FUNGAL ROOT PATHOGENS IN PASTURE SOILS, AND EFFECTS OF FUNGICIDES

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SUMMARY

Red clover (*Trifolium pratense*) seedlings grown in pots in field soil containing pathogenic fungi were used to study effects of seed- and soil-applications of benomyl and metalaxyl fungicides on root diseases. Treatments containing metalaxyl (active against *Pythium* spp.) increased seedling emergence and reduced post-emergence damping-off. Seed treatment with benomyl increased seedling mortality, probably by inhibiting fungi antagonistic to *Pythium* spp. Soil treatments containing benomyl reduced infection of roots by benomyl-sensitive fungi including red clover pathogens (*Fusarium solani*, *F. oxysporum* and *Verticillium dahliae*). Pot tests can be useful for monitoring effects of fungicides applied to pasture.

INTRODUCTION

Recent work comparing establishment of forage plant seedlings from fungicide-treated and untreated seed has demonstrated the presence and importance of fungal root pathogens in New Zealand agricultural soils (Falloon 1981; Falloon and Skipp 1982). Soil fungi also invade roots of plants in established pasture (Skipp *et al* 1985) and may contribute to poor persistence and sub-optimal production (Menzies 1973; Labruère 1979). Fungal root diseases of pasture plants are difficult to study because disease symptoms are often indistinct, or may be obscured by the senescent decay of root tissues. There are also problems in measuring effects of root disease on herbage production in mixed species pastures. Furthermore, populations of soilborne fungi, and root disease levels, cannot easily be manipulated or monitored.

Fungicides have been applied to pastures in recent field trials to study effects of foliage and/or soilborne pathogens (Labruère 1979; Clements *et al* 1985; Watson *et al* 1985). Information from field trials can be increased by using several fungicides each active against different fungal pathogens (e.g. metalaxyl to control Oomycetes and benomyl to control Deuteromycetes (Greenhalgh and Clarke 1985)). However since some fungicides become bound to organic matter they may not reach roots growing in soil beneath pasture. Methods are required to monitor fungicide penetration of pasture soil, and the effect of fungicides on root infection by different pathogens.

Pot experiments with seedlings grown in field soil in the glasshouse have been used to detect root-invading fungi and nematodes in pasture soils (Skipp and Christensen 1981) and to study the effectiveness of fungicide seed treatments for controlling seedling diseases of forage plants (Falloon *et al* 1986). They could also help interpret effects of field applications of fungicides.

Many potentially damaging root-invading fungi have been detected in roots of white clover and ryegrass from New Zealand pastures (Skipp *et al* 1985 and unpubl.). The fungi included *Chalara elegans* (= *Thielaviopsis basicola*), *Codinæa fertilis*, *Fusarium* spp., *Gaeumannomyces graminis*, *Pythium* spp., and *Thanatephorus cucumeris* (= *Rhizoctonia solani*). None is known to cause a specific, easily-recognisable disease of white clover or ryegrass roots. However, several clearly characterised root diseases of red clover were found on plants from field plots examined during studies on poor persistence in a plant breeder's evaluation block at Palmerston North (Skipp *et al* 1986). In these plots *Pythium* spp. caused seedling death, *C. elegans* caused a black cortical rot of tap roots, and *Verticillium dahliae*, *F. solani*, *F.*

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oxysporum and *Cylindrocladium scoparium* were associated with browning of the root stele (*Pythium* spp. are Oomycetes whereas the other pathogens are Deuteromycetes).

This paper describes experiments with red clover seedlings grown, in pots, in soil from the plant breeder's red clover evaluation block, which examined the effects of treatment of seed and field soil (*in situ* and in pots) with the fungicides metalaxyl and benomyl. It illustrates how simple pot tests can be used to monitor effects of fungicides on fungal pathogens in soil.

METHOD

Soil obtained from trial plots at DSIR Grasslands Division, Palmerston North was used in three pot experiments to detect effects of fungicide treatments on red clover seedling root disease. The trial plots (11.4 m²; four replications per treatment) had been sown with 'Grasslands Hamua' red clover and had received either a fungicide drench containing benomyl (1.0 g ai/m² as Benlate 50WP) and metalaxyl (0.15 g ai/m² as Ridomil 25WP) in 4 litres of water/m², or water only, on 16.2.84, 3.4.84, and 17.5.84.

Unperforated plastic pots (65 mm diam. x 95 mm) were filled with soil from samples collected from the top 100 mm of plots and sown with 20 seeds of 'Grasslands Hamua' red clover (laboratory germination 95%) to a depth of 10 mm. Ten replicate pots were prepared per treatment and placed in thermostatically controlled water baths in a glasshouse (14-24 °C air temperature). Pots were weighed daily and water added to adjust pots to a pre-determined percentage of soil water holding capacity (PHC).

Seedlings showing post-emergence damping-off were removed from pots and counted. At the end of experiments, surviving seedlings were washed free of soil, counted and examined with a stereomicroscope (x 40 mag.) for symptoms of root disease. Numbers of seedlings per pot which emerged (final no. present + no. damped-off), damped-off, or showed root lesions were converted to a percentage of viable seed sown and analysed after arcsine transformation.

Methods described by Skipp *et al* (1986) were used to isolate fungal colonies on antibiotic potato dextrose agar at 20 °C from washed (three changes of sterile water) roots of damped-off seedlings, and from surface-sterilised (0.3% NaHClO₃ for 1 min) roots of representative samples of surviving seedlings.

Individual experiments were conducted as follows:—

1. Effects of fungicide seed treatment on seedling root disease

Benomyl and metalaxyl (formulated as above) were applied as single (3 g ai/kg seed), or combined (1.5 g ai/kg seed each fungicide), treatments to red clover seed. Pots of soil from untreated plots were sown with fungicide-treated or untreated seed and maintained for 2 weeks at 80% PHC in water baths at 15, 20, and 25 °C.

2. Effects of fungicide treatment of field plots on seedling root disease

Soil from fungicide-treated and untreated field plots was collected on 16.5.84 and 18.5.84. Ten pots were filled with soil for each treatment/collection date, sown with untreated red clover seed and maintained for 6 weeks at 80% PHC in a water bath at 20 °C.

3. Effects of fungicide treatment of soil in pots on seedling root fungi

Forty pots of soil from untreated plots were sown with untreated red clover seed and maintained at 60% PHC in a water bath at 20 °C. Half the pots were treated with a drench containing benomyl and metalaxyl (1.0 and 0.15 g ai/m² of soil surface) immediately after sowing and 5 weeks later; the remaining pots received no fungicide treatment. Seedlings were harvested from 10 pots of fungicide-treated soil and 10 pots of untreated soil 3 and 6 weeks after sowing. After each harvest, 10 root segments from each of 60 randomly chosen seedlings per treatment were plated for fungal isolation. The percentage of segments yielding colonies of particular fungi were analysed after arcsine transformation.

RESULTS AND DISCUSSION

1. Effects of fungicide seed treatment on seedling root disease

Treatments containing metalaxyl increased emergence and reduced post-emergence damping-off whereas without metalaxyl, benomyl seed-treatment reduced emergence

TABLE 1: Effect of fungicide seed treatments on emergence and damping-off of red clover seedlings grown in pots of field plot soil at three temperatures for 2 weeks.

Fungicide treatment	% seedlings emerged			% seedlings damped-off		
	15 °C	20 °C	25 °C	15 °C	20 °C	25 °C
untreated	70	66	41	8	14	15
benomyl	53**	48**	30*	16**	18	11
metalaxyl	81	89**	88**	1**	0**	0**
benomyl + metalaxyl	85*	88**	93**	0**	0**	0**

* and ** indicates significant differences between seedlings grown from fungicide-treated and untreated seed ($P < 0.05$ and $P < 0.01$).

and increased post-emergence damping-off (15 °C) (Table 1). This indicated that metalaxyl treatments controlled the fungi which killed emerging seedlings while benomyl treatments resulted in increased activity by these pathogens.

Fewer seedlings from untreated seed emerged at 25 °C than at 15 and 20 °C ($P < 0.01$) and more damped-off at 20 and 25 °C than at 15 °C ($P < 0.05$); soil temperature did not affect emergence or damping-off of seedlings grown from metalaxyl-treated seed.

Twenty of 55 fungal isolates obtained from roots of damped-off seedlings from untreated and benomyl-treated seed were *Pythium ultimum*, 31 were sphaerosporangiate *Pythium* spp., and four were sterile in culture.

The effectiveness of metalaxyl in controlling damping-off of red clover seedlings caused by *Pythium* spp. in this soil confirms findings of previous experiments using combined benomyl/metalaxyl seed treatments (Skipp *et al* 1986). Increased seedling disease resulting from benomyl treatment presumably occurred because soil fungi antagonistic to *Pythium* spp. were inhibited by this chemical. Results also indicate that the sensitivity of pot tests for detecting the presence of root pathogens (e.g. *Pythium* spp.) in soil, and the selective effect of fungicides, may be increased by using high soil temperatures (25 °C).

2. Effects of fungicide treatment of field plots on seedling root disease

Applications of benomyl/metalaxyl drenches to field plots increased emergence, and decreased damping-off and root necrosis, of seedlings grown in pots of the treated soil (Table 2). These effects were demonstrated using soil collected from plots 6 weeks after the second fungicide application and 1 day after the third fungicide application. Root necrosis on seedlings examined at the end of the experiment was similar to tap root rotting seen in damped-off seedlings or dark cortical rots in which chlamydospores of *C. elegans* were seen.

TABLE 2: Effect of fungicide treatment of plots on emergence and root disease of red clover seedlings grown in pots of field plot soil at 20 °C for 6 weeks.

Date last treatment	Date soil collected	Drench applied	Percentage seedlings		
			emerged	damped-off	with root necrosis
3.4.84	16.5.84	water	55	13	24
		benomyl + metalaxyl	75**	2**	10*
17.5.84	18.5.84	water	58	17	24
		benomyl + metalaxyl	85**	0**	2**

* and ** indicates significant differences between seedlings grown in soil collected on the same date from fungicide-treated and untreated plots ($P < 0.05$ and $P < 0.01$).

TABLE 3: Effect of fungicide soil treatment on fungi present in primary roots of red clover seedlings grown in pots of field plot soil at 20 °C for 3 or 6 weeks.

Fungus	% root segments yielding fungal colonies			
	3 weeks		6 weeks	
	untreated	treated	untreated	treated
<i>Chalara elegans</i>	2.6	2.0	3.7	1.5
<i>Cylindrocladium scoparium</i>	0	0	0	0.3
<i>Fusarium oxysporum</i>	7.3	3.3**	13.0	2.7**
<i>F. solani</i>	4.1	1.8*	6.6	2.2**
<i>Verticillium dahliae</i>	1.3	0*	2.3	2.8
Total	23.4	11.2**	42.5	20.3**

* and ** indicates significant differences between seedlings grown in fungicide-treated and untreated soil, at the same harvest date ($P < 0.05$ and $P < 0.01$).

These results demonstrate the effects of metalaxyl in reducing damping-off and tap root rots caused by *Pythium* spp., and of benomyl in reducing root lesions caused by *C. elegans*. The effect of fungicide treatment of plots persisted in soil for at least 6 weeks.

Effects of fungicide treatment have persisted for at least 3 months in other pot experiments (Skipp and Watson unpubl.), which used seedlings of lucerne in pasture soil to detect effects of metalaxyl on *Pythium* spp. at a series of trial sites (Watson *et al* 1985).

3. Effects of fungicide treatment of soil in pots on seedling root fungi

The red clover pathogens, *F. oxysporum*, *F. solani*, and *V. dahliae* were isolated less frequently from roots of plants grown in soil treated with benomyl and metalaxyl than from roots from untreated soil (Table 3).

Detection of effects of soil-applied fungicides on infection of roots by fungi sensitive to benomyl, rather than metalaxyl, required the use of techniques involving the culture and isolation of fungi from root tissue. Cultural techniques have also been used to demonstrate the effects of thiram and captafol on root-invasion by fungi during establishment of ryegrass (Labruère 1979). It should be noted that although fungicides can reduce levels of root infection by some pathogenic fungi they may not necessarily prevent plants from becoming diseased (Skipp *et al* 1986).

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