at 0.42 l/ha provided a control of leaf rollers comparable or even better than that obtained with fenitrothion. Especially good results were obtained in the experiments where two applications were made, one at the beginning of the pink bud (timing A), and the second at the time when larvae begin to hatch from eggs laid by the first generation of leaf roller moths (usually in the second half of July - timing B). Good results can also be obtained with a dose of 0.3 l/ha but at both timings the application should be performed twice at an interval of around 7 days.

Considering: a) the good results obtained with spinosad by us and by other authors (e.g. Bylemans and Schoonejans 2000); b) its novel mode of action (Salgado *et al.* 1997); and c) its selectivity towards beneficial insects (Hassan 1994, Bylemans and Schoonejans 2000) we expect that in the forthcoming years the application of this insecticide will be recommended for the control of the pests mentioned in this paper and probably for some other lepidopteran as well as for some hemipteran pests, e.g. *Psylla pyri*. These results indicate that this compound will be especially useful in integrated fruit pest management.

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An evaluation of the efficacy of aldicarb and alternative nematicides against plant-parasitic nematodes in carrots

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ABSTRACT

Plant-parasitic nematodes have been implicated in the reduction of yield and quality in carrots. To date there has been widespread prophylactic use of aldicarb to control nematodes. This approach is not sustainable and a programme of research is underway to develop an IPM strategy for nematode control in carrots and parsnips. Work reported here is part of that programme and involves an investigation of the efficacy of aldicarb and also an evaluation of naturally-occurring alternatives to this nematicide. Reduction in nematode numbers due to aldicarb ranged from 39% to 73%. Despite apparently very poor nematode control at one site, treated carrots produced 16% more yield than untreated. This suggested that although not killed, nematodes were sufficiently affected by aldicarb to prevent root damage. In a field experiment neither chitin or garlic had any effect on numbers of plant-parasitic nematodes

INTRODUCTION

It is estimated that 50 % of the carrot and parsnip crop in the UK is treated with aldicarb (Garthwaite *et al.*, 1999). Much of this use is prophylactic rather than being based on accurate risk assessments. This approach is contrary to the requirements of approved produce schemes. Excessive use of aldicarb may lead to residue problems which are environmentally unacceptable, or may result in enhanced microbial degradation and reduced nematicide efficacy.

There is considerable industry concern about the potential loss of aldicarb as a result of the current Pesticides Safety Directorate anticholinesterase review, the EU 2003 Review and the current EU harmonisation of Maximum Residue Limits (MRL's). Although the issue of the MRL for aldicarb has been resolved for the time being, it is clear that the use of this product on carrots and parsnips must be rationalised. To do this, growers need much more information on plant-parasitic nematodes and their potential to damage carrot or parsnip crops. Our current research will go some way towards providing this information and in particular will attempt to develop a basis for an IPM strategy. This paper presents work investigating the efficacy of aldicarb for nematode control to help determine the cost effectiveness of nematicide use. Work

to evaluate the effects of chitin and garlic, naturally occurring alternatives to aldicarb, is also reported.

MATERIALS AND METHODS

An investigation of the efficacy of aldicarb for nematode control

Three experimental sites were selected at Edwinstowe, Nottinghamshire, Palgrave, Norfolk and Sutton on Derwent, North Yorkshire. At each site extraction of pre-cropping soil samples showed that 3125, 925 and 1040 stubby root nematodes (*Trichodorus* spp.) /l soil were present at Edwinstowe, Palgrave and Sutton on Derwent respectively. At all sites nematode numbers exceeded a guideline threshold of 200-250 stubby root nematodes/l soil at which nematicide treatment is advised. Therefore, root damage would be expected in the absence of treatment.

At each site a field experiment was established comprising two treatments, an untreated control and aldicarb (as Temik 10G, RP, Agric) at 38 g product/100 m row. Each treatment was replicated six times to give 12 plots in total per site. Plots were arranged as six randomised blocks and each treatment appeared once in each block. Plots (20 m long x three beds wide, 1 bed = 1.8 m) were marked out pre-drilling of the carrot crop (cv. Nairobi) and the aldicarb was applied at drilling in May 2001 by the host farmer using a Horstine Farmery granule applicator. Plots were sown on 4 May 2001 at Edwinstowe, 9 May 2001 at Sutton on Derwent and 21 May 2001 at Palgrave. All other agrochemical applications were as per normal farm practice.

Soil samples for nematodes were taken from each plot at the seedling stage (two cotyledons + true leaf), seedling + four weeks, seedling + three months and at harvest. These comprised 30 cores taken within the carrot row with a 15 cm deep x 2 cm diameter cheese corer. A total of 50 randomly selected plants were taken at harvest to assess yield.

Soil samples were extracted using the Whitehead tray (Whitehead & Hemming, 1965) plant-parasitic nematodes identified to genus and counted. All nematode counts were expressed as numbers/200 g soil. All data were subjected to an analysis of variance.

An investigation of alternatives to aldicarb for nematode control

A field experiment was undertaken at Goodiston, Norfolk. Soil samples showed that this site had 925 *Trichodorus* spp./l soil and this level of pest infestation would be expected to reduce crop yield. The efficacy of two novel compounds, garlic granules (at 6g/m^2) and chitin as crushed crab waste (at 540g/m^2), were compared with the standard nematicide aldicarb (at 3.8g/100m row) and an untreated control. There were four replicate plots (3 beds x 3 m (1 bed = 1.8 m)) of each treatment arranged in four randomised blocks. Each treatment appeared once in each block and there were 16 plots in total. The chitin and the garlic granules were broadcast as evenly as possible over the soil surface and incorporated by raking. The aldicarb was applied at drilling using a Horstine Farmery granule applicator. Carrot seed (cv. Nairobi) was drilled on 21 May 2001 using a standard three bed drill. All agrochemical applications to the experimental area were as per normal farm practice.

Nematodes were sampled, extracted, identified and counted as previously described. This was done pre-drilling (21.5.01), at the seedling stage (26.6.01), and at harvest (4.10.01). A total of

25 randomly selected seedlings were also removed at the seedling + two weeks stage (10.7.01) and assessed for incidence of root fanging. At harvest 25 roots were randomly sampled per plot and assessed for fanging and weighed.

All data were subjected to an analysis of variance. Values for percentage of roots showing root fanging were transformed to arcsine values before analysis.

RESULTS

An investigation of the efficacy of aldicarb for nematode control

Analyses of variance were undertaken to assess the effect of aldicarb on numbers of *Trichodorus* spp., *Pratylenchus* spp. and *Tylenchorynchus* spp. and total number of plant-parasitic nematodes at each growth stage at each site. This provided a total of 60 analyses. In general, aldicarb had limited effect on reducing plant-parasitic nematode numbers. In only four of 60 analyses was there a significant difference ($P < 0.05$) between nematode numbers in the untreated and aldicarb treated plots (Table 1). In one of these, Palgrave at the seedling + three months stage, numbers of *Trichodorus* spp. were higher in the treated than untreated plots. No data are presented for sampling occasions when there was no significant difference in nematode numbers between treatments.

Table 1. The effect of aldicarb on nematode numbers (number/200 g soil): Assessments at which nematodes numbers differed significantly between treatments, $P < 0.05$

Nematode group	Site	Growth stage	Nematode numbers/200 g soil	
			Untreated	Aldicarb treated
Total plant parasites	Edwinstowe	Seedling	117.7	72.2
	SED (5 DF) = 14.96			
Tylenchorynchus spp.	Sutton on Derwent	Seedling + 4 weeks	37.8	10.3
	SED (5 DF) = 10.59			
Total plant parasites	Sutton on Derwent	Seedling + 4 weeks	93.0	43.8
	SED (5 DF) = 18.79			
Trichodorus spp.	Palgrave	Seedling + 3 months	1.80	7.80
	SED (5 DF) = 1.24			

There was no significant effect of aldicarb on root yield at any site (Table 2). However, at Edwinstowe the yield of roots from aldicarb treated plots was 16% higher than in untreated plots ($P = 0.082$).

Table 2 Effect of aldicarb on yield of 50 roots (g) at harvest

	Untreated	Aldicarb treated	SED (5 df)
Edwinstowe	4160	4838	312.5
Palgrave	2913	3014	278.6
Sutton on Derwent	5520	5410	564.1

There was no significant effect of treatment on nematode numbers at any assessment date (Table 3). Only at three months post-seedling stage for *Tylenchorynchus* spp., was there any trend for a difference between treatments and on this occasion least nematodes were recovered in the untreated control ($P = 0.114$).

The proportion of fanged roots did not differ significantly between treatments two weeks after the seedling stage or at harvest (Table 4).

Table 3. Mean numbers of nematodes (numbers/200 g soil) following treatment with aldicarb, chitin or garlic granules at Goodiston, May - October 2001.

Date	Nematode group	Treatment				SED
		Untreated	Aldicarb	Chitin	Garlic	(15 df)
21.5.01	*Trichodorus* spp.	0.3	0	0.3	0	0.23
	Pratylenchus spp.	29.7	28.8	27.3	24.8	8.39
	Total plant parasites	50.3	63.2	44.8	43.0	8.72
26.6.01	*Trichodorus* spp.	7.2	7.3	8.3	6.8	2.36
	Pratylenchus spp.	53.0	43.8	43.7	59.8	10.94
	Tylenchorynchus spp.	107.0	101.3	92.3	113.2	24.26
	Total plant parasites	221.0	207.0	207.0	240.0	33.60
17.10.01	*Trichodorus* spp.	4.0	5.3	3.3	6.0	2.14
	Pratylenchus spp.	21.5	26.8	27.7	22.3	6.20
	Tylenchorynchus spp.	66.8	75.3	73.8	90.3	9.67
	Total plant parasites	139.0	147.3	150.7	171.2	20.65

Table 4. Mean percentage root fanging, following treatment with aldicarb, chitin or garlic granules at Goodiston, 2001. Percentages have been transformed to arcsine values, figures in brackets are back transformed data.

Assessment and timing	Treatment				SED
	Untreated	Aldicarb	Chitin	Garlic	(15 df)
Two weeks post seedling					
Percentage root fanging	11.1 (3.7)	13.2 (5.2)	10.0 (3.0)	9.4 (2.7)	3.05
Harvest					
Percentage root fanging	19.0 (10.6)	21.6 (13.5)	22.3 (14.4)	15.9 (7.5)	3.10

DISCUSSION

In general, aldicarb had a limited effect on numbers of plant-parasitic nematodes. Reductions in nematode numbers were recorded at all sites up to four weeks after the seedling stage but control was variable and ranged from 39% to 73%. Also there were no statistically significant and consistent reductions in nematode numbers between the seedling and seedling + four week growth stages. From three months after the seedling stage, where there were differences in nematode numbers between treatments, most nematodes were found in aldicarb treated plots.

The poor performance of aldicarb is difficult to explain. Release of the active ingredient is dependent upon soil moisture and if this is low the efficacy of the nematicide would be reduced. Meteorological data shows that April 2001 was approximately 60% wetter than average. May and June were drier, with rainfall in Norfolk, Nottinghamshire and North Yorkshire being 80%, 76% and 55% of average. Therefore it seems unlikely that soil moisture levels would have significantly influenced the efficacy of aldicarb. It is also possible that the soils at the sites showed enhanced microbial degradation of aldicarb. These fields regularly grow carrots and all have been treated with aldicarb on a number of occasions in the past. Although it was not possible to assess whether there was enhanced degradation of aldicarb at any of the trial sites, it is an important consideration when formulating a nematode control strategy.

Although aldicarb had a variable and limited effect on nematode mortality, it is possible that it was reducing crop damage. Aldicarb has a nematistatic effect on nematodes, although affected individuals may eventually use up their food reserves and die (Hague, 1979). However, at concentrations below those necessary for a nematistatic effect aldicarb may interfere with sensory behaviour and this may contribute to crop protection by interfering with attraction to the host crop. At Edwinstowe aldicarb treated plots produced 16% more yield than untreated plots. Although this difference was not statistically significant ($P = 0.082$) there was a trend to suggest that aldicarb treatment had an effect on crop yield. This supports the contention that although nematodes were not killed they were sufficiently affected by the treatment to reduce root damage.

Despite relatively high numbers of stubby root nematodes, very little root fanging was recorded. The lack of fanging was most noticeable at Edwinstowe where 3125 stubby root nematodes/l soil were recovered. This result suggests that aldicarb had a limited effect on nematodes or alternatively that current thresholds are set well below the level at which root damage occurs and/or that stubby root nematodes are not primarily responsible for root fanging

In the investigation of alternatives to aldicarb for nematode control there was no effect of chitin, garlic granules or aldicarb on nematode numbers and no differences in the level of root fanging between these treatments. This result is interesting and difficult to explain as chitin has been shown to have dramatic effects on nematode numbers in pots (Ellis *et al.*, 1998).

The true seedling + two weeks stage was achieved six to eight weeks after treatment. Aldicarb should persist over this period so nematode counts would be expected to show differences between treatments. In the case of chitin, it is possible that the degree of soil incorporation affected the efficacy of the treatment. In pots it is relatively easy to ensure that chitin is well mixed throughout the volume of soil. In field plots incorporation was by raking and therefore

may have been confined to the top 10 cm of soil whereas soil cores for nematodes were taken to a greater depth (15 cm).

Numbers of stubby root nematodes were much lower than anticipated at the site, particularly at drilling. At no stage during the experiment did numbers approach the 200-250/l soil guideline threshold at which a nematicide treatment would be applied. The site had been sampled on 14 March 2001 when 925 stubby root nematodes were recovered. It is difficult to account for the sudden reduction in numbers of this species approximately one month later. It may be due to cultivations undertaken pre-drilling.

In summary, aldicarb had a limited and variable effect on nematode numbers in both experiments. Despite this, at Edwinstowe carrot yield was increased by 16%. Further work is required to determine the conditions under which this product is most cost effective.

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