testing. Although glasshouse facilities may limit the number of lines which can be tested, the method will be useful for determining reactions of parents or advanced lines in breeding programmes.

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Subterranean Clover Foliage Fungi as Root Pathogens

M.J. Barbetti
Department of Agriculture,
Jarrah Road, South Perth, W.A. 6151

In Western Australia, *Fusarium avenaceum* (Fr.) Sacc., *Leptosphaerulina trifolii* (Rostrup) Petrak, *Myrothecium verrucaria* (Fr.) Sacc., and *Phoma medicaginis* malbr. and Roum. are commonly isolated from cotyledons, leaves and petioles of subterranean clover (*Trifolium subterraneum* L.); (M.J. Barbetti, unpublished data). *L. trifolii* and *P, medicaginis* have been isolated from the foliage of almost all subterranean clover sampled from the lower southwest of Western Australia (M.J. Barbetti, unpublished data). Although common on subterranean clover foliage, *F. avenaceum* and *M. verrucaria* occur at a lower frequency than *L. trifolii* and *P. medicaginis.* Both *F. avenaceum* and *P. medicaginis* have been isolated from rotted subterranean clover roots (10, M.J. Barbetti, unpublished data). *F. avenaceum* is a serious root pathogen of subterranean clover in eastern Australia (4, 5), and is associated with some root rots of subterranean clover in Western Australia (9, 10). *P. medicaginis* has recently been shown to be a weak pathogen on subterranean clover in Western Australia (10). Overseas, *M. verrucaria* is known to cause root rot of red clover and alfalfa (6). *L. trifolii* has not been reported as a root pathogen. The present study investigated the pathogenicity of foliage isolates of each of the fungi described above to subterranean clover roots.

For a single isolate of each fungus, inoculum was prepared by growing the fungus on moist sterile wheat for 3-4 weeks at room temperature. Sterile wheat was prepared by adding 150 ml of distilled water to 200 g wheat in a 1 l flask, soaking for 8 hr. then autoclaving at 100 kPa for 30 min on two consecutive days. The required amount of colonised wheat (0.5, 2.0 or 5.0% w/w) was thoroughly mixed with pasteurized potting mix (sawdust:sand, 50:50 v/ v mix, treated with aerated steam for 90 min at 60 C). Twenty seeds of the cultivar Yarloop (root rot susceptible) were placed on top of the inoculum/soil mix in a 10 cm pot and covered with 1 cm of pasteurized soil. Treatments were replicated six times in a fully randomised design. Pots were watered daily with de-ionized water. Tests were made in growth cabinets each with a 10.5 hr day, a 13.5 hr night, a light intensity of 35,000 lux, and set at day/night temperatures of 15/10, 18/13 and 21/16 C, respectively. Four weeks after sowing plant survival was recorded, and plants were washed and rated for disease severity as previously described (2), viz.

1. Healthy — tap root with 0 — 10% of tap root affected by root rot.
2. Moderate tap root rot — 10 — 70% of tap root affected by root rot.
3. Severe tap root rot — 70 — 100% of tap root affected by root rot.

Plants were similarly rated for severity of damage to the lateral roots. The average percentage root disease index, based on the above disease ratings, was then calculated for both tap and lateral roots using the method described by McKinney (7). The foliage dry weight of each plant was also recorded. The test fungi were re-isolated from affected roots to confirm that they produced the disease symptoms.

Results (Tables 1 and 2) show that each of the four fungi caused root disease. *F. avenaceum* was more severe than *P. medicaginis, M. verrucaria,* or *L. trifolii. F. avenaceum* and *M. verrucaria* caused most root rot at the two lower temperatures while *L. trifolii* caused most root rot at the two higher temperatures. All fungi reduced ($p \leqslant 0.05$) plant weight at one or more inoculum rate/temperature combinations. The fungi had little or

Table 1. Effect of fungal isolate, inoculum rate, and temperature on root rot severity.

Fungus	Inoculum rate % (w/w)	Tap root rot disease index at temperature regimes (C)			Lateral root rot disease index at temperature regimes (C)		
		15/10	18/13	21/16	15/10	18/13	21/16
Fusarium avenaceum	0.5	5.5	7.8	3.8	18.5	16.5	5.2
	2.0	27.7	26.0	7.2	41.4	35.2	20.8
	5.0	58.9	54.8	40.5	65.7	57.1	44.1
Leptosphaerulina trifolii	0.5	0.3	0	0.3	1.7	4.3	6.7
	2.0	0	0.3	0.6	2.9	6.9	12.9
	5.0	1.2	0.9	2.9	6.1	20.6	22.0
Myrothecium verrucaria	0.5	2.3	2.4	1.8	7.9	14.7	1.7
	2.0	4.4	4.4	2.3	15.8	15.7	3.0
	5.0	20.0	9.0	2.2	28.2	26.1	15.3
Phoma medicaginis	0.5	2.7	0.5	1.3	5.7	4.6	2.4
	2.0	2.0	2.0	7.7	14.5	17.2	3.7
	5.0	7.1	5.3	23.6	21.8	20.0	17.5
Control		0	0	0	0.8	0	3.9
Significance (fungus x inoculum rate)		***	***	***	***	***	***
LSD ($p \leq 0.05$)		3.9	4.3	3.4	4.5	8.5	4.4
Significance (fungus x inoculum rate x temperature)		***				***	
LSD ($p \leq 0.05$)		4.8				7.6	

no effect on plant survival.

This investigation has shown that *F. avenaceum, L. trifolii, M. verrucaria* and *P. medicaginis*, common on subterranean clover cotyledons, leaves and petioles, are pathogenic on roots in pasteurized soil. Their pathogenicity needs to be further investigated in field soil. Both *L. trifolii* (1, 3, 8) and *P. medicaginis* are known to cause leaf and petiole lesions but are normally considered pathogens of little consquence in subterranean clover in Western Australia (M.J. Barbetti, unpublished data). *F. avenaceum* and *M. verrucaria* can cause leaf lesions in subterranean clover (M.J. Barbetti, unpublished data). The temperature regimes selected in this investigation are representative of the average growing season maximum and minimum temperatures of the hottest, coldest and intermediate parts of the lower south-west of Western Australia. If both *F. avenaceum* and *M. verrucaria* are pathogenic on subterranean clover under field conditions then they are likely to cause most root damage in the colder and intermediate areas of the lower south-west of Western Australia. Wong *et al.* (10), working with *F. avenaceum,*

Table 2. Effect of fungal isolate, inoculum rate, and temperature on foliage weight and plant survival.

Fungus	Inoculum rate	Top dry weight per plant at temperature regimes (C)			Mean plant survival at at temperature regimes (C)		
		15/10	18/13	21/16	15/10	18/13	21/16
Fusarium avenaceum	0.5	32.6	67.2	39.6	14.0	13.7	13.2
	2.0	29.8	57.9	42.4	13.7	14.0	13.7
	5.0	19.1	44.1	38.0	13.8	13.7	11.3
Leptosphaerulina trifolii	0.5	35.3	70.5	61.5	13.7	14.2	13.0
	2.0	31.2	57.1	66.9	13.7	13.7	13.7
	5.0	23.7	52.7	82.0	13.7	14.0	14.0
Myrothecium verrucaria	0.5	35.1	74.7	35.2	14.3	13.5	14.7
	2.0	35.4	78.2	39.8	14.5	14.2	12.2
	5.0	31.6	63.7	32.9	13.7	13.8	14.5
Phoma medicaginis	0.5	34.4	73.0	32.7	13.8	13.7	12.0
	2.0	28.2	54.1	22.6	14.5	14.3	14.2
	5.0	28.2	46.4	18.0	14.0	14.2	13.3
Control		31.5	83.0	61.0	14.2	14.2	14.0
Significance (fungus x inoculum rate)		**	*	*	NS	NS	***
LSD ($p \leq 0.05$)		3.9	14.3	14.7			1.4
Significance (fungus x inoculum rate x temperature)			***			NS	
LSD ($p \leq 0.05$)			10.2				

F. oxysporum, P. medicaginis, Pythium irregulare Buisman, and *Rhizoctonia solani* Kuhn, showed that root disease in subterranean clover was more severe with combinations of fungi than with individual fungi. It is therefore possible that combinations of the fungi used in the present investigation may cause more severe root rot than individual fungi. Foliage infection may be an important means of disease carryover and inoculum buildup for fungi such as *F. avenaceum*, that cause root rot in subterranean clover. Conversely, the soil may be a reservoir of inoculum for foliar disease.

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Effects of Spray-Seed® on the Growth of Subterranean Clover and its Susceptibility to Root Rot Fungi

M.J. Barbetti
Department of Agriculture
Jarrah Road, South Perth, W.A. 6151

In the south west of Western Australia root rot causes substantial losses in annual pastures of subterranean clover (*Trifolium subterraneum* L.) (4, 6, 10). The subterranean clover content is replaced by weeds and grasses and eventually affected areas require reseeding. Until recently most reseeding relied on cultivation for seedbed preparation. Cultivation is known to give short term (one or two seasons) reductions in root rot severity (3). More recently chemicals, such as Spray-Seed® [paraquat (1, 1'-dimethyl-4, 4' bipridyliumion) 125 g/L/diquat (6, 7-dihydrodipyridol (1, 2-a:2, 1-c) pyrazinediium dibromide) 75 g/L], have been used as an alternative to cultivation. Spray-Seed® is applied before seeding and makes cultivation for weed control unnecessary. It also reduces the possibility of soil erosion and allows earlier planting. An investigation was conducted into the effects of time of seeding and Spray-Seed® application on root disease, plant growth, and the incidence of root fungi.

In late May, 1983, 2-3 weeks after the first major rain (20 mm or more) which broke the summer drought, undistrubed field cores (10 cm diameter, 12 cm depth) were taken from root rot affected pastures at Albany (400 km SE of Perth), and Scott River (300 km S/SW of Perth), on the south coast of Western Australia using the methods described by MacNish *et al.*, 1973 (7). The soil at both sites was a leached sand soil with a Northcote Convention code of Uc 2.2 (9). After sampling, the following treatments were applied:

1. Control, (no Spray-Seed® application);
2. Seeded then immediate Spray-Seed® application;
3. Spray-Seed® application and sown one day later;
4. Spray-Seed® application and sown one week later;
5. Spray-Seed® application and sown two weeks later.

Spray-Seed® was applied by hand sprayer at the rate of 3.5 1/ha onto the foliage and the soil surface. Ten holes 1 cm deep were made by inserting a pencil into each core, two subterranean clover seeds of cv. Woogenellup placed in each hole and the holes closed over. For each treatment there were 10 and 8 single core replications for the Albany and Scott River sites, respectively. Cores were watered every second day and were maintained in a growth cabinet with a 12 hr photoperiod (light intensity 35,000 lux) at temperatures of 15 C day and 10 C night. Four weeks after sowing, seedling

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