Effects of Three Foliar Diseases on Biomass and Seed Yield for 11 Cultivars of Subterranean Clover

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ABSTRACT

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The losses in subterranean clover sward production and seed yield from foliar disease caused by *Cercospora zebrina*, *Kabatiella caulivora*, and *Pseudopeziza trifolii* were determined for 11 cultivars at a single field site in southwestern Western Australia. Benomyl sprays gave excellent control of *C. zebrina* and *K. caulivora* and often good control of *P. trifolii*. Foliar disease resulted in significant decreases (9–32%) in biomass (sward) production in five of the cultivars examined. All three pathogens were shown to be able individually to decrease sward production in at least one of the susceptible cultivars tested. In all but one cultivar, there were large decreases (25–91%) in seed yield from foliar disease, and overall mean seed yield decrease was 38%. *K. caulivora* or *P. trifolii* individually, or *C. zebrina* in combination with *P. trifolii*, were shown to decrease seed yield. This is the first record of *P. trifolii* causing yield losses in subterranean clover.

Subterranean clover (*Trifolium subterranean* L.) is an important pasture legume not only in its native Mediterranean region but also in other areas of the world. In the United States and New Zealand, it is an important species for improving pastures (15,18). It is also cultivated in areas of Israel, Portugal, Spain, USSR, and South Africa (13,16) and in Argentina, Chile, Uruguay, Japan, Kenya, and Venezuela (10). It is the most important pasture legume species in southern and eastern Australia (13,16,17), where it has been sown over an estimated 16 million hectares of dryland pasture (9,10,12).

Diseases have been considered to be one of the major limitations to the production of subterranean clover, affecting both the amount of plant matter and the quantity of seed produced. The most important foliar disease is clover scorch (M. J. Barbetti, *unpublished*), caused by the fungus *Kabatiella caulivora* (Kirchn.) Karak., which causes large losses in New South Wales (19), Victoria (14), South Australia (5), and Western Australia (6,7). Rust (*Uromyces trifolii-repentis* Liro) and "Cercospora disease" (*Cercospora zebrina* Pass.) are also important in some situations (1,2). A number of other fungi cause minor diseases (except for occasional epidemics) and include *Pseudopeziza trifolii* (Fr.) Fuckel, *Phoma medicaginis* Malb. & Roum., *Leptosphaerulina trifolii* (Rostrup) Petrak, *Erysiphe polygonii* DC., *Physoderma trifolii* (Pat.) Karling,

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Synchytrium aureum Schroeter, *Stemphylium botryosum* Wallr., *Colletotrichum trifolii* Bain & Essary, and *Ascochyta pisi* Lib. (M. J. Barbetti, *unpublished*). All but *S. aureum* have been recorded on subterranean clover in Western Australia (4). However, with the exception of *K. caulivora*, there is little or no information on the effects of foliar disease on biomass (sward or green herbage) production and seed yields. This paper reports the results of investigations to determine losses in clover sward production and seed yield associated with *C. zebrina*, *K. caulivora*, and *P. trifolii*.

MATERIALS AND METHODS

The experiments were conducted at Vasse, Western Australia, where a number of subterranean clover cultivars (Table 1) were growing in unreplicated plots, with a minimum 4 m bare ground buffer between each sward, in almost pure stands for seed production. Infection was natural, and *C. zebrina*, *K. caulivora*, and *P. trifolii* were prevalent on one or more of the cultivars at the site for the two seasons before the experiment. For each cultivar, four plots 4 × 30 m were sprayed with the fungicide benomyl (Benlate) at 1 kg a.i. ha^{-1}, and four paired plots were left untreated. Benomyl sprays were applied on 14 August, 5 and 24 September, and 10 October to all treated plots, except cultivar Woogenellup, which received only the last two sprays applied. As the site was ungrazed, plots were closely mowed and raked clean on 1 April and topdressed with 200 kg/ha of 5:1 superphosphate:potash. Subterranean clover germinated in early April with the commencement of winter rains that follow the hot dry summer. All swards were mowed to 5 cm high on 29 July, and for the later maturing cultivars, Esperance, Junee, Karridale, Larisa, Woogenellup, DMN12, and GD56, mowing was repeated in the spring (14 September).

Plots were assessed for disease caused by *C. zebrina*, *K. caulivora*, or *P. trifolii* on 24 September, 15 October, and 12 November at two locations per plot on a scale of 0–10, where 0 = no disease and 10 = stand completely collapsed. Plots were rated 1 when symptoms could be found only after an exhaustive search, 2 when symptoms were readily found within the plant canopy but not apparent from above the canopy, and 3 when symptoms were mild but visible from above the canopy. Ratings of 4–10 reflected an increasing incidence and severity of damage and, ultimately, complete collapse of the sward. Lesions produced by the three fungi were easily distinguishable from each other and were also confirmed periodically by culture and isolation. On 15 October, pasture production was assessed at five locations per plot with a calibrated capacitance pasture meter (8). Seed reserves from previous seasons were estimated on 17 July by sampling 20 cores 8.3 cm in diameter per cultivar and sieving out seed. Plot seed yields were estimated with the same technique in mid-January after all swards had senesced. Treatment seed yields were obtained by subtracting estimates of seed reserves.

The area under the disease progress curve (AUDPC) was calculated for each of the three diseases for the period between 24 September and 12 November. Analysis of variance for fungicide effects was carried out on the combined data from all cultivars using the appropriate strata structure. Correlations were computed separately within each cultivar between disease parameters, clover sward production, and seed yield. Percent yield loss was calculated as 100 × (fungicide treatment yield − control yield/fungicide treatment yield).

RESULTS

For each of the three diseases individually, the disease levels occurring at the three dates of assessment were highly correlated. The AUDPC was also highly correlated with the three dates of assessment for each of the three diseases. AUDPC was found to be the best means of expressing disease.

The benomyl sprays gave excellent control of *C. zebrina* and *K. caulivora* (Table 1). Benomyl was less effective in controlling *P. trifolii*. Cultivars 8B40, Esperance, Daliak, and GD56 had the most disease from *C. zebrina*, whereas cultivars Woogenellup, Dalkeith, and Larisa had the least. *K. caulivora* was recorded on Larisa, Trikkala, and Woogenellup only. Cultivars Dalkeith, DMN12, and Junee had the most disease from *P. trifolii*. By early November, all cultivars except Larisa, Trikkala, and Woogenellup had high levels of disease from *P. trifolii* as natural senescence developed. With all three fungi combined, 8B40, Esperance, Daliak, and Dalkeith had the most disease and Karridale and Trikkala the least.

Foliar disease resulted in significant decreases in subterranean clover sward production for Junee (9%), Karridale (11%), Larisa (32%), DMN12 (10%), and 8B40 (12%) (Table 2). Foliar disease decreased clover sward production by 10% when averaged over all cultivars. There was significant negative correlation of subterranean clover sward production

Table 1. Severity of disease (measured as area under the disease progress curve [AUDPC] caused by *Cercospora zebrina*, *Kabatiella caulivora*, *Pseudopeziza trifolii*, individually or combined, for 11 cultivars of *Trifolium subterraneum* either unsprayed or sprayed with benomyl

	Disease severity (measured as area under curve)							
	C. zebrina		*K. caulivora*		*P. trifolii*		All three fungi combined	
T. subterraneum cultivar	Fungicide-treated	Control	Fungicide-treated	Control	Fungicide-treated	Control	Fungicide-treated	Control
Daliak	7.3	305.0	0.0	0.0	69.3	164.5	76.5	469.5
Dalkeith	0.0	1.3	0.0	0.0	330.3	460.0	330.3	461.3
Esperance	19.0	356.5	0.0	0.0	27.5	185.3	46.5	541.8
Junee	0.0	76.0	0.0	0.0	35.8	264.5	39.5	340.8
Karridale	0.0	83.8	0.0	0.0	54.5	163.0	54.5	246.8
Larisa	0.0	3.0	0.0	316.0	3.0	0.0	3.0	319.0
Trikkala	0.0	58.8	0.0	176.0	0.0	14.0	0.0	248.8
Woogenellup	0.0	0	54.5	321.0	0.0	7.0	54.5	328.0
DMN12.3.3.3 (DMN12)	0.0	30.0	0.0	0.0	80.3	346.8	80.3	376.8
GD56.8 (GD56)	1.3	251.8	0.0	0.0	42.5	120.3	43.8	372.0
8B40.2.1.1 (8B40)	35.3	460.0	0.0	0.0	41.5	133.8	76.8	593.8
Cultivar significance	$P<0.001$		$P<0.001$		$P<0.001$		$P<0.001$	
Fungicide significance	$P<0.001$		$P<0.001$		$P<0.001$		$P<0.001$	
Cultivar × fungicide significance	$P<0.001$		$P<0.001$		$P<0.001$		$P<0.001$	
LSD ($P<0.05$)	25.17		7.99		21.42		32.77	

with AUDPC of *C. zebrina* for Junee, Trikkala, and 8B40, with AUDPC of *K. caulivora* for Larisa and Trikkala, with AUDPC of *P. trifolii* for Junee, Karridale, DMN12, and 8B40, and with AUDPC of all pathogens combined with Junee, Karridale, Larisa, Trikkala, DMN12, and 8B40 (Table 3).

Except for Trikkala, foliar disease caused large decreases in seed yields of all the cultivars, particularly for Daliak (87%), 8B40 (70%), Larisa (62%), and Junee (51%). Averaged over all cultivars, foliar disease decreased seed production by 38%. There was significant negative correlation of seed yield with AUDPC of *C. zebrina* for Daliak and Esperance, with AUDPC of *K. caulivora* for Larisa, with AUDPC of *P. trifolii* for Daliak, Esperance, DMN12, and 8B40, and with AUDPC of all pathogens combined for Daliak, Esperance, Larisa, and DMN12. With one exception, a significant correlation between seed yield and total disease from all three fungi occurred only when at least one of the pathogens showed significant correlation with yield. Regression coefficients varied between diseases and also between cultivars within a disease. Regression equations involving yield as the dependent variable and total disease from the three pathogens as the independent variable were used to project the potential seed yield in a disease-free situation. Potential percentage yield loss was calculated using these projected yields (Table 2). Potential yield losses in most cultivars were in proportion to the losses recorded in the fungicide trials except for cultivars Esperance and GD56. Actual loss in cultivar Esperance was almost half of the potential loss, indicating some measure of tolerance to foliar disease. Significant correlation indicated that increased clover sward production increased seed yield for cultivars Junee, DMN12, and 8B40.

Table 2. Effects of three foliar diseases on subterranean clover biomass (sward) production (dry weight) and seed yield in 11 cultivars of *Trifolium subterraneum*

T. subterraneum cultivar	Clover sward production (kg ha^{-1} dry wt)		Seed yield (kg ha^{-1})		Seed yield loss (%)	
	Fungicide-treated	Control	Fungicide-treated	Control	Actual	Potential
Daliak	3,190	3,148	385	51	87	89
Dalkeith	656	637	777	494	36	52
Esperance	2,429	2,540	526	336	36	76
Junee	4,596	4,190	692	337	51	54
Karridale	5,507	4,909	1,268	772	39	49
Larisa	4,872	3,328	1,087	410	62	62
Trikkala	3,167	2,972	1,026	976	5	4
Woogenellup	1,398	1,301	659	475	28	33
DMN12.3.3.3 (DMN12)	5,875	5,300	1,178	888	25	39
GD56.8 (GD56)	4,911	4,831	1,465	1,022	30	18
8B40.2.1.1 (8B40)	3,324	2,936	602	178	70	67
Cultivar significance	$P<0.001$		$P<0.001$		$\overline{X} = 43$	$\overline{X} = 49$
Fungicide significance	$P<0.001$		$P<0.001$			
Cultivar × fungicide significance	$P<0.001$		Not significant			
LSD ($P<0.05$)	382					

DISCUSSION

The significant increases in subterranean clover sward production from the benomyl sprays and the fact that all significant disease versus clover sward correlations were negative are clear indications that these foliar diseases can markedly reduce sward production in subterranean clover cultivars. All three pathogens were shown to be able individually to decrease sward production in at least one susceptible cultivar. The decreased production in cultivar Larisa (32%) is almost entirely from *K. caulivora*, a fungus known to be able to cause complete collapse and loss in swards of highly susceptible cultivars (7). The decreased sward production in cultivar 8B40 (12%) is mainly from *C. zebrina*, and this is the first time that losses in sward production from *C. zebrina* have been demonstrated. The decreased sward production in cultivars DMN12 (10%) and Junee (9%) is primarily due to *P. trifolii*, because by 15 October, *P. trifolii* incidence was high and *C. zebrina* incidence was extremely low. This is the first record of *P. trifolii* being able to cause losses in subterranean clover sward production. The sward production losses in cultivar Karridale (11%) are probably due to a combination of moderate *P. trifolii* and low *C. zebrina* infection levels.

The significant increases in seed production from disease control obtained with benomyl sprays and the fact that all significant disease versus seed yield correlations were negative are clear indications that these foliar diseases are the cause of the seed yield losses recorded. Except for cultivar Trikkala, foliar disease resulted in large decreases in seed yield of all subterranean clover cultivars tested. Averaged over all cultivars, the level of foliar disease occurring in these tests resulted in a seed yield decrease of 38%. *K. caulivora* is known to reduce seed yield markedly in susceptible cultivars, up to 92% in Woogenellup

Table 3. Correlation of clover sward production or seed yield with severity of disease (measured as area under the disease progress curve [AUDPC]) caused by *Cercospora zebrina*, *Kabatiella caulivora*, or *Pseudopeziza trifolii*, individually or combined, for 11 cultivars of *Trifolium subterraneum*

T. subterraneum cultivar	Correlation of clover sward production with AUDPC of:				Correlation of seed yield with AUDPC of:				Correlation of clover sward production with seed yield
	C. zebrina	*K. caulivora*	*P. trifolii*	All pathogens	*C. zebrina*	*K. caulivora*	*P. trifolii*	All pathogens	
Daliak	−0.30	···[a]	−0.23	−0.28	−0.81**[b]	···	−0.78*	−0.81**	0.21
Dalkeith	0.21	···	−0.18	−0.18	−0.25	···	−0.48	−0.48	0.08
Esperance	0.18	···	0.05	0.14	−0.76*	···	−0.70*	−0.74*	−0.48
Junee	−0.88**	···	−0.91***	−0.91***	−0.45	···	−0.56	−0.54	0.72*
Karridale	−0.47	···	−0.73*	−0.64*	−0.48	···	−0.41	−0.45	0.37
Larisa	−0.51	−0.90***	0.48	−0.90***	−0.10	−0.75*	−0.07	−0.75*	0.48
Trikkala	−0.71*	−0.65*	−0.59	−0.67*	−0.14	−0.21	−0.12	−0.19	0.21
Woogenellup	···	−0.50	−0.43	−0.52	···	−0.51	0.14	−0.48	−0.16
DMN12.3.3.3	−0.57	···	−0.69*	−0.69*	−0.39	···	−0.67*	−0.64*	0.91***
GD56.8	−0.17	···	−0.37	−0.22	−0.51	···	−0.32	−0.47	−0.52
8B40.2.1.1	−0.89***	···	−0.73*	−0.88**	−0.58	···	−0.63*	−0.60	0.63*

[a]··· = Insufficient data.

[b]Correlations significant at * = $P<0.05$, ** = $P<0.01$, and *** = $P<0.001$.

severely affected by *K. caulivora* (11), but seed yield losses from *K. caulivora* have not been measured previously in Larisa, a cultivar known to have some field resistance to the disease. It is likely that most, if not all, the seed yield decreases in Larisa (62%), Woogenellup (28%), and Trikkala (5%) are solely from *K. caulivora*. *C. zebrina* is also known to reduce seed yield markedly in severely affected commercial stands of cultivar Esperance (M. J. Barbetti, *unpublished*). Because cultivars with high levels of susceptibility to *C. zebrina* (8B40, Esperance, and Daliak) also had moderate to high levels of susceptibility to *P. trifolii*, it is not possible to clearly distinguish which disease has caused most of these observed yield reductions. It is likely, however, that most loss in 8B40 is from *C. zebrina*. All the yield decrease observed in Dalkeith (36%) and most of the seed yield decreases in Junee (51%) and DMN12 (25%) are probably caused by *P. trifolii*. This is the first record that *P. trifolii* can cause seed yield losses in subterranean clover. On cultivars where more than one pathogen is present, it is likely that the presence of one disease affects the damage caused by the other. Such effects are known to occur when *C. zebrina* and *K. caulivora* are both present (3).

Foliar diseases have been shown, in this study, to reduce seed yields in susceptible subterranean clover cultivars in ungrazed or lightly grazed stands. The loss of seed yield is not only of economic importance to seed producers. If disease levels are severe enough to reduce seed yield to below the levels needed to maintain a dominance of subterranean clover in a sward, there can be a steady shift from subterranean clover dominance to that of a mixed-species pasture. The disease effect on seed production is associated with both reduced productivity and persistence of susceptible subterranean clover cultivars. The seed yield losses from *P. trifolii* indicate that this and other fungi previously considered to be only minor or unimportant pathogens of subterranean clover may be much more important than first thought.

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