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Effects of Soil Temperature and Moisture on the Pathogenicity of Fungi associated with Root Rot of Subterranean Clover

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Abstract

The effects of soil temperature (10, 15, 20 and 25°C) and moisture (45% water holding capacity (WHC), 65% WHC, and flooding) on the pathogenicity of five fungi, both alone and in combinations, were investigated to determine the involvement of these fungi in a severe root rot disorder of subterranean clover in Western Australia. *Fusarium avenaceum*, *Pythium irregulare*, and *Rhizoctonia solani* were highly pathogenic while *Fusarium oxysporum* and *Phoma medicaginis*, particularly when used singly, were only weakly pathogenic. Compared with individual fungi, fungal combinations increased the severity of root disease and decreased plant survival and plant fresh weight. While the fungi investigated caused root rot over the range of soil temperatures and moisture conditions of this investigation, the most severe root rot occurred at 10°C, with less at 15 and 25°C, and least at 20°C. Temperature had a marked effect on the disease severity and its effect varied with individual fungi and their combinations, in particular, combinations involving *P. irregulare* (severest root rot at 10 and 15°C). The most severe root rotting, compared with the control, occurred at 65% WHC, with less at 45% WHC, and least under flooding conditions. There was often a significant interaction between temperature and moisture for the various fungi and fungal combinations tested.

Introduction

Subterranean clover, *Trifolium subterraneum* L., is the most important pasture legume in many regions of Australia, particularly in Western Australia where 6·5 million ha of annual pastures are based on subterranean clover (Gladstones 1975). Root rots of subterranean clover in Australia have been recognized in Victoria (Kellock 1972; Burgess *et al.* 1973; McGee and Kellock 1974; Kellock *et al.* 1978), New South Wales (Valder 1954; Stovold 1974), South Australia (Ludbrook *et al.* 1953) as well as Western Australia (Shipton 1967*b*; Barbetti and MacNish 1978).

Fusarium oxysporum Schlecht. and *F. avenaceum* (Fr.) Sacc. were reported to be highly pathogenic to subterranean clover roots in Western Australia (Shipton 1967*a*). Barbetti and MacNish (1978) investigated the extent of root rots in the irrigation areas of the south-western regions of Western Australia and showed that the main fungi associated with the root rots were *Pythium irregulare* Buisman, *P. acanthicum* Drechsler, *F. oxysporum*, *P. middletonii* Sparrow, *P. debaryanum* Hesse, and *Rhizoctonia* sp. The *Rhizoctonia* spp. have also been shown to cause root rot in white clovers in Western Australia (Maughan and Barbetti 1983). *Rhizoctonia solani* Kuhn was found to be the main cause of the 'bare-patch disease' and associated problems in subterranean clover pastures in South Australia (Ludbrook *et al.* 1953). *F. avenaceum* and *F. oxysporum* were found to be

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associated with root rots of subterranean clover as seed-borne pathogens in Victoria (McGee and Kellock 1974; Kellock *et al.* 1978). In New South Wales *P. irregulare* was reported to be the main cause of root rot of subterranean clover (Stovold 1974). *Phoma medicaginis* Malb. et Roum has been reported to cause root rot and damping-off of red clover in Europe (Wojciechowska 1969; Rufelt 1978) and Egyptian clover in Egypt (Michail and El Khatib 1976). *Codinea fertilis* Hughes & Kendrick has been reported as a pathogen of white clover in New Zealand (Menzies 1973) and in the U.S.A. (Campbell 1980).

Environmental factors such as temperature and soil moisture may significantly affect the expression of root rots. In general, *Fusarium* species are most active and survive best in dry soils (Stover 1953) and *Fusarium* diseases are commonly more important under dry rather than wet conditions (Cook and Papendick 1972). Graham *et al.* (1957) and Graham and Newton (1960) showed that, for damping-off of Ladino clover, *Fusarium* was most active in drier soils and had little effect in saturated soils. Temperature is a significant factor in *Fusarium* disease expression in various legume crops (Kraft *et al.* 1981); Siddiqui (1968) found that red clover root rot, caused by *F. roseum*, was more prevalent at temperatures of 28–38°C. Generally, high soil moisture is reputedly necessary for disease development involving *Pythium* species (Hendrix and Campbell 1973), and Graham *et al.* (1957) showed that, for damping-off of Ladino clover, *Pythium* was most active in wet soils. Soil temperature is also a determining factor in *Pythium*-related diseases, and certain *Pythium* species are favoured by either high or low soil temperatures (Campbell and Hendrix 1973). Soil moisture and temperature affect the severity of disease caused by *Rhizoctonia* species. Crowder and Craigmiles (1960) showed that disease caused by *R. solani* was less in irrigated than in unirrigated white clover, and *R. solani* is known to cause more severe disease in spinach at higher (>16°C) soil temperatures (Sumner *et al.* 1976).

This paper reports the results of glasshouse studies to determine the effects of soil temperature and moisture on the pathogenicity, singly and in combinations, of those fungi commonly associated with root rot of subterranean clover and to determine their involvement in root rot in Western Australia.

Materials and Methods

Isolates of *F. avenaceum*, *F. oxysporum*, *Phoma medicaginis*, *Pythium irregulare* and *R. solani* were obtained from the roots of rot-affected subterranean clover seedlings sampled in August 1982 from Augusta and Albany in the south-west of Western Australia. The inoculum levels for each test fungus for root rot development were determined in a separate preliminary experiment by testing the effectiveness of a range of inoculum concentrations in a free draining Augusta field soil maintained at 15/10°C (day/night).

The following isolates and combinations were used in pathogenicity studies: (1) *F. avenaceum* (IMI 275551) 0·5% w/w; (weight of inoculum/weight air-dried soil); (2) *F. oxysporum* 0·5%; (3) *P. medicaginis* 2·0%; (4) *P. irregulare* (IMI 274963) 0·5%; (5) *R. solani* 0·2%; (6) *F. avenaceum* + *P. medicaginis* 0·5% + 2·0%; (7) *F. avenaceum* + *P. irregulare* 0·5 + 0·5%; (8) *F. avenaceum* + *R. solani* 0·5 + 0·2%; (9) *F. oxysporum* + *F. avenaceum* 0·5 + 0·5%; (10) *F. oxysporum* + *P. medicaginis* 0·5 + 2·0%; (11) *F. oxysporum* + *P. irregulare* 0·5 + 0·5%; (12) *F. oxysporum* + *R. solani* 0·5 + 0·2%; (13) *P. medicaginis* + *R. solani* 2·0 + 0·2%; (14) *P. irregulare* + *P. medicaginis* 0·5 + 2·0%; (15) *P. irregulare* + *R. solani* 0·5 + 0·2%; (16) Combination of all five fungi at inoculum rates above; and (17) control (3·7% w/w sterile millet seeds only).

Cultures of the above *Fusarium* spp. were stored on ceramic beads using the method described by Lange and Boyd (1968). All other fungi were stored on potato-dextrose agar (PDA) slopes at 4°C for 8–11 months prior to use as inoculum. Inoculum was prepared by growing the test fungi on moist sterile

millet (*Panicum milipaceum* L.) seeds for 2–3 weeks. The inoculum was then mixed with pasteurized (60°C for 30 min) Augusta field soil that had a history of severe root rot in subterranean clover. The chemical characteristics of this soil (in mg/kg) were:

	P	K	N as NH_4	N as NO_3	Fe	Mn	Al	Cl	Organic C(%)	pH
Unsteamed soil	10	36	24	15	755	0·7	0·4	43	2·0	6·2
Pasteurized soil	11	37	36	16	740	0·8	0·6	54	1·7	6·5

Plastic pots (7·5 cm diameter), containing 200 g of inoculated soil, were sown with 10 surface-sterilized germinated seeds of subterranean clover cv. Woogenellup. Germinated seeds were used to avoid possible germination failures due to dryness of the soil. The seeds were then covered with a thin layer of pasteurized soil and subsequently with a layer of inert plastic beads to reduce both soil drying and the possibility of cross contamination between pots. There were six replicate pots for each treatment. The pots were placed in temperature-controlled tanks in an air-conditioned glasshouse and maintained at constant temperatures of 10, 15, 20 and 25°C. Three moisture levels were used, viz. 45% WHC (water holding capacity), 65% WHC, and flooding to a depth of 1–1·5 cm above soil level. The pots were watered daily to a constant weight with deionized water according to the required moisture level. Owing to the large number of treatments this investigation was conducted as four sequential experiments during May–August 1983, beginning with 10°C and ending with 25°C.

Four weeks after sowing, the surviving seedlings were counted, removed, washed and rated for root rot severity on tap and lateral roots. The rating was carried out on a 0–5 scale as follows: 0, tap and lateral roots healthy; 0·5, parts of tap root light brown, a few lateral roots may also be affected; 1·0, whole tap root light brown, usually some lateral roots affected; 1·5, part of tap root brown; 2·0, whole tap root brown, usually lateral roots affected; 2·5, tap root brown with visible lesions, 3·0, as for 2·5 with tip of the tap root constricted, very few lateral roots present; 3·5, tap root brown and stunted, few lateral roots present and these are brown; 4·0, tap root dark brown to black, discrete lesions may be present; 4·5, tap root dark brown to black, tissues beginning to slough off; 5·0, whole tap root rotted off or seedling is dead. The average percentage root disease index, based on the above disease ratings was then calculated using the method described by McKinney (1923).

Fresh shoot weights were recorded for all plants.

Analysis of variance was conducted on all data. The 40 treatments showing the largest reductions in plant survival were ranked in a descending order of reduction. Rankings were made by dividing the number of plants surviving in the control by the number of plants surviving in the treatment. Similarly rankings were made for the largest reductions in fresh shoot weight. The largest increases in root disease index were calculated by dividing the treatment disease index by the control disease index.

Twelve randomly selected root pieces (0·5–1·0 cm long) from the six replicate pots of each treatment were surface-sterilized (in sodium hypochlorite (1·25% available chlorine) for 2–3 min), rinsed three times in sterile distilled water, blotted dry, and plated onto PDA to check for the presence of treatment fungi in rot-affected tissue.

Field soil temperatures were measured at 2, 5 and 10 cm depths in five root-rot-prone areas of Western Australia during the expected period of severe root rot.

Results

All treatment fungi were readily re-isolated from rot-affected tissue to fulfil Koch's postulates.

The survival rates, root disease indexes, and fresh shoot weights of the inoculated plants are shown in Table 1. The fungal treatments giving the largest reductions in plant survival, root disease index and fresh shoot weight are listed in Tables 2, 3 and 4 respectively.

Discussion

The results of this investigation suggest that root rot of subterranean clover pastures in Western Australia is induced by a complex of interacting fungal pathogens. Many fungi have been isolated from diseased roots collected in the field in southern Western Australia, but, in preliminary studies, only *F. avenaceum*, *F. oxysporum*, *P. medicaginis*, *P. irregulare*, and *R. solani* were pathogenic (D.

Table 1. The effect of temperature and moisture (%WHC) on the survival rate, root disease index, and the fresh shoot weight of subterranean clover (cv. Woogenellup) inoculated with selected fungi

Fungus	WHC (%)	Mean survival rate (%)				Root disease index (%)				Shoot weight (mg)			
	Temp. (°C):	10	15	20	25	10	15	20	25	10	15	20	25
Control	45	28·3	46·7	53·3	78·3	17·8	39·2	56·4	33·1	151	271	635	1233
	65	73·3	70·0	48·3	86·7	15·1	29·9	62·8	26·2	603	722	732	1754
	Flood	71·7	60·0	50·0	73·3	48·8	26·7	88·9	86·9	642	783	234	273
F. avenaceum	45	0	43·3	38·3	18·3	100·0	76·2	33·5	78·9	0	355	482	175
	65	21·7	35·0	40·0	18·3	87·4	65·0	36·4	72·8	102	558	660	284
	Flood	30·0	40·0	40·0	30·0	76·4	50·7	86·9	93·7	138	592	270	92
F. oxysporum	45	5·0	65·0	53·3	43·3	93·3	46·5	21·6	54·1	11	785	753	354
	65	16·7	60·0	48·3	20·0	35·3	23·3	15·9	51·7	45	767	1220	149
	Flood	28·3	23·3	50·0	55·0	50·0	75·3	84·9	91·6	110	320	580	250
P. medicaginis	45	36·7	60·0	45·0	60·0	71·3	81·9	45·2	33·0	146	433	605	1107
	65	70·0	55·0	68·3	61·7	70·1	69·2	55·2	20·3	427	742	935	1557
	Flood	66·7	18·3	55·0	36·7	80·5	90·0	94·3	92·6	472	157	319	165
P. irregulare	45	6·7	36·7	20·0	46·7	85·8	90·8	88·3	70·3	35	223	134	520
	65	45·0	23·3	16·7	28·3	91·4	93·6	84·2	69·3	171	100	160	418
	Flood	26·7	10·0	25·0	23·3	95·6	95·0	94·4	98·8	114	82	183	211
R. solani	45	8·3	3·3	0	13·3	66·7	96·7	100·0	71·7	0[A]	32	0	125
	65	21·7	21·7	1·7	13·3	95·8	45·8	100·0	74·2	44	252	14	197
	Flood	23·3	10·0	0	5·0	71·7	75·6	100·0	99·2	151	122	0	13
F. avenaceum +	45	6·7	45·0	41·7	16·7	50·0	83·8	41·8	76·4	8	245	563	103
F. oxysporum	65	0	41·7	40·0	11·7	100·0	47·5	68·5	90·5	0	528	606	56
	Flood	11·7	23·3	40·0	16·7	90·0	90·0	92·1	95·4	32	203	181	86
F. avenaceum +	45	0	11·7	10·0	3·3	100·0	97·8	83·3	93·3	0	48	69	36
P. irregulare	65	1·7	8·3	21·7	0	100·0	95·0	78·9	100·0	3	27	143	0
	Flood	1·7	5·0	10·0	0	100·0	98·3	99·2	100·0	120	48	96	0
F. avenaceum +	45	5·0	26·7	35·0	13·3	97·8	84·3	61·0	85·0	13	80	467	97
P. medicaginis	65	11·7	55·0	50·0	3·3	93·3	73·8	54·0	93·3	28	398	638	33
	Flood	26·7	51·7	35·0	6·7	83·3	84·6	92·8	96·1	63	275	141	16

F. avenaceum +	45	6·7	8·3	0	0	92·8	88·3	100·0	100·0	34	55	0	0
R. solani	65	10·0	38·3	0	0	96·7	82·5	100·0	100·0	50	383	0	0
	Flood	35·0	16·7	0	0	82·8	76·7	100·0	100·0	90	122	0	0
F. oxysporum +	45	5·0	15·0	26·7	25·0	83·3	86·7	82·5	74·6	7	87	251	210
P. irregulare	65	1·7	11·7	23·3	16·7	100·0	92·8	83·6	85·8	0[A]	30	192	185
	Flood	0	1·7	16·7	11·7	100·0	100·0	95·8	98·3	0	10	139	44
F. oxysporum +	45	6·7	61·7	0	36·7	96·7	79·4	100·0	61·5	0[A]	268	0	294
P. medicaginis	65	6·7	56·7	10·0	50·0	86·7	77·3	85·3	60·7	25	312	91	778
	Flood	23·3	10·0	3·3	18·3	80·0	93·3	98·3	94·5	81	50	26	45
F. oxysporum +	45	5·0	13·3	0	5·0	83·3	86·9	100·0	85·0	0[A]	85	0	26
R. solani	65	11·7	40·0	0	1·7	82·2	54·5	100·0	91·7	42	445	0	58
	Flood	33·3	28·3	0	0	70·7	59·0	100·0	100·0	13	270	0	0
P. irregulare +	45	10·0	23·3	28·3	23·3	100·0	84·8	84·5	86·1	34	69	193	133
P. medicaginis	65	15·0	13·3	30·0	11·7	100·0	100·0	81·4	88·3	28	43	282	71
	Flood	8·3	1·7	23·3	13·3	100·0	100·0	93·2	98·9	30	7	150	45
P. irregulare +	45	5·0	0	0	0	97·8	100·0	100·0	100·0	8	0	0	0
R. solani	65	21·7	10·0	0	0	97·2	91·7	100·0	100·0	47	30	0	0
	Flood	11·7	1·7	1·7	0	95·0	96·7	100·0	100·0	36	12	13	0
P. medicaginis +	45	1·7	1·7	43·3	0	91·7	98·3	35·4	100·0	0[A]	5	964	0
R. solani	65	21·7	6·7	45·0	0	84·4	90·0	31·4	100·0	45	35	898	0
	Flood	21·7	26·7	5·0	0	75·4	83·3	95·0	100·0	73	270	34	0
Combination of	45	5·0	0	11·7	0	80·0	100·0	81·7	100·0	16	0	22	0
the five fungi	65	0	1·7	0	0	100·0	100·0	100·0	100·0	0	3	0	0
	Flood	0	6·7	5·0	0	100·0	100·0	98·3	100·0	0	22	20	0
l.s.d.(P = 0·05), treatment × moisture		13·1	17·2	16·4	12·5	21·6	14·3	13·0	13·8	68	177	200	126
l.s.d.(P = 0·05), treatment × temperature	45		13·3				20·0				128		
	65		15·1				14·9				179		
	Flood		16·2				12·4				144		

[A] Shoot weight was negligible and not recorded as emergence was delayed until just prior to assessment.

Wong, unpublished data). Other non-pathogenic fungi isolated included *Alternaria* sp., *Drechslera* sp., *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. heterosporum* Mees. ex Fr., *F. sambucinum* Fuckel, *F. sulphureum* Schlecht, *Mortierella* sp., *Mucor* sp., *Penicillium* sp., *Phialophora* sp., *Pythium coloratum* Vaartaja, *P. splendens* Braun, *Pythium* sp. (sterile), *Rhizopus* sp., *Trichoderma* sp., *Truncatella* sp., and *Waitea circinata* Warcup & Talbot. *F. avenaceum*, *P. irregulare*, and *R. solani* were the most pathogenic on subterranean clover roots, while *F. oxysporum* and *P. medicaginis*, in particular when acting singly, were only weakly pathogenic. While *F. avenaceum*, *F. oxysporum*, *P. irregulare*, and *R. solani* have been previously shown to be pathogenic on subterranean clover

Table 2. The 40 fungal treatments showing the largest reductions in plant survival, ranked in a descending order of reduction

Treatments within vertical brackets have identical values

There was a significant temperature × moisture interaction for all treatments except *F. avenaceum*, *F. avenaceum* + *F. oxysporum*, *F. avenaceum* + *P. irregulare*, *F. oxysporum* + *P. irregulare*, and *P. irregulare* + *P. medicaginis*

Pathogen(s)	Temp. (°C)	Moisture (% WHC)	Pathogen(s)	Temp. (°C)	Moisture (% WHC)
F. avenaceum + *P. irregulare*	25	65	*R. solani*	20	45
F. avenaceum + *R. solani*	25	65	*F. avenaceum* + *R. solani*	20	45
P. irregulare + *R. solani*	25	65	*F. oxysporum* + *P. medicaginis*	20	45
P. medicaginis + *R. solani*	25	65	*F. oxysporum* + *R. solani*	20	45
Combination of five fungi	25	65	*P. irregulare* + *R. solani*	20	45
F. avenaceum + *R. solani*	25	45	*F. oxysporum* + *R. solani*	25	65
P. irregulare + *R. solani*	25	45	*R. solani*	20	flooded
P. medicaginis + *R. solani*	25	45	*F. avenaceum* + *R. solani*	20	flooded
Combination of five fungi	25	45	*F. oxysporum* + *R. solani*	20	flooded
F. avenaceum + *F. oxysporum*	10	65	*F. avenaceum* + *R. solani*	20	65
F. avenaceum + *P. irregulare*	25	flooded	*P. irregulare* + *R. solani*	20	65
F. avenaceum + *R. solani*	25	flooded	Combination of five fungi	20	65
F. oxysporum + *R. solani*	25	flooded	*P. irregulare* + *R. solani*	15	45
P. irregulare + *R. solani*	25	flooded	Combination of five fungi	15	45
P. medicaginis + *R. solani*	25	flooded	*F. avenaceum* + *P. irregulare*	10	65
Combination of five fungi	10	65	*F. oxysporum* + *P. irregulare*	10	65
Combination of five fungi	25	flooded	*F. avenaceum* + *P. irregulare*	10	flooded
F. oxysporum + *P. irregulare*	10	flooded	Combination of five fungi	15	65
Combination of five fungi	10	flooded	*F. oxysporum* + *P. irregulare*	15	flooded
			P. irregulare + *P. medicaginis*	15	flooded
			P. irregulare + *R. solani*	15	flooded

(Kellock 1972; Burgess *et al.* 1973; McGee and Kellock 1974; Stovold 1974; Barbetti and MacNish 1978; Kellock *et al.* 1978), the pathogenicity of *P. medicaginis* has not been previously demonstrated in Australia. Unfortunately most previous investigations into subterranean clover root rots in Australia were limited to isolating and testing single fungi, or to testing only very few combinations (Barbetti and McNish 1978; Kellock *et al.* 1978). However, our investigations have shown that the severity of root disease is often enhanced by the application of fungal combinations rather than of individual fungi. Even fungi such as *F. oxysporum* and *P. medicaginis*, which are only weakly pathogenic on their own, can markedly

increase root rot when added in combinations with other root pathogens under certain temperature and moisture conditions.

The most severe root rot occurred at 10°C, with less at 15 and 25°C and least at 20°C. Williams (1972) has shown that temperatures of 10 and 15°C are less favourable for subterranean clover growth than are 20 and 25°C, and our results suggest that the fungi we investigated, with the exception of *R. solani*, are taking advantage of reduced plant vigour at the lower temperatures. Leach (1947) showed that susceptibility of spinach to *Pythium ultimum* was also related to the effect of temperature on host vigour. We found that temperature markedly affected the severity of disease, particularly in diseases arising from combinations involving

Table 3. The 40 fungal treatments showing the largest increases in root disease index, ranked in a descending order of effect

Treatments within vertical brackets have identical values

There was a significant temperature × moisture interaction for all treatments except *P. irregulare*, *F. avenaceum* + *P. irregulare*, *F. avenaceum* + *R. solani*, *F. oxysporum* + *P. irregulare*, *F. oxysporum* + *R. solani*, *P. irregulare* + *R. solani*, and the combination of five fungi

Pathogen(s)	Temp. (°C)	Moisture (% WHC)	Pathogen(s)	Temp. (°C)	Moisture (% WHC)
F. avenaceum + *F. oxysporum*	10	65	*F. oxysporum*	10	45
F. avenaceum + *P. irregulare*	10	65	*F. avenaceum* + *R. solani*	10	45
F. oxysporum + *P. irregulare*	10	65	*P. medicaginis* + *R. solani*	10	45
P. irregulare + *P. medicaginis*	10	65	*P. irregulare*	10	45
Combination of five fungi	10	65	*F. oxysporum* + *P. irregulare*	10	45
F. avenaceum + *R. solani*	10	65	*F. oxysporum* + *R. solani*	10	45
P. irregulare + *R. solani*	10	65	*P. medicaginis*	10	65
R. solani	10	65	Combination of five fungi	10	45
F. avenaceum + *P. medicaginis*	10	65	*P. medicaginis*	10	45
P. irregulare	10	65	*P. avenaceum* + *P. irregulare*	25	65
F. avenaceum	10	65	*F. avenaceum* + *R. solani*	25	65
F. oxysporum + *P. medicaginis*	10	65	*P. irregulare* + *R. solani*	25	65
F. avenaceum	10	45	*P. medicaginis* + *R. solani*	25	65
F. avenaceum + *P. irregulare*	10	45	Combination of five fungi	25	65
P. irregulare + *P. medicaginis*	10	45	*R. solani*	10	45
F. avenaceum + *P. medicaginis*	10	45	*F. avenaceum* + *P. irregulare*	15	flooded
P. irregulare + *R. solani*	10	45	*F. oxysporum* + *P. irregulare*	15	flooded
F. oxysporum + *P. medicaginis*	10	45	*P. irregulare* + *P. medicaginis*	15	flooded
F. oxysporum + *R. solani*	10	45	Combination of five fungi	15	flooded
			P. irregulare	15	flooded
			P. irregulare + *R. solani*	15	flooded

P. irregulare or *R. solani*. Fungal combinations containing *P. irregulare* generally caused the severest root rot at 10 and 15°C, with less at 25°C and markedly less at 20°C. It has been reported that the pathogenicity of *P. irregulare* is greater at low temperatures in peach trees (Biesbrook and Hendrix 1970), cotton (Roncardori and McCarter 1972), and subterranean clover (Stovold 1974). Root rot caused by fungal combinations containing *R. solani* were generally most severe at 20 and 25°C. These results support those of Leach (1947) who showed that pre-emergence damping-off by *R. solani* in spinach and silverbeet was more severe at 20–25°C than at 4–12°C, and the results of Sumner *et al.* (1976) who showed that *R. solani*

caused severe damping-off and root rot of spinach only at soil temperatures of 16°C and above.

Severe root rot usually occurred at 65% WHC, with less at 45% WHC and least under flooding. This result was not expected for *P. irregulare* as high soil moisture (i.e. field capacity or above) is usually associated with increased incidence of root disease in lucerne caused by *Pythium* sp. (Stanghellini and Burr 1973). Flooding did not provide a clear ecological advantage for *Pythium*, although an advantage has been shown by other researchers (Griffin 1963; Hock *et al.* 1975). Perhaps *P. irregulare* on its own was not sufficiently pathogenic to take full advantage of the flooding conditions, or perhaps it was hampered by its inability to readily produce

Table 4. The 40 fungal treatments showing the largest reductions in fresh shoot weight, ranked in a descending order of reduction

Treatments within vertical brackets have identical values

There was a significant temperature × moisture interaction for all treatments except *F. avenaceum*, *F. avenaceum* + *P. irregulare*, *F. oxysporum* + *P. irregulare*, *P. irregulare* + *P. medicaginis*, *P. irregulare* + *R. solani*, and the combination of five fungi

Pathogen(s)	Temp. (°C)	Moisture (% WHC)	Pathogen(s)	Temp. (°C)	Moisture (% WHC)
P. irregulare + *R. solani*	25	65	*F. avenaceum* + *F. oxysporum*	10	65
F. avenaceum + *R. solani*	25	65	*F. oxysporum* + *P. irregulare*	10	65
F. avenaceum + *P. irregulare*	25	65	Combination of five fungi	10	65
P. medicaginis + *R. solani*	25	65	*F. avenaceum* + *P. irregulare*	25	flooded
Combination of five fungi	25	65	*F. avenaceum* + *R. solani*	25	flooded
F. avenaceum + *R. solani*	25	45	*F. oxysporum* + *R. solani*	25	flooded
P. irregulare + *R. solani*	25	45	*P. irregulare* + *R. solani*	25	flooded
P. medicaginis + *R. solani*	25	45	*P. medicaginis* + *R. solani*	25	flooded
Combination of five fungi	25	45	Combination of five fungi	25	flooded
F. avenaceum + *R. solani*	20	65	*P. irregulare* + *R. solani*	15	45
F. oxysporum + *R. solani*	20	65	Combination of five fungi	15	45
P. irregulare + *R. solani*	20	65	*F. avenaceum* + *R. solani*	20	flooded
Combination of five fungi	20	65	*F. oxysporum* + *R. solani*	20	flooded
F. oxysporum + *P. irregulare*	10	flooded	*F. avenaceum*	10	45
Combination of five fungi	10	flooded	*R. solani*	10	45
R. solani	20	45	*F. avenaceum* + *P. irregulare*	10	45
F. avenaceum + *R. solani*	20	45	*F. oxysporum* + *P. medicaginis*	10	45
F. oxysporum + *P. medicaginis*	20	45	*F. oxysporum* + *R. solani*	10	45
F. oxysporum + *R. solani*	20	45	*P. medicaginis* + *R. solani*	10	45
P. irregulare + *R. solani*	20	45	*P. irregulare* + *P. medicaginis*	15	flooded

zoospores (Hendrix and Campbell 1973). Some combinations with *P. irregulare* were more pathogenic than *P. irregulare* on its own, but then the flooding was probably unfavourable to the other fungus in the combination, thus preventing the *P. irregulare* combination from taking advantage of the flooding. Also, the effects of fungal treatment under flooding conditions may have been masked by the discoloration of the roots, which was evident in the control treatment. Isolations from rot-affected control plants did not suggest the presence of any pathogenic fungi as the cause of this rotting. Our results for *R. solani* agree with those of Crowder and Craigmiles (1960), who showed that, when white clover was irrigated, the influence of root disease from *R. solani* was less than if the clover was not irrigated.

The various fungi and their combinations greatly reduced seedling survival under the range of temperature and moisture conditions used in this study. Seedling survival in fields with root rot is very poor in Western Australia (M. J. Barbetti, unpublished data; Gillespie 1975) and our results suggest that this poor field survival is due to pre-emergence root rotting by these fungi. There is also a good negative correlation between seedling survival and disease severity (D. Wong, unpublished data).

Plant shoot weight was greatly reduced by the various fungi and their combinations under the range of temperature and moisture conditions used in this study. There is a good negative correlation between plant weight and disease severity (D. Wong, unpublished data). These results agree with those of Barbetti (1984) who showed that, in fields with root rot, there is a high negative correlation between the severity of root rot and plant size.

F. oxysporum, *P. irregulare* and *R. solani* are closely associated with field symptoms of subterranean clover root rot in Western Australia and we believe that these fungi are major pathogens involved in this root rot disorder. *F. avenaceum* and *P. medicaginis* may not be important in all root rot situations as they are not always associated with field symptoms. In addition to the five fungi used in this study other fungi may be involved in this root rot complex. The complex of fungi examined produced severe root disease over a wide range of both temperature (10–25°C) and moisture (45% WHC–flooding) conditions. Field soil temperature (9–23°C) and moisture (48% WHC–flooding) levels monitored in some root-rot-prone areas closely approximated the levels used in this investigation, although allowances must be made for the fluctuations that occur in the field. Therefore our glasshouse results reflect the field situation and explain the occurrence of severe field root rot in Western Australia over a wide range of temperature and moisture conditions. This study also explains why both plant survival and the growth of surviving plants are markedly reduced in fields with root rot.

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