SESSION 3A

DEVELOPMENTS IN HORTICULTURAL CROP PROTECTION FOR THE 1990s

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BIOLOGICAL CONTROL OF *FUSARIUM* WILT OF CARNATION

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ABSTRACT

Saprophytic *Fusarium* isolates derived from the rhizosphere of carnations grown in soils suppressive to *Fusarium oxysporum* f.sp. *dianthi*, effectively controlled *Fusarium* wilt of carnation. Dipping rooted cuttings of carnation directly before transplanting in a spore suspension (4-5 x 10^7 cfu/ml) of the antagonists gave the best control. Soil applications of the antagonists, as chlamydospores diluted in Talc (5-20 x 10^4 cfu/g of soil), or as infested wheat kernels (200 g/m^2), gave some of control but were less effective. Combination treatments of antagonists with benomyl or prochloraz further increased control but treatments that included prochloraz were phytotoxic.

INTRODUCTION

Wilt, caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *dianthi*, is a major problem in the cultivation of carnation Garibaldi & Gullino (1987). Disease control measures include partial soil disinfestation, use of partially resistant cultivars, raised bench propagation and fungicide applications. Although these methods may reduce disease incidence, control is often unsatisfactory and more effective alternatives are needed.

In french and italian soils known to be suppressive to the pathogen, disease suppression has been associated with the presence of antagonistic non-pathogenic species of *Fusarium* (Garibaldi *et al.*, 1985; Tramier *et al.*, 1983). Non-pathogenic *Fusarium* spp. also play a major role in disease suppression in North America, where the effect has been attributed to interactions between the bacterium *Pseudomonas putida* and saprophytic *Fusarium* spp. (Park *et al.*, 1988). Antagonistic species of *Fusarium*, isolated from the rhizosphere of carnation plants grown in suppressive soils in Italy, are rhizosphere competent with a high ability to colonize roots (Garibaldi *et al.*, 1990). Competition with the pathogen for occupation of infection sites is one of the suggested mechanisms of the action of these antagonists (Cugudda & Garibaldi, 1987). Studies made in France and Italy over several years suggest the possibility of using antagonistic *Fusarium* spp. for the practical control of several *forma speciales* of *F. oxysporum* (Alabouvette, 1986).

In this paper we present results of experiments made in 1988 and 1989 to test the efficacy of different preparations of antagonistic *Fusarium* spp. for the control of *Fusarium* wilt on carnations.

MATERIALS AND METHODS

All experiments were made at the Centro Orticolo Sperimentale of the Chamber of Commerce, Albenga, North Italy. Carnations were grown in steam-sterilised soil in accordance with local commercial practice. The cultivars used were 'Manon', 'Lena' and 'Nevada', highly susceptible to race 2 of Fusarium oxysporum f.sp. dianthi and 'Cantalupo', moderately susceptible to this race (Garibaldi & Rossi, 1987). Rooted cuttings, obtained by an operation producing certified propagation material, were transplanted at a density of 35 plants/m^2 on raised benches in a glasshouse or on ground benches in the field.

Inoculum of Fusarium isolate Fod 75, race 2 was prepared by growing the fungus on autoclaved wheat kernels for 10 days at 25^{0}C, or by preparing a dilution of chlamydospores in talc following the method described by Locke and Colhoun (1974). The soil used in the experiments was infested with the pathogen by uniformly mixing the inoculum with the soil to a depth of 25 cm. Infested wheat kernels were applied at 20 g/m^2 of soil; chlamydospores were applied at 1.5-7.5 x 10^3 cfu/ml of soil. Two groups of antagonistic Fusarium isolates were used in the experiments. One group (WT) was sensitive to benzimidazoles and was composed of isolates 141, 233, 245, 251, and 257. The other group (WB) was composed of mutant isolates 233/1, 233/2, 245/1, 251/1, 251/4, 251/5, and 251/6. The latter group had previously been obtained for use in combination with fungicide treatments (Garibaldi et al., 1988b). Both groups had known activity as biocontrol agents. The antagonists were grown on autoclaved wheat kernels for 15-20 days at 25^{0}C or on a shaker in casein hydrolysate medium for 5-7 days at room temperature. The antagonists were applied by dipping rooted carnation cuttings in a spore suspension (4-5 x 10^7 spores/ml) prepared from cultures grown on casein hydrolysate or by soil infestation with colonised wheat kernels (200 g/m^2) or chlamydospores prepared following the same technique used for the pathogen. Soil treatments with the antagonists were applied at planting. Four experiments were made in the glasshouse and two in the field. In all glasshouse experiments the soil used had been steam-sterilised. In the field experiments the soil was sterilised with Di-Trapex at 100 ml/m^2 (Table 5) or was steam-sterilised (Table 6) In all but one experiment fungicide (benomyl or prochloraz) treatments were tested either alone or in combination with antagonists. Fungicides were applied at planting or at planting and at intervals after planting. 'Mycostop', a biofungicide based on Streptomyces griseoviridis (Kemira, Finland), was used in one trial as a root dip (10^6 cfu/ml) and soil drench (10^8 cfu/ml). The design of all experiments was a randomised block with three replicates of each treatment. Disease severity at the end of each experiment was calculated using an index of 0-100 (Garibaldi, 1966). Differences between treatments were determined using Duncan's multiple range test.

RESULTS

The use of colonised wheat kernels or chlamydospores diluted in talc to infest soil with F. oxysporum f.sp. dianthi was successful in all experiments with disease severity indices on untreated plants ranging from 55 to 96. In glasshouse tests both methods were equally effective.

In glasshouse tests, dipping rooted cuttings of carnation in a conidial suspension (4-5 x 10^7 cfu/ml) of antagonistic Fusarium isolates before planting generally gave satisfactory control of wilt. When this treatment was combined with soil treatment with the antagonists, increased disease control was obtained in two experiments (Tables 1 and 2) but not in a third (Table 5). When benzimidazole-sensitive and resistant isolates were used in the same experiments there were no significant differences in disease control between the two (Tables 1 and 3). Combination treatments involving pre-planting root applications of antagonists and soil treatment with the fungicides benomyl or prochloraz consistently gave the best disease control (Tables 2 and 3). A benomyl pre-planting soil treatment also increased the disease control obtained by soil infestation with the antagonists (Table 4). Further soil applications of benomyl after planting did not result in increased disease control (Tables 1, 2 and 3). Although prochloraz was also effective it produced phytotoxicity symptoms. The level of disease control obtained by antagonists, applied as root dips or soil incorporations, varied with the cultivars used (Table 4).

The efficacy of the antagonistic Fusarium spp. in controlling wilt under field conditions was confirmed in two experiments in 1988 and 1989 (Tables 5 and 6). In the 1988 field experiment, application of the benzimidazole-sensitive antagonists to the roots gave significantly better disease control than soil application (Table 5). Combining the two treatments did not increase the level of control. In 1989, a combination treatment of root dip and soil application with benzimidazole-resistant antagonists significantly reduced disease severity (Table 6). The degree of control was not increased by the application of fungicides. In this trial, Mycostop, applied as combination of root dip and soil drench was not effective.

TABLE 1. Effect on wilt severity of antagonists applied as root dips (root) and/or as a chlamydospore soil incorporation (soil). The soil was infested with a chlamydospore suspension of the pathogen (5x10^3 cfu/ml). Cv. 'Manon'.

Antagonists[1]	Application method	Benomyl (g/m^2) at planting	Benomyl (g/m^2) post-planting[2]	Disease index (0-100)	% healthy plants
-	-	-	-	66 d	13 d
RB	Root	-	-	31 c	41 c
RB	Root	5	5	28 c	46 c
RB	Root	5	2.5	29 c	39 c
RB	Root + soil	-		14 b	72 b
WT	Root + soil	-		15 b	72 b
RB	Root + soil	5	5	7 a	83 a

[1] RB = resistant to benzimidazole, WT = sensitive to benzimidazole.
[2] Applications made 20 and 40 days after planting.
Values followed by the same letter are not significantly different (P=0.05).

TABLE 2. Effect on wilt severity of antagonists applied as root dips (root) and/or as a chlamydospore soil incorporation (soil). The soil was infested with wheat kernels (200 g/m^2) colonised by the pathogen. Cv. 'Manon'.

Antagonists[1]	Application method	Benomyl (g/m^2) At planting	Benomyl (g/m^2) Post-planting[2]	Disease index (0-100)	% plants dead	% plants healthy
-	-	-	-	90 c	86 c	2 d
RB	Root	-	-	33 b	19 b	39 c
RB	Root	20	-	3 a	1 a	92 a
RB	Root	10	5	7 a	3 a	88 ab
WT	Root + soil	-	-	10 a	3 a	76 b

[1] RB = resistant to benzimidazole, WT = sensitive to benzimidazole.
[2] Applications made 20 and 40 days after planting.
Values followed by the same letter are not significantly different (P=0.05).

TABLE 3. Effect on wilt severity of antagonists applied as root dips (root) and/or as a chlamydospore soil incorporation (soil). The soil was infested with a chlamydospore suspension of the pathogen (1.5x10^3 cfu/ml). Cv. 'Manon'

Antagonists[1]	Application method	Fungicide	Rate(g/m^2) At planting	Rate(g/m^2) Post-planting[2]	Disease index (0-100)
	-	-	-	-	96 c
RB	Root	Benomyl	10	-	40 ab
RB	Root + soil	Benomyl	10	-	34 a
RB	Root + soil	-	-	-	47 b
WT	Root + soil	-	-	-	39 ab
RB	Root	Prochloraz	2	1	32 a
RB	Root + soil	Benomyl	5	2.5	37 ab

[1] RB = resistant to benzimidazole, WT = sensitive to benzimidazole.
[2] Post-planting soil treatments 20, 40 and 60 days after planting.
Values followed by the same letter are not significantly different (P=0.05).

TABLE 4. Effect on wilt severity of antagonists applied as a soil incorporation. The soil was infested with a chlamydospore suspension of the pathogen ($5x10^3$ cfu/ml).

Cultivar	Antagonists[1]	Benomyl (g/m^2)	Disease index (0-100)	% plants dead	% plants healthy
Manon	-	-	69 d	65 d	27 d
Cantalupo	-	-	49 c	42 c	38 cd
Manon	WT[2]	-	45 c	40 c	47 c
Cantalupo	WT[2]	-	20 b	15 b	70 b
Manon	RB[3]	-	40 c	34 c	47 c
Cantalupo	RB[3]	-	25 b	17 b	65 b
Manon	WT[2]	20	10 a	6 ab	86 a
Cantalupo	WT[2]	20	4 a	2 a	95 a

1 RB = resistant to benzimidazole, WT = sensitive to benzimidazole.
2 Applied as a chlamydospore suspension at 10^5 cfu/g soil.
3 Applied as infested wheat kernels at $200g/m^2$.
Values followed by the same letter are not significantly different ($P=0.05$).

TABLE 5. Effect on wilt severity of benzimidazole-sensitive antagonistic Fusarium spp. (WT). 1988/89 field experiment. Cv. Nevada.

Antagonists[1]	Application method	Disease index[1] (0-100)
-	-	55 c
WT	Root	22 a
WT	Root + soil	23 a
WT	Soil	34 b

1 Values followed by the same letter are not significantly different ($P=0.05$).

TABLE 6. Effect on wilt severity of Mycostop and of benzimidazole-resistant antagonistic Fusarium spp (RB). Field experiment 1989. Cv. 'Lena'

Antagonists[1]	Application method	Fungicide	Rate (g/m^2) at planting	Rate (g/m^2) post-planting[2]	Disease index (0-100)	% healthy plants
-	-	-	-	-	75 b	16a
Mycostop	Root + soil	-	-	-	72 b	15 a
RB	Root + soil	-	-	-	21 b	66 b
RB	Root	Benomyl	5	-	15 a	75 b
RB	Root + soil	Benomyl	5	-	17 a	73 b
RB	Root	Prochloraz	2.5	-	15 a	75 b
RB	Root + soil	Benomyl	5	2[1]	21 a	71 b

[1] Applications made 20, 40 and 60 days after planting.
Values followed by the same letter are not significantly different (P=0.05).

DISCUSSION

The studies described here confirm previous findings on the efficacy of antagonistic Fusarium isolates in controlling carnation wilt caused by Fusarium oxysporum f.sp. dianthi (Garibaldi et al., 1989a). In the present investigation application of the antagonists as a spore suspension to the roots gave the highest degree of control. However, although the former technique is simple and quick, it is not practicable for large scale applications. Infestation of soil with the antagonists was less effective than root application, especially in the presence of high disease pressure or when very susceptible varieties were grown. Despite the reduced efficacy of soil treatments, the incorporation into soil of chlamydospores diluted in talc offers an easy and attractive means of applying the antagonists. Moreover, in the case of fungicide resistant antagonists of Fusarium, the antagonists may be applied with fungicide at transplanting. Further work is required to determine the most effective means of producing inoculum and the most effective formulation for soil application.

The combination of antagonistic Fusarium isolates, applied to the soil or to the roots of cuttings, and a single chemical treatment at planting significantly reduced disease incidence. However, although both benomyl and prochloraz were effective, the latter fungicide was phytotoxic in some experiments. This observation has been reported previously (Garibaldi et al., 1988a). Chemical treatment alone, either with benomyl or prochloraz, only partially controls F. oxysporum f.sp. dianthi (Garibaldi et al., 1989a). The availability of antagonistic benzimidazole-resistant isolates of Fusarium allows the integrated use of chemical and biological control measures. The benzimidazole-resistant isolates used in the present study were selected for this very purpose (Garibaldi et al., 1988b); mutants of Fusarium resistant to prochloraz are being investigated at present.

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EVALUATION OF PHOSPHONIC ACID AS A FUNGICIDE IN AUSTRALIA

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ABSTRACT

Phosphonate, as Foli-R-Fos, a 20% solution of phosphonic (phosphorous) acid neutralised with potassium hydroxide has been evaluated for several years as a fungicide to control diseases in a wide range of crops in Australia. Foli-R-Fos is registered or registration is pending in Australia for the control of downy mildew on grape vines, and Phytophthora root rots in avocado, clover pastures, citrus, pineapple and ornamentals. Phosphonate has been evaluated as a foliar spray and trunk injection for the control of *Phytophthora* related diseases in almond, apple, cherry and peach.

Phosphonate is also being evaluated for the control of *Phytophthora* root rot of tomato, downy mildew of crucifer, lettuce and onion and *Phytophthora* related diseases in potato crops.

INTRODUCTION

Phosphonic (phosphorous) acid (H_3PO_3) the active ingredient of fosetyl-Al, is a widely used fungicide released in 1977 for the control of downy mildew and the diseases caused by *Phytophthora* spp.

Phosphonic acid and other phosphonate compounds are patented for use as fungicides by Rhone-Poulenc, the manufacturers of fosetyl-Al. However in Australia a formulation of phosphonic acid was developed as a fungicide by a Queensland company and as a result of a court decision phosphonic acid can now be legally used and marketed as a fungicide throughout Australia. Phosphonic acid as a 20% solution partially neutralised with potassium hydroxide was released in 1987 as Foli-R-Fos and Fos-ject 200 by U.I.M. Agrochemicals (Aust.) Pty. Ltd., Rocklea, Queensland for use as a foliar spray and trunk injection respectively.

This development encouraged many Australian scientists to evaluate these formulations for the control of Oomycetes on a wide range of crops.

This paper briefly describes the development of phosphonic acid as a fungicide in Australia. Much of this work was presented at a workshop on phosphonic acid held in 1989 at the 7th Australasian Plant Pathology Conference - Brisbane, and further details will appear in Australasian Plant Pathology Volume 20(1).

EVALUATIONS AND RECOMMENDATIONS

Avocado

Phosphonic acid was first evaluated on avocado in Australia in Queensland in 1983. The dramatic results in the control of *Phytophthora* root rot in avocado led eventually to the commercial development of phosphonic acid as a fungicide. Pegg *et al.* (1985 and 1987) showed that avocado trees severely affected by *Phytophthora cinnamomi* recovered rapidly following injection of a 20% solution of potassium phosphonate directly into the trunk. This work showed that injected trees had higher yields (48-58 kg fruit/tree compared to 4 kg) and lower leaf chloride (0.8-2.4% wt/wt compared to 4.2%) than controls in the third season after treatment. Treated trees also had phosphite residues in fruit ranging from 23 to 83 mg/kg which is below the maximum residue limit of 100 mg/kg set by Australian health authorities.

As a result of these studies trunk injections of phosphonic acid are widely used by avocado growers throughout Queensland. Avocado trees showing aerial symptoms of root rot are injected with 15 mls of Fos-ject per m of canopy diameter in early spring and again after the spring growth flush has matured. Trees are injected before mid-day when the transpiration rate is highest and the uptake most rapid.

Micronutrient deficiencies have also been overcome by the use of compatible formulations of nutrients such as zinc-chelate and phosphonate injected into the trunks of diseased avocado trees.

Apple

Phosphonic acid has been evaluated extensively in Victoria for the control of collar rot caused by *Phytophthora cactorum*. Preliminary work showed that trunk injections of partially neutralised 10% or 20% solutions of phosphonic acid were phytotoxic, causing chlorosis and necrosis of leaves and death of flowers. Evaluation of foliar sprays and trunk injections are continuing.

Citrus

Root rot caused by *Phytophthora nicotianae* var *parasitica* and trunk canker caused by *P. citrophthora* are common in citrus orchards and nurseries throughout Australia. In Queensland foliar sprays of phosphonic acid (32-64 g/tree/year) have been compared with foliar sprays of fosetyl-Al and soil sprays of metalaxyl for the control of these diseases on mandarin trees over a period of five years. Compared to the phosphonic acid and metalaxyl treated trees, untreated and fosetyl-Al treated trees showed significant reduction in tree canopy density, increased trunk girdling, increased canker area and reduced fruit yields (de Boer *et al.* 1990). While these experiments were conducted on mature trees, experience from South Australia has indicated that lower rates of application may be required for young trees (Walker 1989). Young container-grown mandarin nursery trees exhibited leaf burn and growth retardation following a foliar application or soil drench of phosphonic acid at rates recommended for established trees.

Grapevine

Preliminary investigations showed that phosphonic acid controlled *Plasmopara viticola* on grape (Wicks *et al.*, 1987; Magarey & Wachtel, 1987; Magarey *et al.*, 1987). Extensive investigations conducted over a period of 4 years resulted in

registration throughout Australia of a formulation of phosphonic acid for the control of grapevine downy mildew. Brief details of some of this work has been published (Magarey *et al.* 1989) while other details are in press (Wicks *et al.* 1990, Magarey *et al.* 1990). Overall these studies showed that 1.2 g/l phosphonic acid effectively controlled downy mildew when applied within 7 days after infection and in some experiments up to 13 days after infection. On the other hand, the same rate of phosphonic acid was less effective when applied before infection. Using innoculated leaf disks as a means of measuring efficacy of phosphonic acid and analysing the phosphite content in the leaves from which the disks were taken, showed that the poor protectant activity of phosphonic acid was due to translocation of phosphite from the sprayed leaves. The movement of phosphite from sprayed leaves to unsprayed leaves on the same and adjacent shoot, as well as soil uptake, was demonstrated in field and glasshouse studies, but was not sufficient to control downy mildew in the field away from the application site.

As a result of these studies foliar applications of 1.2 to 1.6 kg/ha of phosphonic acid are recommended for use on vines as a curative spray applied within 7 days from infection. Tank mixes with either copper oxychloride or mancozeb are also recommended for pre-infection control. Phosphite residues on vines sprayed at least four times per season have not been detrimental to fermentation and have been within the maximum residue level of 80 ppm set by the Australian health authorities.

Pineapple

Phosphonic acid has been evaluated in Queensland for the control of root and heart rots of pineapples caused by *Phytophthora cinnamomi*. Since pineapples are grown from the crowns (tops) of mature fruit, pre-harvest and post-planting foliar sprays were tested for the control of root rot.

These experiments showed that a single pre-harvest (pre-plant) spray of phosphonic acid at 2.5, 5.0, 10.0 or 15.0 kg/ha protected pineapple roots for 2 years after planting. The single pre-plant treatment was as effective as four post-planting applications of phosphonic acid (de Boer *et al.* 1990).

Stone Fruit

Investigations into the use of phosphonic acid for the control of *Phytophthora cambivora* were initially carried out in South Australia on potted juvenile almond and cherry tress (Wicks and Hall 1988). These studies showed phosphonic acid applied as a foliar spray or drench inhibited the development of stem lesions on plants previously inoculated with *Phytophthora cambivora* and were as effective as metalaxyl or fosetyl-Al drenches.

Similar results were obtained when phosphonic acid was evaluated on mature trees over several season (Wicks and Hall 1990). In these studies foliar sprays of 2 g/l phosphonic acid applied in autumn and the following spring were the most effective in controlling the disease.

Injections of 60 ml of a 10% solution of phosphonic acid directly into the trunk of either almond or cherry trees inhibited the development of lesions on shoots inoculated with *P.cambivora*.

The inhibitory effect of phosphonic acid on lesion growth persisted for at least 48 weeks after treatment irrespective of the method of application.

Experiments have also been conducted on peaches in Victoria to evaluate

phosphonic acid for the control of collar rot caused by *Phytophthora cactorum* (Lim *et al.* 1989). This work showed that trunk injections of 1.4 g phosphonic acid per tree or two foliar sprays of 1 g phosphonic acid per tree inhibited the development of lesions when applied before inoculation.

Vegetables

The evaluation of phosphonic acid on vegetable crops has recently been stimulated by several reports of metalaxyl failing to control downy mildew on a number of vegetable crops throughout Australia.

Cauliflower

Systemic infections of downy mildew caused by *Peronospora parasitica* result in significant economic losses to growers of export cauliflower curds in Western Australia.

Two applications of 2.4 kg/ha phosphonic acid at 21 and 7 days before harvest controlled downy mildew, and reduced disease incidence from 97% in untreated plots to 7.5% in treated plots (McKay *et al.* - In press). Phosphonic acid had no deleterious effect on crop appearance or maturity and maximum phosphite residues in the curds at harvest were 12 mg/kg which are below the maximum acceptable levels.

Tomato

Root rot caused by *Phytophthora nicotianae var nicotianae* is a serious disease of processing tomatoes grown in the irrigation areas of Northern Victoria. Phosphonic acid has been evaluated extensively on tomatoes in this area, and has reduced the severity of root rot when applied at 10 kg/ha as a foliar spray at flowering (Flett *et al.* 1989). Fruit yield was also increased by 62% compared to the untreated controls.

Other vegetables

Phosphonic acid has been evaluated throughout Australia for the control of downy mildew on cucurbits, onions, peas and lettuce in both glasshouse and field experiments, but few details of the results are available. Phosphonic acid is also being evaluated for the control of *Phytophthora* related diseases in potatoes.

Pastures

Phytophthora clandestina is now recognised as the cause of root rot and subsequent decline of subterranean clover (*Trifolium subterranean*) an annual legume widely used in pastures in southern Australia. The problem is particularly severe in irrigated pastures in Victoria where phosphonic acid has been extensively evaluated as a means of controlling the problem.

Outstanding results have been achieved with foliar sprays of phosphonic acid applied to seedlings at 300 g/ha in 200 l of water/ha (de Boer *et al.* 1990). This work showed that sprays were most effective when applied 8 to 9 days after the first autumn irrigation and just before the second irrigation. Dry matter increases of over 2 tonnes/ha have been measured following application of phosphonic acid. As a result of this work a formulation is now registered for use on clover throughout Australia.

Ornamentals and native trees

Root and crown rots caused by *Phytophthora cinnamomi* are major problems on a wide range of native and introduced ornamental plants in nurseries and on established plants in the field. Most experiments evaluating phosphonic acid on ornamentals have been conducted in the glasshouse with artificially inoculated plants. Foliar sprays of 1 g/l phosphonic acid have controlled root rot of azaleas, and inhibited the development of stem lesions on inoculated *Rhododendron* and *Leucodendron* (Marks and Smith 1990). In these experiments phosphonic acid gave superior disease control to fosetyl-Al.

CONCLUSIONS AND FUTURE DEVELOPMENTS

Extensive field evaluation of phosphonic acid for the control of a wide range of diseases on a number of subtropical and temperate crops has been conducted throughout Australia since the mid 1980's. As a result of this work phosphonic acid is registered, or registration is pending, for use on avocado, citrus, grapes, pineapple, ornamentals and subterranean clover.

Further uses will no doubt be discovered as phosphonic acid is evaluated on other crop and pathogen combinations. Phosphonate fungicides are likely to increase in use on horticultural crops in Australia as they are cost effective and relatively easy to manufacture and use.

While investigations into the mode of action of phosphonates may shed some light on the behaviour at the cellular level and lead to the development of new compounds, studies on the dynamics of phosphite levels and movement in plants may be of more practical use in the short term. For example the critical phosphite levels that inhibit the development of a pathogen in plant tissue need to be determined. If these levels are known it may be possible to develop field test kits to monitor phosphite levels in plants and use this to decide when to apply the fungicide. Further work is needed to evaluate phosphonates as a means of enhancing the natural defence systems of plants and to better utilise the systemic activity of the fungicide.

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ONION WHITE ROT CONTROL - STERILANT OR STIMULANT?

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ABSTRACT

The effects of different soil sterilants and sclerotial stimulants on white rot disease and yield were investigated. Significant disease control was obtained with the sterilants 1,3 dichloropropene, metham sodium and dazomet. Autumn-applied dazomet gave better disease control than spring treatments. Dazomet at 570 kg product/ha autumn-applied gave complete control in one trial in the two subsequent years of cropping but in another trial no significant control was achieved in the second crop. No significant differences in disease levels were found between various metham sodium and dazomet treatments applied in the spring. The sclerotial stimulant diallyl disulphide gave up to 50% disease control and in combined treatments with metham sodium and dazomet disease control was similar to that given by the sterilant alone. The effect of treatments on marketable yields was variable with significant yield increases following dazomet treatments. However, no significant marketable yield increases were recorded following spring-applied metham sodium or a low rate diallyl disulphide treatments.

INTRODUCTION

White rot disease caused by the fungus Sclerotium cepivorum remains a major disease of bulb onions in the UK. It is of increasing importance in all the main areas where the crop is grown. The majority of UK bulb onion crops are direct-drilled and in these crops fungicide treatments have been of limited value (Gladders et al., 1984). However, more recently triadimenol and tebuconazole applied as foliar sprays prior to irrigation have given good disease control in trials in Southern Germany (Krauthausen, 1990). A high degree of control was obtained in trials on module-raised bulb onions with pre-planting drenches of myclozolin and procymidone (Gladders et al., 1987). However, these two fungicides are not registered for use in the UK and therefore at present, there is no effective fungicide control of white rot in bulb onions.

The white rot pathogen can survive in the soil for many years. Treatment with partial soil sterilants based on products generating methyl isothiocyanate such as metham sodium or dazomet have given variable results (Adams & Johnson, 1983; Entwistle et al., 1985; Kerr, 1986). At Kirton Experimental Horticulture Station (EHS), autumn-applied dazomet at 380 kg product/ha, metham sodium at 300 l product/ha and 1,3 dichloropropene at 220 l product/ha and sheeted over after application with 500 g polythene gave mean reductions of 75%, 73% and 53% respectively in the number of viable sclerotia artificially buried. Only dazomet gave good disease control and increased yields. At Moulton, Lincs, metham sodium, 1,3 dichloropropene and dazomet were applied as in

the previous trial and in addition dazomet at 570 kg product/ha. Only the dazomet treatments gave a good kill of artificially buried sclerotia. Moderate disease was recorded in the metham sodium and 1,3 dichloropropene treated plots, very low levels in the 380 kg product/ha dazomet plots and none in the 570 kg product/ha plots (Davies & Coley-Smith, 1986). However, no significant yield differences were recorded between treatments with 19% modules affected in the untreated plots (J M Ll Davies, unpublished).

Sclerotia of S. cepivorum only germinate in the presence of Allium host plants (Coley-Smith, 1960). The roots of these plants exude compounds which are metabolised by the soil microflora to produce a mixture of thiols and sulphides which stimulate the sclerotia to germinate (Coley-Smith & King, 1969). Sclerotia germinate only once and in the absence of an onion crop they die. Synthetic stimulants offer control opportunities. Substantial reductions in the numbers of sclerotia and disease incidence have been recorded in Australia, (Merriman et al., 1981) and Canada, (Rahe & Utkhede, 1982). The major constituents of artificial onion oil have been tested and diallyl disulphide (DADS) has proved to be the most active (Coley-Smith et al., 1981). At Moulton, Lincs, onion oil soil-injected at 500 l/ha gave some reduction in the numbers of viable sclerotia artificially buried and 36% disease control but did not significantly increase yield (Davies & Coley-Smith, 1986). This paper reviews the recent ADAS trials sponsored both by the Government and by the Horticultural Development Council (HDC).

MATERIALS AND METHODS

All the trials were located on sites in parts of fields where a severe attack of white rot had been noted in the past.

Government-funded trials

Moulton, Lincs

This trial was made to see if the effects of treatments applied in the first year (as previously reported, Davies & Coley-Smith, 1986), persisted into the second year. Metham sodium (BASF Metham Fluid 510 g/l) at 300 l product/ha, 1,3 dichloropropene (Telone 11 94% Dow Agriculture) at 220 l product/ha and onion oil (Bush, Boke & Allen, London) 500 l/ha (diluted 10X) were applied by Rumpstadt Combijet 225 soil injector on 28 October 1985. This machine was also driven through the control plots without any chemical prior to application of treatments. Dazomet (Basamid 98-99%, BASF) at 380 and 570 kg product/ha was applied using a MJF Basamid Incorporator (a spade digger with a hopper) on 5 November 1985. All plots were covered on the day of application with 500 g polythene which was removed prior to planting the first crop on 8 May 1986. The trial design was a randomised block with five replicates. Each plot measured 4 m x 1.83 m with four rows of module-raised plants. The plots were separated from each other by guard beds on each side and a 2 m wide unplanted strip at each end. The plots were located on the no fungicide half of the first year plots. Module-raised onions, cv. Hyton were planted on 5 April 1987 and all subsequent husbandry was according to farm practice. The trial was harvested on 15 October 1987 and assessments were made of disease incidence, marketable and unmarketable yields.

Kirton EHS "quarantine site" - Year 1

Using a MJF Basamid Incorporator dazomet was applied at 380 kg and 570 kg product/ha covered with 125 g polythene, or at 570 kg product/ha and covered with 500 g polythene on 7 November 1986. The untreated control plots were also dug by the Basamid Incorporator but no chemical was applied and plots were covered with 125 g polythene. A light wind blew across the trial at the time of application and some of the Basamid drifted across the plots, especially in two replicates of treatments 5 and 6. More precise application was achieved subsequently by using a hand-held wind shield. Vinclozolin (Ronilan 50% w/w, BASF) was applied pre-planting as a drench at 6.16 g product in 200 ml/tray to a duplicate set of treatments. The experiment was a randomised block design with four replicates, each plot measuring 6 m x 1.83 m. The plots were separated from each other by guard beds on each side and a 2 m wide unplanted strip at each end. The trial was isolated from other onion crops by a 2 m wide unplanted strip at the sides and headlands. Artificially-produced sclerotia of S. cepivorum were supplied by and viability tests made by Professor John Coley-Smith of Hull University. Twenty-five sclerotia mixed with sand and placed in a nylon bag were inserted in a "Netlon String", three bags to each string being spaced out so that they could be buried at depths of 2.5, 10 and 20 cm. Two strings were buried at the two ends of each plot immediately after treatment and were removed on the day of planting. Module-raised onions cv.Hyton were planted on 22 April 1987 and subsequent husbandry was according to farm practice. The trial was harvested on 29 September 1987.

Kirton EHS "quarantine site" - Year 2

This trial was a continuation of the previous trial to investigate whether the effect of soil sterilants had persisted into the second year. No further dazomet treatments were made but all other treatments were identical to those of the first year. The trial was planted on 26 April 1988 and harvested on 14/15 September 1988. All other treatments were identical to the ones of the first year.

HDC-funded trials

In five trials, treatments were applied in the spring at Northorpe, Lincs on 13 May 1987, Kirton EHS (1) on 8 May 1987 (mineral soils) and Methwold Fen, Norfolk (organic peat) on 29 March 1989 and in the autumn at Kirton EHS (2) on 7/8 November 1988 and Arthur Rickwood EHF Mepal Cambs (organic peat) on 27 October 1988. The treatments were identical in all trials and consisted of dazomet at 380 and 570 kg product/ha, metham sodium at 600 and 1200 l product/ha, DADS (Phillips Petroleum and Oxford Organic Chemicals) at 50 l/ha and 200 l/ha, dazomet at 380 kg product/ha plus DADS at 50 l/ha, metham sodium at 600 l product/ha plus DADS at 50 l/ha and metham sodium at 1200 l product/ha plus DADS at 200 l/ha. The metham sodium and the DADS treatments were applied by a Rumpstadt Combijet soil injector. Dazomet was applied using a MJF Basamid Incorporator at Northorpe and Kirton EHS and by hand at Arthur Rickwood EHF and Methwold. At sites where treatments were made by hand, plots were rotovated after application. In order to prevent contamination by DADS in other treatments, the DADS treatments were the last to be applied. All plots, including the untreated, were covered with 125 g polythene which was left on for approximately 6 months at each site. Each trial was a randomised block design with 4 replicates. The plots were separated from each other by unplanted guard beds on each side and a 2 m unplanted strip at each end. In four of the trials each plot

measured 4 m x 1.83 m in a treated area of 6-10 m x 2-3 m, with four rows of module-raised plants of cv. Hyton or cv. Caribo. At Methwold three double rows of pickling onions cv. Plastro were drilled in a 1.52 m wide bed. All subsequent husbandry was according to farm practice. The trials were sown or planted in March/April and harvested in August/ September. All data were subjected to analysis of variance. Treatment means followed by the same letter within any one column do not differ significantly at P = 0.05 (Duncan's Multiple Range Test).

RESULTS

TABLE 1 Effect of treatments on white rot disease and yield - Moulton

Treatment (product/ha)	% Modules affected	Yield (t/ha) Total	Marketable
1. Untreated	29.0^{c}	23.6^{a}	22.1^{a}
2. Metham sodium 300 l	13.2^{b}	26.6^{a}	25.7^{ab}
3. 1,3 dichloropropene 220 l	11.1^{ab}	30.5^{ab}	29.6^{abc}
4. Onion oil 500 l	12.5^{ab}	30.0^{ab}	29.2^{abc}
5. Dazomet 380 kg	2.1^{ab}	32.0^{ab}	31.6^{bc}
6. Dazomet 570 kg	0.0^{a}	37.0^{b}	36.7^{c}
SED (20 df)	5.7	3.8	3.9

At Moulton (Table 1) all the chemical treatments gave significant control of white rot. These results reflect the previous year's disease scores. The two dazomet treatments significantly increased marketable yield but only the high rate significantly increased total yield.

TABLE 2 Effect of dazomet on artificially buried sclerotia - Kirton EHS

Treatment (product/ha)	Polythene gauge	Mean no. of viable sclerotia/25 depth of burial (cm) 2.5	10	20
1. Untreated	125	17.2^{b}	21.0^{c}	17.4^{b}
2. Untreated	125	21.6^{b}	23.3^{c}	21.9^{b}
3. Dazomet 380 kg	125	0.75^{a}	1.8^{ab}	0.6^{a}
4. Dazomet 380 kg	125	4.9^{a}	6.1^{b}	2.6^{a}
5. Dazomet 570 kg	125	0.0^{a}	0.0^{a}	2.6^{a}
6. Dazomet 570 kg	125	0.0^{a}	0.0^{a}	0.1^{a}
7. Dazomet 570 kg	500	3.1^{a}	0.0^{a}	3.3^{a}
8. Dazomet 570 kg	500	0.0^{a}	0.0^{a}	0.0^{a}
SED (21 df)		3.3	2.1	2.5

TABLE 3 Effect of dazomet on white rot and yield - Kirton EHS Year 1

Treatment (product/ha)	Polythene gauge	Pre-planting drench	% Modules affected	Yield (t/ha) Total	Yield (t/ha) Marketable
1. Untreated	125	-	52.6^{b}	22.5^{a}	20.3^{a}
2. Untreated	125	Vinclozolin	43.6^{b}	30.9^{b}	26.9^{ab}
3. Dazomet 380 kg	125	-	13.0^{a}	44.3^{cd}	41.1^{d}
4. Dazomet 380 kg	125	Vinclozolin	10.0^{a}	46.5^{d}	43.7^{d}
5. Dazomet 570 kg	125	-	17.0^{a}	35.9^{bc}	31.1^{bc}
6. Dazomet 570 kg	125	Vinclozolin	4.6^{a}	38.6^{bcd}	35.6^{bcd}
7. Dazomet 570 kg	500	-	6.6^{a}	42.9^{cd}	40.6^{cd}
8. Dazomet 570 kg	500	Vinclozolin	5.6^{a}	44.2^{cd}	41.9^{d}
SED (21 df)			8.6	3.9	4.4

In the first year at Kirton EHS moderate to high levels of white rot developed. All the dazomet treatments gave a significant kill of sclerotia at the three depths (Table 2), and significant disease control and increases in total and marketable yields (Table 3). Dazomet at 570 kg product/ha and covered with 125 g polythene gave a significant lower yield than dazomet at 380 kg product/ha and covered with 125 g polythene.

TABLE 4 Effect of dazomet on white rot disease and yield - Kirton EHS, Year 2

Treatment (product/ha)	Polythene gauge	Pre-planting drench	% Modules affected	Yield (t/ha) Total	Yield (t/ha) Marketable
1. Untreated	125	-	26.5^{a}	20.8^{a}	18.9^{a}
2. Untreated	125	Vinclozolin	21.5^{a}	21.6^{a}	21.4^{a}
3. Dazomet 380 kg	125	-	22.5^{a}	28.4^{a}	26.8^{a}
4. Dazomet 380 kg	125	Vinclozolin	14.0^{a}	28.3^{a}	26.5^{a}
5. Dazomet 570 kg	125	-	10.5^{a}	29.5^{a}	27.9^{a}
6. Dazomet 570 kg	125	Vinclozolin	15.0^{a}	28.4^{a}	27.5^{a}
7. Dazomet 570 kg	500	-	17.0^{a}	28.1^{a}	26.2^{a}
8. Dazomet 570 kg	500	Vinclozolin	22.0^{a}	25.0^{a}	23.6^{a}
SED (21 df)			8.8	4.1	4.1

Low to moderate levels of white rot developed in the second year of the trial and no significant effects of treatments were recorded (Table 4).

TABLE 5 Effect of treatments on disease and yield - HDC funded trials

Treatment (product/ha)	% Disease incidence[1]	Ang trans[2]	Yield (t/ha)[3] Total	Marketable
1. Untreated	42.2*	40.4	31.7	30.2
2. Dazomet 380 kg	19.8	22.4	38.0	36.1
3. Dazomet 570 kg	17.4	19.3	38.9	37.0
4. Metham sodium 600 l	20.7	21.8	36.1	34.5
5. Metham sodium 1200 l	13.8	15.8	33.4	21.4
6. DADS 50 l	30.1	29.4	35.9	34.4
7. DADS 200 l	21.1	23.0	38.4	37.0
8. Dazomet 380 kg + DADS 50 l	17.5	19.5	39.6	37.3
9. Metham sodium 600 l + DADS 50 l	17.7	18.7	38.5	36.4
10. Metham sodium 1200 l + DADS 200 l	11.3	16.5	39.9	37.9
SED		3.67	2.15	2.19
df		81	135	135

1 Mean of three trials, Northorpe, EHS(I) and Methwold.
2 Disease data angular transformed.
3 Mean of five trials.

Low, moderate and high white rot disease levels developed at Northorpe, Kirton EHS(1) and Methwold sites respectively (Table 5). Little disease was recorded at the other two sites with the exception of one plot of treatment 5 at the Kirton EHS(2) site which was severely affected. Results from the three sites showed that all treatments gave significant control of white rot with the best control (approximately 70%) achieved from treatments which included metham sodium at 1200 l product/ha. The control from the low rate and high rate DADS treatments was 28% and 50% respectively. The combined treatments of sterilants plus DADS gave similar results to those given by the sterilants alone. Data from the five sites showed that total yields were significantly increased by all treatments except metham sodium at 1200 l product/ha and DADS at 50 l product/ha. Marketable yields were significantly increased by all treatments except by metham sodium at 600 l or 1200 l product/ha and DADS 50 l product/ha.

DISCUSSION

These investigations have confirmed the variability in white rot control achieved with soil sterilants. Overall, dazomet applied in the autumn gave approximately 85% while spring applications gave only 56%. No significant differences in disease control were found between the 380 and 570 kg/product/ha rates of dazomet but lower disease scores were recorded following the latter. At Kirton EHS there were no significant differences in disease control between the two thicknesses of polythene sheet used to seal the dazomet treatments. In the same trial, although there was up to 100% kill of artificially buried sclerotia this did not

result in comparable disease control. However, at Moulton, following the 570 kg/product/ha rate of dazomet there was 100% kill of buried sclerotia and complete disease control (Davies & Coley-Smith, 1986). The persistency of dazomet treatments was studied in two subsequent crops at two sites. At Moulton a high degree of control was achieved with the 380 kg/product/ha rate with complete control following the 570 kg product/ha treatment. However, at Kirton EHS the control achieved in the first year did not persist into the second year. It was likely that this lack of persistence resulted from the unsatisfactory method of application. Signficant yield increases were recorded following the dazomet treatments. In the HDC sponsored trials the effect of treatments on disease control could only be evaluated at three sites. Unfortunately, the effect of autumn-applied treatments on disease control could not be assessed as little disease developed on these sites despite a history of white rot, a situation similar to that encountered by Entwistle et al., (1986). Dazomet and metham sodium gave significant disease control, although, inexplicably, no significant increase in marketable yields were detected following metham sodium treatments. The effects of sclerotial stimulants on disease control were disappointing. Onion oil at Moulton gave a mean of 46% control over two years and DADS at the low and high rates gave 28% and 50% control respectively in the HDC trials. Possibly, DADS was not applied in sufficient volume (the highest rate used was 200 l/ha) as the greater the quantity and volume the better the results (Coley-Smith et al., 1986). In higher volumes DADS gave a high degree of control (72-92%) in module-raised bulb onions with drenches of 6.25-25 l/m^2 (Davies & Coley-Smith, 1990). It was unfortunate that the effect on disease of autumn-applied DADS could not be evaluated. The disease scores following combined treatments of sterilants and DADS were similar to those of the sterilant alone with no significant advantages resulting from the dual treatment.

The control of white rot remains a problem for the bulb onion growers and they will ultimately run out of disease-free land. Growers are concentrating their crops on land known to be free of white rot. The use of sterilants alone cannot be relied upon to give good disease control. DADS gave insufficient control in these trials and there was no advantage of the dual treatment applied with sterilants. It may be worthwhile evaluating different formulations of DADS, such as a granule which would not require specialist equipment and could be applied at low rates in the rotation in the absence of an Allium crop.

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BIOLOGICAL STUDIES ASSOCIATED WITH FORECASTING THE TIMING OF ATTACKS BY THE LARGE NARCISSUS FLY, *MERODON EQUESTRIS*

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ABSTRACT

A method is described that enabled laboratory cultures of the large narcissus fly (*Merodon equestris*) to be maintained efficiently throughout the year on a pollen-free diet. Each narcissus bulb used in the culture was inoculated with either two newly-emerged large narcissus fly larvae or four eggs. The incidence of larval establishment in the bulbs was not increased by cutting or drilling holes in the bases of bulbs before inoculation. In field-cages, 50% of the flies emerged by 31 May in 1989 and by 24 May in 1990. The spread of emergence extended from 22 May to 16 June in 1989 and from 11 May to 3 July in 1990. In laboratory experiments, female flies required temperatures in excess of 19°C to lay eggs. The base temperature for egg hatch was 8°C.

INTRODUCTION

The large narcissus fly (*Merodon equestris*) is the major insect pest of the UK bulb industry and could cause considerable damage to crops each year if chemical control methods failed to work effectively. In the UK most narcissus bulb crops are left in the ground for 2-3 seasons, as this improves bulb yield and quality and helps to produce blooms with long stalks and good colour. In the past, protecting crops against the fly throughout this period was achieved by the use of aldrin, a highly persistent insecticide. However, this chemical has now been withdrawn and only less persistent insecticides are available. It seems likely, therefore, that control of the fly during the second and third seasons on well-established crops may prove difficult in future.

Adult large narcissus flies resemble small bumble bees. They emerge from the soil in May or June and lay their eggs in the soil, either close to narcissus bulbs or on senescing narcissus foliage. After a few days, the eggs hatch and the newly-emerged larvae tunnel through the soil and enter the bulbs via the basal plate. The larvae feed and grow inside the bulbs and destroy the centres. Although several larvae may enter a single bulb, usually only one survives and, by the onset of winter, it is fully grown and about 15-20 mm long. The larvae overwinter inside the bulbs before they emerge in April/May and pupate just below the surface of the soil. The aim of present research at this Institute is to develop a practical system for forecasting the time of appearance of the large narcissus fly so that the timing and effectiveness of control measures may be optimized.

Pest forecasting systems are produced from information collected during extensive and detailed studies of insect behaviour and development. Hence, an essential pre-requisite of such studies is the development of

methods suitable for rearing large numbers of insects under laboratory conditions. This is particularly important for species like the large narcissus fly which have only one generation each year in the field. This paper concentrates on methods for establishing laboratory cultures of the fly and on the rate of development of the insect.

REARING INSECTS IN THE LABORATORY

Factors affecting the fecundity of the large narcissus fly

During 1969-71, Conn (1971) made several attempts to rear more than one generation of the large narcissus fly per year in the laboratory. Although he produced adults in January/February, he was unable to induce the females to lay eggs and he concluded that, until a proteinaceous food could be found that the flies would accept in place of fresh pollen, producing two generations of the fly per year would not be feasible.

Based on the conclusions of Conn (1971), we provided large narcissus flies at Wellesbourne with flowering hedgerow plants during the summer months and with flowering pot plants (e.g. *Cineraria*), or cut plants (e.g. chrysanthemums and narcissi) during the winter. The flies were housed in cages in a controlled environment room with an 18:6 h L:D phase, light of 50 watts/m, a day temperature of 22.5±0.5°C and a night temperature of 15.5±0.5°C. Once the culture was established, experiments were done to test whether fresh pollen was essential for egg-laying, or whether the diet used for rearing other flies at Wellesbourne (Finch & Coaker, 1969) may be suitable also for the large narcissus fly.

Flies were presented with the standard Wellesbourne diet (Finch & Coaker, 1969) in two Petri dishes, one containing 10% sucrose solution absorbed on a pad of cotton wool and the other containing powdered brewer's yeast and yeast hydrolysate. Flies in one half of the cages were provided also with bunches of flowers that included species of *Prunus* (flowering cherry), *Anthriscus* (cow parsley), *Heracleum* (hogweed) and *Ranunculus* (buttercup).

The results of these experiments indicated that fresh pollen was not an essential food, as large narcissus flies provided with the standard Wellesbourne diet laid as many eggs (81±7 eggs per female) as those provided with the standard diet supplemented with flowers (90±12 eggs per female). However, although the flies were provided with a narcissus bulb resting on damp filter paper, sand or peat in a shallow dish, most eggs were not laid at such sites. Instead, they were laid wherever gaps occurred that were large enough to accommodate the telescopic probing ovipositor. For example, in wooden-framed glass cages, many eggs were pushed down between the glass and the wooden supporting frame and hence could not be recovered easily. To prevent this occurring, flies were held in cages made from 45 cm^3 cubed rod- and ball-frames covered with sleeves of Terylene net. Each cage rested on a white plastic tray. In these cages, most eggs were laid under the feeding containers in gaps between the finely-ridged bottoms of the containers, the Terylene mesh and the white tray. When the flies started to lay, eggs were found stuck to the bottom of the feeding containers and to the Terylene net directly below the edges of the containers. Eggs sticking to the Terylene of the cage bottom were brushed through the mesh and were then either picked or washed from the

collection tray. Each female produced about 24 eggs on the first day of laying.

Factors affecting larval establishment

Collecting eggs of the large narcissus fly is labour-intensive. Therefore, every effort was made to ensure that the return of insects from inoculated eggs was as high as possible. The cost of rearing insects under laboratory conditions is high, particularly for insects like the large narcissus fly that have a 4-5 month period of larval development. Therefore it is important to try to ensure that every bulb inoculated for the culture becomes infested with a larva.

The viability of the eggs in the culture ranged from 61 - 88%. Therefore, with only one egg inoculated on each bulb, many bulbs would not contain larvae. When one, two or four eggs were placed alongside bulbs potted in Levington compost, larvae developed successfully in 41±8%, 76±6% and 102±3% of the bulbs, respectively. Figures in excess of 100% indicate that, in some pots, a few larvae established in offsets as well as in the main bulb. It is likely that inoculating three eggs on each bulb may be sufficient, but this treatment was not tested. In similar tests, in which bulbs were inoculated with either one or two newly-emerged larvae, fully-grown larvae were found subsequently in 78±7% and 106±3% of the bulbs, respectively.

Previous information indicated that bulbs are usually attacked through the tough basal plate. In attempts to increase the establishment of larvae in narcissus bulbs, holes were made in bulbs by cutting a groove across the basal plate or by drilling through the plate, using a fine scalpel. Some of the holes were smeared with the waste products from large larvae of the large narcissus fly to test if bacteria associated with larval feeding help small larvae to establish in bulbs. Test bulbs were inoculated with larvae that had emerged from eggs of large narcissus flies maintained in incubators. Larvae were placed on the soil surface alongside each bulb or directly in the cuts made in the base of the bulbs.

None of the treatments increased larval establishment in the bulbs. When larvae were placed directly into the cuts or holes were made in the base of the bulbs, the larvae did not enter through the artificially damaged tissues. By carefully paring away the base of the bulbs, it was apparent that larvae initially by-passed the physical barrier of the basal plate by chewing into and up through roots that had already emerged through the basal plate. Why the larvae move subsequently into the tough basal plate to feed, remains a mystery. When two four, eight or 16 larvae were inoculated on to each bulb, only one individual survived within the main bulb. However, if the bulb was large with a number of offsets, larvae were frequently found in the offsets. No potted "bulb" contained more than three larvae.

STUDIES ASSOCIATED DIRECTLY WITH FORECASTING ATTACKS

Emergence of flies in field cages

Bulbs infested with larvae of the large narcissus fly were placed

into dumpy 15 cm diameter pots of Levington compost, with 5 larvae in each pot. Pots were placed into one of two 5.0 x 2.0 m field plots so that the rims of the pots were just below soil level. In late April, each plot was covered with a 6.0 x 3.0 x 1.8 m high field-cage (Finch, 1971). When the first fly was observed, the cages were inspected daily and all emerging large narcissus flies were hand-picked from the cage walls, sexed and counted. The flies were active only during sunny periods.

In 1989, a total of 174 flies was recovered from the field cages. The first fly was caught on 22 May, 50% of the flies had emerged by 31 May and the last fly was caught on 16 June. In 1990, 817 flies were recovered from the cages; the first fly was caught on 11 May, 50% of the flies had emerged by 24 May and the last fly was caught on 3 July. Both sexes emerged at more or less the same time.

Threshold temperature for egg-laying

Pairs of adult flies, placed in 220 mm x 120 mm x 70 mm deep ventilated plastic boxes (Conn, 1971), were provided with the standard diet and a narcissus bulb to stimulate oviposition. The boxes were maintained in a series of cooling incubators at 17, 19, 21.5 and 24°C.

Flies at the two lower temperatures did not lay eggs, whereas those at the two higher temperatures laid large numbers of eggs. It was concluded that the temperature threshold for egg-laying by this fly lies between 19-21.5°C.

Base temperature for egg hatch

The basic assumption of the accumulated temperature, or day-degree, concept is that, within limits, the rate of insect development is directly proportional to temperature. Although this is true in the mid-temperature range of insect development, it is not true at low and high temperatures (Sharpe & DeMichele, 1977). Hence, the usual way to determine the base temperature is to record the rate of insect development at about every 2°C over a 20°C range (Baker, 1980) so that several points occur on the linear part of the curve. A linear regression is then fitted to these points only and is extrapolated to provide a theoretical base temperature where the line intercepts the abscissa (Sharpe & DeMichele, 1977; Collier & Finch, 1985). This approach was used in the present study.

To determine the base temperature for egg hatch in the large narcissus fly, batches of 100 freshly-laid eggs were maintained in Petri dishes lined with damp filter papers in cooling incubators (Gallenkamp Ltd., London, England) at 9, 11.5, 14, 16.5, 19, 21.5 and 24°C (all ± 1°C). Three dishes of eggs were used in each incubator and the numbers of eggs that hatched were recorded daily.

The time for 50% egg-hatch ranged from 4.5 days at 24°C to 43.5 days at 9°C. The rates of egg-development are shown in Fig. 1. When extrapolated, the linear regression (r = 0.97; P = 0.05) indicated that the base temperature for egg-hatch in the large narcissus fly was about 8°C.

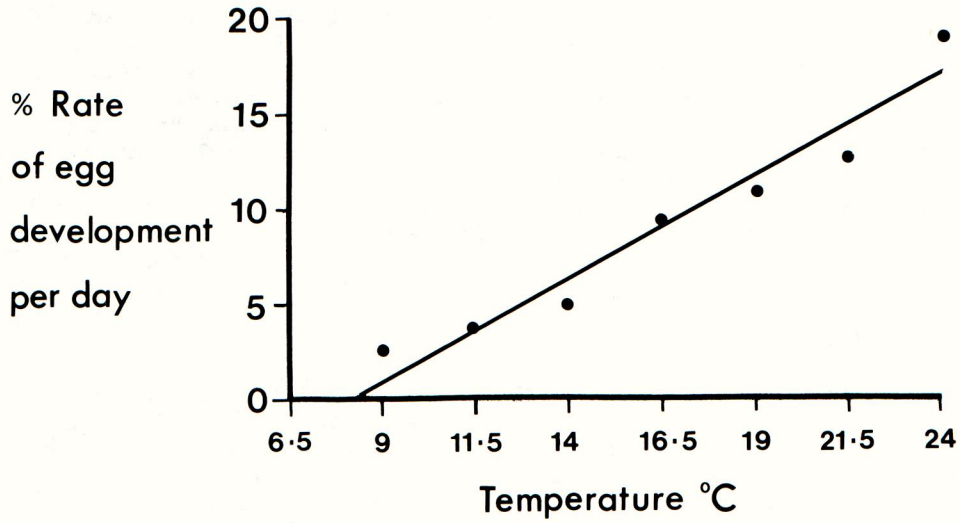

Fig. 1. The rates of development of eggs of the large narcissus fly maintained at 9-24°C.

DISCUSSION

Two major problems face the researcher attempting to develop an insect pest forecast: the need for large numbers of insects and the large amount of space and expensive equipment required. The present study occupies two rooms, each with a floor space of 16 m^2. One room houses ten cooling incubators and the other is used to rear the large numbers of insects required for the experiments. Without such facilities, the time-scale involved for the entire series of experiments required would be prohibitive. Despite the availability of such facilities, problems arise when working at temperatures close to the base temperatures of insect development because of the considerable time required to obtain data. In the present experiments, egg-hatch (the shortest stage in the life-cycle of the insect) took 43.5 days at the lowest temperature tested. The base temperature for the other stages in the life-cycle of the large narcissus fly have not yet been determined, but it is probable that some tests are likely to last as long as a year. In such tests, large numbers of insects have to be included from the start to ensure that sufficient insects survive the protracted period of development to obtain a reasonable estimate of the variation in the rate of development.

It is important, when developing pest forecasting models, to obtain as much field data as possible on the activity of the insect; it takes several seasons to collect sufficient data to validate a model when the pest species being considered has only one generation a year in the field. Collecting weather data is initially not as important. Until the appropriate base temperature has been identified, all that can be recorded of value are daily maximum and minimum temperatures in the locality in which the flies are active. This information can be obtained retrospectively from local standard meteorological stations.

One phase of the life-cycle of the large narcissus fly that needs to be studied intensively is the pre-oviposition period. The present laboratory studies show that, at high-temperatures, newly-emerged females require only 90 day-degrees, under optimum conditions, before they start to lay. Further research is needed to determine: if females can obtain the nutrients for egg maturation as quickly under field as under laboratory conditions; if females can resorb their eggs when conditions become inclement; if the basal temperature for egg maturation is high; and if the female flies are capable of retaining fully-developed eggs for long periods when days with suitable high temperatures and sunny periods do not occur.

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PRE-PLANTING PREDICTION OF STRAWBERRY WILT (*VERTICILLIUM DAHLIAE*) RISK AS AN AID IN DISEASE MANAGEMENT

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ABSTRACT

The soil-borne disease verticillium wilt (*Verticillium dahliae*) is a serious problem of strawberry production in southern Britain. The causal fungus is widespread in soil, and may be present where no strawberries have been grown previously. Chemical control must be implemented before the disease starts to develop, but there is currently no means of knowing whether the risk of wilt is sufficient to justify these measures. A method has been devised for soil analysis that has a maximum sensitivity of 0.1 units of *V. dahliae*/g soil. When this method was used to analyse soils from sites with different levels of strawberry wilt, a good correlation between the amount of *V. dahliae* and the incidence of disease was obtained. The amount of the pathogen in soil corresponding to 5% wilt was about 0.5-1 unit/g. This method of pre-planting soil analysis for *V. dahliae* could therefore be useful in the management of strawberry wilt.

INTRODUCTION

Wilt caused by *Verticillium dahliae* is a serious problem of strawberry cultivation in Britain, particularly on light soils in the south where most of the crop is grown, and affects fruiting and runner beds (Keyworth and Bennett, 1951). The disease has become more significant in the last decade because of the increasing dependence of growers on cultivars with good agronomic qualities but which are very susceptible to *V. dahliae* (Harris, 1989).

Strawberries may be produced intensively on smallholdings or on larger farms as part of a rotation with other crops. Runner production is a highly specialised activity and, in order to meet the requirements of the Ministry of Agriculture, Fisheries and Food Plant Health Propagation Scheme (MAFF PHPS), growers may be obliged to rent or buy new land for runner production.

The severity of wilt in strawberries depends on several factors: the susceptibility of the cultivar; the soil type; the weather conditions in the first growing season; but the cardinal factor is the amount of inoculum in the soil. Although *V. dahliae* may be disseminated with runners, all available evidence indicates that infested soil is the most important immediate source of inoculum (Keyworth and Bennett, 1951).

V. dahliae has a wide host range among crop and weed species and produces long-lived survival structures (microsclerotia). Once infested

with the pathogen, land may remain a source of wilt disease for many years. It is one of the few soil-borne diseases of strawberry which can be present where no strawberries have previously been grown. Little is known of the effects of cropping practices on the longevity of V. dahliae in Britain, but it is well established that serious wilt problems in strawberries frequently occur on sites with a history of potato cultivation. In general, however, strawberry growers have no knowledge of the degree of infestation of a site before strawberries are planted and therefore of the potential wilt hazard.

Methods of control currently used for strawberry wilt are: chemical disinfestation of soil (Harris, 1989; 1990); drenching plants with carbendazim generating fungicides (Talboys et al., 1976); or crop rotation. Whichever method is employed, a knowledge of the danger from wilt associated with any site before planting would be valuable in managing the disease.

This paper describes work on the pre-planting analysis of soil for V. dahliae as a means of predicting strawberry wilt risk.

MATERIALS AND METHODS

Soil samples

During 1988 samples were collected by officers of the Ministry of Agriculture, Fisheries and Food Agricultural Development and Advisory Service (ADAS) and growers from sites over southern England with various histories of verticillium wilt. Each sample was a bulk of sub-samples taken across the site. During 1989, samples were obtained by ADAS officers from a number of sites where wilt susceptible cultivars were being propagated. The samples were bulked from sub-samples taken with a 2 cm diameter coring auger down to 20 cm on a 20 x 10 m grid pattern.

Soil preparation for analysis

Bulked samples were air-dried, ground and passed through a 2 mm diameter sieve. Refined samples were thoroughly mixed and stored outside in a polythene bag until analysed.

Estimating V. dahliae in soil

An agar plate method for enumerating V. dahliae in soil was used. Procedures and media described in the literature for this purpose were evaluated for sensitivity and reproducibility in a series of preliminary experiments, as a result of which the following technique was adopted. Twenty-five g of soil were thoroughly dispersed by shaking in 100 ml sterile distilled water and the size fraction 20-160 µm was recovered by washing through nested sieves. This fraction was resuspended in 50 ml distilled water and 1 ml aliquots were dispensed to each of 20 Petri dishes containing a semi-selective medium based on that of Isaac et al. (1971). Free moisture was removed from the plates by surface drying and the plates were incubated at 22°C for 4 weeks. Plates were then washed, drained and scanned under a dissecting microscope for colonies of V. dahliae which were distinctive because of the presence and

disposition of microsclerotia. The sensitivity end-point of the procedure is 0.1 colony forming units (cfus)/g soil.

The identity of a proportion of colonies on the soil plates of the 1989 samples identified presumptively as V. dahliae were confirmed by a procedure in which individual microsclerotia were extracted from the agar, surface sterilised and plated on prune lactose agar. The pathogenicity of these isolates to strawberry is currently under test.

Incidence of verticillium wilt

For the 1988 samples the site of origin was classified arbitrarily by the ADAS officer or grower as having a recent history of little or no wilt, moderate wilt or severe wilt. There were five sites in each category. In 1989, wilt incidence in the runner sites was determined by surveying the whole area and counting the number of mother plants with unequivocal wilt symptoms. The percentage of plants affected by wilt was determined from the planting density and the area of the site. The crops at sites 6 and 7 were so badly affected by wilt that the plants were ploughed in before records could be taken: the wilt incidence was estimated to be about 90%.

RESULTS AND DISCUSSION

The results of analyses of the 1988 samples are summarised in Figure 1.

There was a good correlation between the amount of V. dahliae in the sample and the wilt history of the site, suggesting that the soil analysis may have good predictive value. The results also indicate that the inoculum threshold for significant wilt is about 1 cfu/g soil which is well above the level of sensitivity of detection.

The results of soil analyses and wilt incidence in runner production sites in 1989 are shown in Table 1.

For predictive purposes the relationship between the amount of V. dahliae in soil and wilt incidence at critical thresholds must be established. The 5% disease level is a practical threshold in this context because it is the maximum permissible incidence for certification of runners under the MAFF PHPS, and it is a level above which control measures become cost-effective for fruiting crops. There are too few data from runner sites at the intermediate levels of wilt incidence to define with any confidence the relationship between wilt incidence and the amount of V. dahliae in soil over the critical range. However, V. dahliae was detected where wilt incidence was 2% or more in the crop, with one exception. There is as yet no explanation for the failure to detect V. dahliae in soil from site 2, or for the comparatively small amount of the pathogen in soil from site 3. These sites were subdivisions of one large field. The critical level of infestation for economic wilt taken overall seems to be between 0.5 and 1 cfu/g soil.

The ability to determine V. dahliae in soil greatly increases the options for management of strawberry wilt. A runner producer can screen

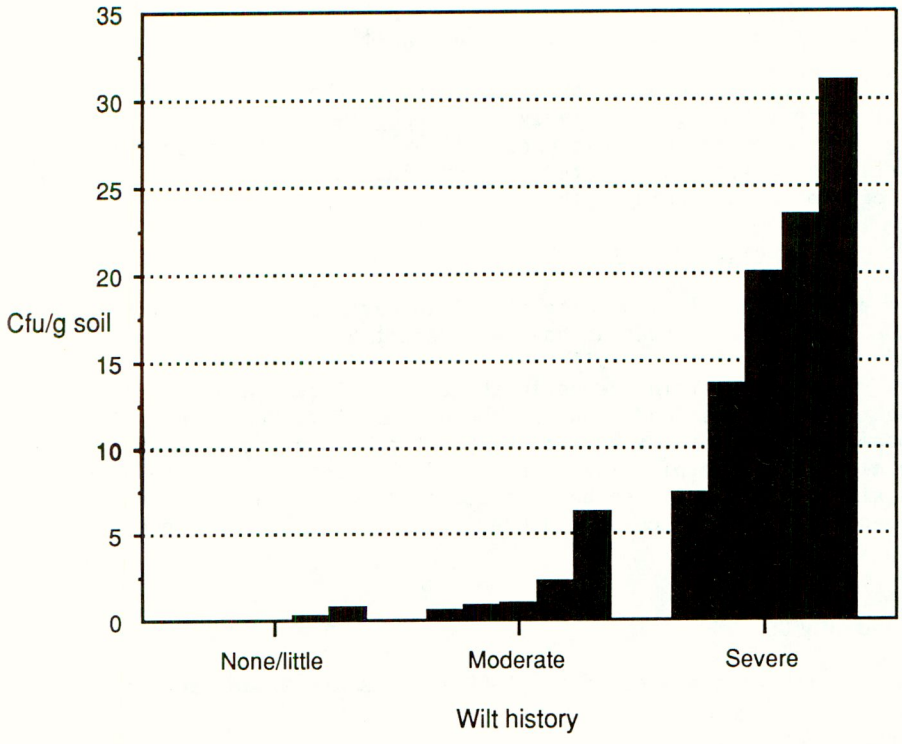

FIGURE 1. The relationship between recent strawberry wilt history of sites and the amount of V. dahliae in soil samples from the sites.

several possible sites and select the one with the least wilt risk. Where only one site is available, the need for chemical treatment can be determined in good time. The effectiveness of soil disinfestation treatments may also be ascertained before planting. Moreover, growers who prefer to use crop rotation for control can determine whether the pathogen has declined sufficiently for susceptible strawberry cultivars to be grown safely.

Clearly the pre-planting soil analysis for wilt described here can give no more than an indication of the danger of significant disease in a situation which favours wilt, that is, where soil type and weather conditions are suitable. However, if sufficient data are obtained, it may be possible to define separate critical levels of infestation for cultivars of intermediate and high susceptibility. Further studies are required to validate the soil analysis method as a means of detecting and quantifying a wilt hazard before planting strawberries. If they are successful, a service could be made available to strawberry growers in Britain through the Agricultural Development and Advisory Service. The value of many strawberry crops is sufficient to make the cost of such a service a good investment for those growing wilt susceptible cultivars.

TABLE 1. Wilt incidence and the amount of V. dahliae in soil at runner production sites.

Site no.	% wilt	cfu V. dahliae/g soil
20	0.0	0.0
12	0.1	0.2
15	0.1	0.0
19	0.1	0.1
13	0.4	0.0
11	0.6	0.1
16	0.9	0.0
4	1.0	0.1
14	1.2	0.0
23	1.5	0.0
5	1.7	0.1
9	2.0	0.5
10	3.4	1.6
8	4.6	0.5
3	14.0	0.6
2	18.3	0.0
22	25.1	6.1
7	90.0	17.3
6	90.0	34.1

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SEEDLING DISEASE OF TURF GRASSES CAUSED BY *FUSARIUM CULMORUM* AND *CLADOCHYTRIUM CAESPITIS* AND THEIR CONTROL BY FUNGICIDE SEED TREATMENTS

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ABSTRACT

In a series of field trials and laboratory investigations over a three year period seedling diseases, caused mainly by *Fusarium culmorum* and *Cladochytrium caespitis* together as a disease complex, were recorded on a total of 21 host turfgrasses. *Cladochytrium caespitis* was identified as a little-recognised zoosporic fungus pathogenic only in turfgrass and responsible for considerable stand reductions. Variations in susceptibility of cultivars of *Poa pratensis* to attack by these pathogens were also recorded. In initial experiments, benomyl seed treatment reduced disease by *F. culmorum* but increased disease by *Cladochytrium* spp. However, in a series of three field trials in 1989/90 combinations of thiabendazole with metalaxyl (APRON T) gave effective control of both *F. culmorum* and *C. caespitis*, and significantly increased seedling establishment.

INTRODUCTION

In newly-seeded turf, losses may occur due to seedling disease at both the pre-emergence and post-emergence stages of seedling growth (Smith *et al.*, 1989). Whilst a well prepared seedbed can do much to prevent seedling disease, significant stand reductions may occur when seed germination and seedling growth is slow, e.g. due to low soil temperatures experienced in early-spring or late-autumn sowings (Baldwin, 1987). Several studies (Smith 1955, 1956, 1957) have indicated that fungicide seed treatments may control pre-emergence seedling disease. The effect of fungicide seed treatments on the emergence and establishment of agricultural grasses has also been investigated (Clements *et al.* 1982, Holmes 1976, Lewis 1987, 1988). In this paper observations are reported on the host range of *Cladochytrium caespitis*, a little recognised zoosporic fungus capable of causing seedling disease and on variation in the susceptibility of cultivars of *Poa pratensis* to *C. caespitis* and *Fusarium culmorum*. A series of field trials to determine the effect of fungicide seed treatments on post-emergence seedling disease of *Agrostis castellana* and *P. pratensis* are described and the role of a broad-spectrum fungicide seed treatment for sports turf and amenity grassland is discussed.

MATERIALS AND METHODS

Field trials were undertaken either on the STRI experiment ground (NGR SE 095 391, altitude 200 m, sandy loam soil pH 5.5) or the Cullingworth trials site (NGR SE 073 372, altitude 160 m, loam soil pH 6.1).

Host range of *Cladochytrium caespitis*.

On 28 April 1989 (STRI trial) and 9 September 1989 (Cullingworth trial) experimental

plots (1 m^2, 4 replicates per treatment, fully randomised design) were marked out on a prepared seedbed and sown according to the details in Table 1. All plots were examined daily for seedling emergence and disease. *C. caespitis* as the causal pathogen was confirmed by optical microscopy according to Cook (1934), and only recorded if resting sporangia of the fungus were observed in seedlings from all replicate plots at least at one of the field sites. Surface sterilised stem base or root tissue of each diseased turfgrass containing *C. caespitis* sporangia was plated onto Potato Dextrose Agar to attempt to isolate any other pathogen. Detailed methodology is given in Baldwin (1990).

TABLE 1. Turfgrasses assessed for susceptibility to *Cladochytrium caespitis*.

Turfgrass species	Cultivar	Seed rate (kg ha^{-1})
Agrostis canina ssp. *canina*	Kingstown	100
A. canina ssp. *montana*	–	100
A. castellana	Highland	100
A. stolonifera	Carmen	100
A. tenuis	Bardot	100
Festuca arundinacea	Bartes	250
F. ovina	Barok	250
F. pratensis	Senu	250
F. rubra ssp. *commutata*	Bellamy	250
F. rubra ssp. *litoralis*	Polar	250
F. rubra ssp. *rubra*	Moncorde	250
F. tenuifolia	Commercial seed	250
Lolium perenne	Mondial	300
Phleum pratense	Barvanti	250
P. bertolonii	Aberystwyth S48	250
Poa annua	Commercial seed	250
P. compressa	Canon	250
P. nemoralis	Barnemo	250
P. pratensis	Ampellia	250
P. trivialis	Ino	250

Susceptibility of *Poa pratensis* cultivars to post-emergence seedling disease by *Cladochytrium caespitis* and *Fusarium culmorum*.

On 17 August 1988, a field experiment was sown on the STRI experiment ground consisting of 30 *P. pratensis* cultivars in 1.5 m^2 plots, 3 replicates per treatment, arranged in a randomised block design using a seed rate of 300 kg ha^{-1}. The percentage area of each plot affected by seedling disease was assessed visually. Post-emergence seedling disease appears as chlorotic seedlings, rotting at their base to form dead individual patches 20-30 mm in diameter. Analysis of variance was applied to logit transformations of the data and least significant differences were calculated using the Waller & Duncan (1969) range test and is quoted for an error-weight ratio of k = 100:1. For 10 of the 30 cultivars, 20 seedlings per plot that exhibited typical disease symptoms were selected randomly. The incidences of *F. culmorum* and *Cladochytrium* spp. on rotted stem bases were determined by mounting 10 seedlings in lactophenol cotton blue and assessment by optical microscopy. Identification of *F.culmorum* and *Cladochytrium* spp. was based on the presence of typical conidia and resting

sporangia respectively. The remaining 10 seedlings were floated on sterile distilled water in sterile Petri dishes for five days at room temperature and emerging *Pythium* and *Rhizoctonia* spp. were identified microscopically by the presence of typical sporangia and mycelium respectively. Analyses of variance were performed on the percentage incidence of *F. culmorum* and *Cladochytrium* spp. following angular transformation. No analysis of the incidences of *Pythium* and *Rhizoctonia* spp. was possible due to the infrequent occurrence of these two pathogens.

Comparison of fungicide seed treatments for control of post-emergence seedling disease.

In a preliminary experiment benomyl was evaluated for effects on post-emergence seedling disease of *A. castellana* and *P. pratensis* (Baldwin 1989). For the main trials, *A. castellana* cv. Highland and *P. pratensis* cv. Cynthia seeds were polymer-coated with the following fungicides.

Treatment	Dose (g a.i./100 kg seed)
1. Control	Untreated
2. Thiabendazole	37
3. Thiram	30
4. Metalaxyl	70
5. Thiabendazole plus metalaxyl	37 + 70
6. Thiabendazole plus metalaxyl plus thiram	37 + 70 + 30

Trials 1 and 3 were undertaken on the STRI experiment ground and trial 2 was undertaken on the Cullingworth experiment ground. For all trials, a seedbed was prepared and plots were marked out, 5 replicates per treatment, 1 m^2 in a randomised block design. Seeds were hand sown (Table 2) at rates of 150 kg ha^{-1} for *A. castellana* and 350 kg ha^{-1} for *P. pratensis*. Plots were examined every few days for emergence and signs of phytotoxicity, and post-emergence damping-off was assessed as percentage plot area affected. The number of viable seedlings per plot was determined by taking three, 50 mm diameter soil cores randomly from each plot and counting the number of shoots 88 days after emergence.

In the control plots of each trial, causing post-emergence damping-off were investigated. The presence of *Cladochytrium* spp. and *F. culmorum* on rotted stem bases was determined by mounting seedlings in lactophenol cotton blue and making observations by optical microscopy. *Cladochytrium* spp. and *F. culmorum* were identified as described previously.

RESULTS

TABLE 2. Sowing and emergence dates, days from sowing to emergence and to disease appearance.

	Sowing date (1989)	Emergence date (1989)	Disease observed date	Disease observed days after emergence
Trial 1	21 September	9 October	20 November	42
Trial 2	25 September	12 October	15 December	54
Trial 3	4 October	23 October	20 November	28

Host range of *Cladochytrium caespitis.*

C. caespitis resting sporangia were recorded in root or stem base tissue from all turf grasses listed in Table 1. Optical microscopy of diseased seedlings containing *C. caespitis* resting sporangia generally did not also detect the presence of *F. culmorum* mycelia or conidia. Also, isolations made from tissue containing *C. caespitis* resting sporangia did not yield *F. culmorum* or any other recognised turfgrass pathogen. Thus, *C. caespitis* was recorded as the primary pathogen responsible for seedling death and not as a saprophyte on dead or senescing plant tissue. No attempt was made to assess visually the severity of post-emergence damping-off by *C. caespitis*.

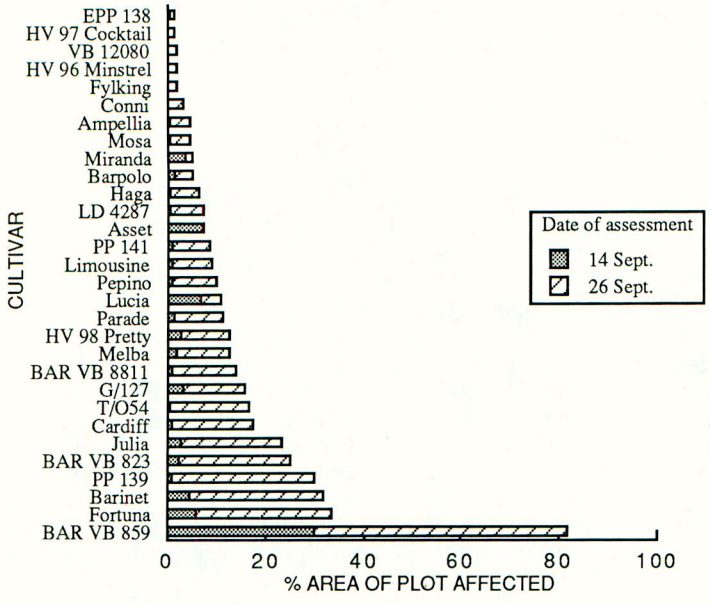

FIGURE 1. Severity of post-emergence damping-off on different cultivars of *Poa pratensis* on two assessment dates.

The seed lots representing the *P. pratensis* cultivars differed significantly with respect to post-emergence damping-off, although the ranking order of increasing susceptibility varied depending on the assessment date (Figure 1.).

Microscopical examination of shoots showing typical symptoms of post-emergence damping-off revealed either *F. culmorum* or *Cladochytrium* spp. in over 95% of cases. On most shoots, only one pathogen was detected per shoot although this was not always the case, resulting in the totals shown in Figure 2 exceeding 10 shoots. There were significant differences in the relative incidences of *F. culmorum* and *Cladochytrium* spp. on diseased shoots. Cultivars with a lower incidence of one pathogen tended to have a higher incidence of the other.

TABLE 3. Effect of fungicide seed treatment on the plot area affected by post-emergence seedling disease. *Agrostis castellana*

	% Plot area affected by post-emergence seedling disease								
	Trial 1			Trial 2			Trial 3		
Assessment date	20 Nov. 1989	21 Dec. 1989	4 Jan. 1990	28 Nov. 1989	7 Dec. 1989	4 Jan. 1990	15 Dec. 1989	21 Dec. 1989	28 Dec. 1989
Control	16.8	15.0	8.4	3.4	4.0	0.0	1.4	0.8	0.0
Thiabendazole	8.0	13.6	8.8	5.0	5.0	0.0	1.2	0.8	0.0
Thiram	12.4	10.4	7.2	5.4	5.6	0.0	1.4	0.8	0.0
Metalaxyl	0.4	1.2	1.2	0.2	0.2	0.0	0.0	0.0	0.0
Thiabendazole + metalaxyl	0.4	1.4	0.8	0.0	0.0	0.0	0.0	0.0	0.0
Thiabendazole + metalaxyl + thiram	0.0	1.4	0.6	0.0	0.0	0.0	0.0	0.0	0.0
LSD (P =0.05)	7.8	5.5	3.0	3.1	3.2	-	1.6	1.6	-

TABLE 4. Effect of fungicide seed treatment on the plot area affected by post-emergence seedling disease. *Poa pratensis*

	% Plot area affected by post-emergence seedling disease								
	Trial 1			Trial 2			Trial 3		
Assessment date	20 Nov. 1989	28 Dec. 1989	4 Jan. 1990	28 Nov. 1989	28 Dec. 1989	4 Jan. 1990	15 Dec. 1989	28 Dec. 1989	11 Jan. 1989
Control	19.4	32.8	32.8	16.8	32.0	40.6	5.6	7.2	8.6
Thiabendazole	13.4	40.6	34.4	11.4	33.2	37.9	15.6	26.0	25.4
Thiram	21.0	52.6	52.6	13.0	30.2	33.0	6.8	6.6	6.4
Metalaxyl	1.0	7.4	9.8	0.0	18.8	28.4	0.0	0.0	0.2
Thiabendazole + metalaxyl	3.0	7.0	11.6	0.0	15.6	24.0	0.0	0.0	1.6
Thiabendazole + metalaxyl + thiram	0.8	9.8	15.0	0.0	16.2	26.0	0.0	0.2	1.4
LSD (P =0.05)	10.7	18.0	23.5	7.8	12.5	16.5	7.2	6.4	6.5

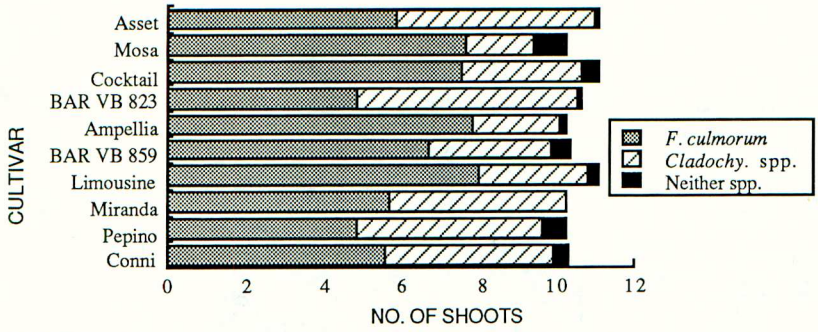

FIGURE 2. Mean incidence of *Fusarium culmorum* and *Cladochytrium* spp. on ten diseased shoots of *Poa pratensis* cultivars.

TABLE 5. Effect of fungicide seed treatments on seedling establishment of *Agrostis castellana*

FUNGICIDE	Number of seedlings m^{-2} Trial 1 4 Jan. 1990	Trial 2 8 Jan. 1990	Trial 3 19 Jan. 1990
Nil	39888	20095	33876
Thiabendazole	36118	29543	36475
Thiram	34895	21368	39002
Metalaxyl	48802	37546	50127
Thiabendazole + metalaxyl	37595	51351	54865
Thiabendazole + metalaxyl + thiram	39002	41878	50586
LSD ($P = 0.05$)	19604	15755	15178

TABLE 6. Effect of fungicide seed treatments on seedling establishment of *Poa pratensis*

FUNGICIDE	Number of seedlings m^{-2} Trial 1 4 Jan. 1990	Trial 2 8 Jan. 1990	Trial 3 19 Jan. 1990
Nil	31737	29224	27967
Thiabendazole	36576	27144	23281
Thiram	32246	26507	26694
Metalaxyl	71625	29690	30412
Thiabendazole + metalaxyl	61335	28686	31635
Thiabendazole + metalaxyl + thiram	57157	27242	30311
LSD ($P = 0.05$)	17409	14713	10877

Comparison of fungicide seed treatments for control of post-emergence seedling disease.

Metalaxyl seed treatments both alone and in combination with thiabendazole and thiram significantly reduced post-emergence seedling disease of *A. castellana* in trials 1 and 2 and *P. pratensis* in trials 1, 2 and 3 (Tables 3 and 4). There were no significant reductions in post-emergence seedling disease between control and thiram treatments for either test grass in any trial. Thiabendazole significantly reduced disease of *A. castellana* on one assessment date (20 Nov. 1989) in trial 1 only. Thiabendazole significantly increased disease of *P. pratensis* in trial

3 only.

Seed treatments containing metalaxyl significantly increased the number of *A. castellana* seedlings m^{-2} in trials 2 and 3 (Table 5) and significantly increased the number of *P. pratensis* seedlings m^{-2} in trial 1 (Table 6).

DISCUSSION

Cladochytrium caespitis has previously been described as a pathogen of grass seedlings (Griffon & Maublanc 1910) and there is also a record of a disease affecting a newly sown lawn caused by *Cladochytrium graminis* Büsgen (Massee 1913). Evidence presented by Cook (1934) indicates that, after a re-examination of material, the fungus described by Massee was in fact *C. caespitis*. To date, *C. graminis* has not been recorded in the UK. Observations made in the present study on resting spore shape and dimensions, wall thickness, colour and ability to absorb cotton blue stain are consistent with those described by Cook (1934). In the present study, the microscopic and macroscopic symptoms and diagnostic features of the disease on a range of seedling turfgrasses are described, and a tentative host range for *C. caespitis* is presented. Smith & Jackson (1965) recorded post-emergence seedling disease by *Cladochytrium* spp. on *A. canina* ssp. *canina, A. cannina* ssp. *montana, A. tenuis, Cynosurus cristatus* L., *F. rubra* ssp. *rubra, F. rubra* ssp. *commutata, F. tenuifolia, L. perenne, Phleum bertolonii, Poa pratensis* and *Poa trivialis*. *C. caespitis* was also recorded on these turfgrasses in the present study, with the exception of *Cynosurus cristatus* which was not included in the field trials. Additionally, *C. caespitis* is first reported on *A. castellana, A. stolonifera, F. arundinacea, F. pratensis, P. annua, P. compressa, P. pratense and P. nemoralis*.

The high proportion of plot area affected by post-emergence seedling disease on some *P. pratensis* cultivars was possibly due to the damp conditions following sowing (Baldwin 1987). These results are consistent with the reports of Smith (1958) and Shildrick (1970) which showed that seed lots representing different cultivars differed in the severity of post-emergence seedling disease. Only cv. Fylking, however, was common to both this investigation and a previous study (Shildrick 1970) and in both experiments this cultivar was not severely affected by post-emergence seedling disease. The differences in cultivar ranking of increasing susceptibility to post-emergence seedling disease between the two assessments of percentage of plot area affected, indicates that cultivars may become resistant to seedling disease at different times. For example, cv. Asset showed relatively severe disease on 14 September but this increased very little over the next 12 days, which contrasted with many other cultivars where disease increased substantially. The differing relative incidence of *Cladochytrium* and *Fusarium* spp. on diseased seedlings suggests that cultivars may show different relative susceptibilities on soils where the two pathogens occur in different proportions. This may explain, for example, why Smith (1958) found cv. Merion to be moderately resistant to seedling disease caused by *Cladochytrium* spp. while Shildrick (1970) observed the same cultivar to exhibit a relatively large degree of damping-off. In the initial benomyl seed treatment experiments (Baldwin 1989) the fungicide caused a significant increase in post-emergence seedling disease and a corresponding increase in the incidence of *Cladochytrium* spp.. The most prominent feature of these preliminary trials was the increase in post-emergence seedling disease of seedlings from benomyl treated seed and the ineffectiveness of benomyl, despite it being recorded as active against *Fusarium* spp., and there being previous reports of control (Lewis 1987, 1988). However, in this study, increases in post-emergence seedling disease were associated with increases in the incidence of *Cladochytrium* spp. and, according to Bollen & Fuchs (1970), benomyl is not effective against zoosporic fungi such as *Cladochytrium* spp.. Previous studies evaluating benomyl for control of post-emergence seedling disease (Lewis 1987, 1988) have identified *Fusarium* spp. as the major pathogen with *Cladochytrium* spp. not recorded. Effective control of post-emergence seedling diseases was achieved by seed treatments consisting of combinations of thiabenndazole with metalaxyl to control *Fusarium* spp. and *Cladochytrium* spp. respectively. In the trials, thiabendazole only did not give consistent

disease control, and in one case it increased disease severity. The importance of the combination of fungicide active ingredients is thus emphasised. In these trials, disease first appeared 4-6 weeks after seedling emergence and effective control was achieved by combinations of systemic fungicides. In contrast, the protectant non-systemic fungicide thiram did not have any effect on disease incidence.

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