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Field performance of subterranean clover germplasm in relation to severity of Cercospora disease

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Abstract. Ninety six genotypes, including 14 cultivars, of *Trifolium subterraneum* var. *subterraneum* and var. *yanninicum* were screened in the field for resistance to Cercospora (*Cercospora zebrina*). Seven genotypes, 84S43-13, 84S43-15, EP132Sub-E, 84Y32-59, 83Y83-23, 84Y32-42 and 83Y79-20, were totally resistant to Cercospora disease. A further 26 genotypes, including the cvv. Meteora and Napier, had an incidence of Cercospora of <1 (0–10 scale) and no consequent leaf collapse from the disease (score 0; 0–10 scale), while 15 genotypes had incidence scores between 1 and 2 without any leaf collapse evident. There was excellent overall correlation between Cercospora incidence and leaf collapse across the genotypes tested, with both strong quadratic ($y = -0.17x^2 + 2.50x + 1.33$; $R^2 = 0.89$) and linear ($y = 0.96x + 1.63$; $R^2 = 0.82$) components to this relationship. There was circumstantial evidence of ecogeographical differences for Cercospora resistance among ecotypes collected from different regions. Of eight overseas introductions with Cercospora incidence scores of <2 and a Cercospora leaf collapse score of 0, six were from Sardinia and one each were from Portugal and Greece. In contrast, all seven Sicilian ecotypes had Cercospora incidence scores of 7.75 or greater. The high degree of resistance observed in many of the genotypes to Cercospora highlights the existence of many excellent sources of resistance that could be exploited in breeding and development programmes to minimise production losses in Australian subterranean clover pastures.

Introduction

Subterranean clover (*Trifolium subterraneum*) is still the most important pasture legume species in Western Australia, where it has been sown over 6.5 million ha (Gladstones 1975). Cercospora disease (caused by *Cercospora zebrina*), has been reported on clovers in Western Australia (Barbetti 1983, 1985, 1991; Bokor 1983), eastern Australia (Valder 1954; Anon. 1964, 1975) and in North America (Hanson 1953; Baxter 1955, 1956; Berger 1962; Berger and Hanson 1963; Pratt 1984). *C. zebrina* is well adapted to the Mediterranean-type environment of south-west Western Australia, where it readily oversummers on infested clover residues to release conidia ~2 weeks after the first substantial rainfall of the winter growing season, there being a significant relationship between numbers of conidia released with field disease incidence (Barbetti 1987*c*). On highly susceptible subterranean clover cultivars, it can cause severe losses in herbage and seed yield (Barbetti 1987*a*, 1987*b*; Pratt 1987, 1989), an indication of the need to ensure that future cultivars have adequate resistance. Variation in resistance to Cercospora disease has been demonstrated under controlled conditions (Pratt 1984; Barbetti 1985) and in the field (Barbetti and Nichols 1994), but Barbetti and Nichols (1994) advocated that all potential new cultivars and parental materials are best screened for Cercospora resistance under field conditions as this gives the most reliable indication of commercial field performance.

This paper reports the results of field screening of 82 advanced breeding lines and 14 cultivars of *T. subterraneum* var. *subterraneum* and var. *yanninicum* clovers for resistance to Cercospora.

Methods

Eighty two breeding lines of *T. subterraneum* var. *subterraneum* and var. *yanninicum* clovers were screened for resistance to Cercospora (Table 1). Their performance was compared with 14 control cvv. (Coolamon, Daliak, Dalkeith, Denmark, Esperance, Gosse, Izmir, Junee, Meteora, Mount Barker, Napier, Nungarin, Seaton Park and Urana). Screening was conducted at the University of Western Australia, Shenton Park Field Station, Perth, Western Australia. Plots consisted of double rows 1 m long with a row spacing of 10 cm. A 1 m bare ground buffer surrounded all double rows. There were two replications arranged

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Table 1. Incidence of leaves affected by, and amount of leaf collapse from, *Cercospora zebrina* at the end of October for 96 genotypes of *Trifolium subterraneum* in the field

Genotype	Subspecies	Origin of genotype	Cercospora incidence[A]	Cercospora leaf collapse
84S43-13	S	Crossbred	0	0
84S43-15	S	Crossbred	0	0
EP132Sub-E	S	Sardinia	0	0
83Y79-20	Y	Crossbred	0	0
83Y83-23	Y	Crossbred	0	0
84Y32-42	Y	Crossbred	0	0
84Y32-59	Y	Crossbred	0	0
84S43-18	S	Crossbred	0.25	0
84S43-20	S	Crossbred	0.25	0
EP125Brachy-D	S	Sardinia	0.25	0
Meteora[B]	Y	Greece	0.25	0
83Y70-06	Y	Crossbred	0.25	0
84Y21-01	Y	Crossbred	0.25	0
84Y25-12	Y	Crossbred	0.25	0
84Y32-51	Y	Crossbred	0.25	0
83S37-01	S	Crossbred	0.5	0
CPI 103906F	S	Portugal	0.5	0
EP142Sub-F	S	Sardinia	0.5	0
84Y25-05	Y	Crossbred	0.5	0
78YYS01-04	Y	Crossbred	0.5	0
83Y79-01	Y	Crossbred	0.5	0
83Y79-07	Y	Crossbred	0.5	0
83Y79-26	Y	Crossbred	0.5	0
80S50-02	S	Crossbred	0.75	0
83S05	S	Crossbred	0.75	0
83S19-07	S	Crossbred	0.75	0
84S43-07	S	Crossbred	0.75	0
EP145Sub-D	S	Sardinia	0.75	0
Napier[B]	Y	Crossbred	0.75	0
83Y83-29	Y	Crossbred	0.75	0
78YYS01-01	Y	Crossbred	0.75	0
83Y86-02	Y	Crossbred	0.75	0
83Y86-13	Y	Crossbred	0.75	0
79S12-02	S	Crossbred	1	0
84S43-08	S	Crossbred	1	0
83Y70-04	Y	Crossbred	1	0
EP158BrachySub-B	S	Sardinia	1.25	0
83Y81-13	Y	Crossbred	1.25	0
83Y70-05	Y	Crossbred	1.25	0
83Y86-04	Y	Crossbred	1.25	0
83Y86-12	Y	Crossbred	1.25	0.25
83S27-02	Y	Crossbred	1.5	0
83Y86-10	Y	Crossbred	1.5	0
EP128Brachy-I	S	Sardinia	1.75	0
Gosse[B]	Y	Crossbred	1.75	0
84Y21-07	Y	Crossbred	1.75	0
83Y83-10	Y	Crossbred	1.75	0
83Y86-03	Y	Crossbred	1.75	0
84S20-08	S	Crossbred	2	0
84S45-11	S	Crossbred	2	0
84Y25-09	Y	Crossbred	2	0.25
Coolamon[B]	S	Crossbred	2.25	0
83S33-02	S	Crossbred	2.25	0.25
83Y86-11	Y	Crossbred	2.25	0.25

(*Continued next page*)

Table 1. Continued

Genotype	Subspecies	Origin of genotype	Cercospora incidence[A]	Cercospora leaf collapse
Junee[B]	S	Crossbred	2.5	0
83Y70-03	Y	Crossbred	2.5	0
Denmark[B]	S	Sardinia	2.75	0
84S20-10	S	Crossbred	2.75	0
84Y25-11	Y	Crossbred	3.25	0
84S20-03	S	Crossbred	3.25	0.25
84Y32-09	Y	Crossbred	3.25	0.25
84S20-11	S	Crossbred	3.25	0.5
84Y32-08	Y	Crossbred	3.5	0.5
84S20-02	S	Crossbred	4.5	0.25
83Y86-15	Y	Crossbred	4.75	0.25
84S20-01	S	Crossbred	4.75	0.5
83S27-01	S	Crossbred	5.5	1
Mt Barker[B]	S	Australian naturalised	6.5	0.75
79S04	S	Crossbred	6.75	2.75
Esperance[B]	S	Crossbred	7.25	1.25
Seaton Park[B]	S	Australian naturalised	7.25	2.25
3486-C	S	Sardinia	7.75	1.75
S3615-B	S	Sicily	8.5	7.5
Dalkeith[B]	S	Australian naturalised	8.5	8.75
Daliak[B]	S	Australian naturalised	8.75	5.5
EP158BrachySub-I	S	Sardinia	8.75	6.75
S3609E	S	Sicily	8.75	7.5
S3623A	S	Sicily	9	7.5
S3615-D	S	Sicily	9	7.75
S3615-H	S	Sicily	9.25	9
S3617H	S	Sicily	9.5	5.5
82S23-15	S	Crossbred	9.5	9
CIZ008Sub-K	S	Turkey	9.5	9.7
80S42.8.1.1	S	Crossbred	9.75	5.75
82S58-11	S	Crossbred	9.75	7
82S58-08	S	Crossbred	9.75	7.5
Izmir[B]	S	Turkey	9.75	9
Urana[B]	S	Crossbred	10	8
S3617D	S	Sicily	10	8.5
81S31.18-1	S	Crossbred	10	8.75
81S04-03	S	Crossbred	10	9.25
CIZ008Sub-E	S	Turkey	10	9.5
CPI 075312	S	Libya	10	9.5
81S03-25	S	Crossbred	10	9.55
Nungarin[B]	S	Crossbred	10	9.65
SY002	S	Syria	10	9.85
Significance of genotypes			$P < 0.001$	$P < 0.001$
LSD ($P < 0.05$)			1.89	1.28

[A]0–10 scale for Cercospora incidence and leaf collapse, where 0 = healthy; 10 = 100% leaves affected. S = var. *subterraneum*, Y = var. *yanninicum*.
[B]Registered cultivars.

in a randomised block design. Plots were sown in mid-May 1997, at a density of 60 kg/ha by sowing seed into 2-cm-deep furrows and lightly raking to cover. Seed was inoculated with *Rhizobium* and lime pelleted prior to sowing. Superphosphate (9.1% P, 10.5% S) at a rate of 200 kg/ha was topdressed prior to seeding and extra applications of superphosphate and potash were made as required for normal plant growth during the season. Dimethoate at a rate of 50 mL/ha was applied at approximately fortnightly intervals throughout the season to control any red-legged earthmite (*Halotydeus destructor*) or aphids present. Plots were hand-weeded and irrigated when necessary.

Thirteen different *C. zebrina* isolates collected from diseased subterranean clover in different areas of Western Australia were used to produce inoculum for this study, WAC2030 collected from Three Springs in 1981, WAC 3883 from Denmark in 1983, WAC isolates 5082, 5088, 5091, 5093, and 5095, all from Shenton Park in 1987, WAC5097 from Denmark in 1987, and WAC isolates 5106, 5108, 5113, 5114 and 5116, collected from Northam in 1987. A mixture of *C. zebrina* isolates, collected from the above areas in which *C. zebrina* regularly occurs in Western Australia, was used to ensure that the test genotypes were exposed to the maximum available range of variation in the pathogen. These isolates are lodged in the Department of Agriculture Western Australia Herbarium Collection. One week after sowing, millet (*Panicum miliaceum*) seed inoculum, grown as separate flasks of the 13 different *C. zebrina* isolates, was mixed and applied at a rate of 400 kg/ha. To prepare the *Cercospora* inoculum, five colonised agar plugs (4-mm-diameter) of each isolate were used to inoculate 1 L conical flasks containing sterilised millet seeds. These were prepared by soaking 200 g of seeds overnight in distilled water, draining excess water and then autoclaving at 121°C for 20 min on three consecutive days. Inoculated seeds were incubated at 25°C on a laboratory bench for 21 days and were shaken daily to facilitate uniform colonisation of the seed.

The disease had established in the experimental plots by early July. Cercospora incidence was assessed in the plots on 29 October 1997 on a 0–10 scale (Barbetti 1987*a*), where 0 = nil disease, 1, = 1–10, 2 = 11–20, 3 = 21–30, 4 = 31–40, 5 = 41–50, 6 = 51–60, 7 = 61–70, 8 = 71–80, 9 = 81–90 and 10 = 91–100% of leaves affected. Swards were also assessed on a 0–10 scale for the amount of leaf collapse from Cercospora infection, where 0 = nil disease, and 10 = >90% of leaves collapsed from Cercospora infection. A score of 10 generally represented total collapse of the sward. For each plot, both for Cercospora incidence and for the amount of leaf collapse, a single 'whole of plot' assessment score was made by two different assessors and subsequently these two scores were averaged to present a single assessment score per plot.

Cercospora incidence and leaf collapse scores were analysed using ANOVA with Genstat 6 Edition (Genstat Procedure Library Release PL14). Treatment means were compared using least significant difference (LSD) tests. Regression analysis was conducted to determine the relationship between Cercospora incidence and leaf collapse across the genotypes tested.

Results

Seven genotypes, 84S43-13, 84S43-15, EP132Sub-E, 84Y32-59, 83Y83-23, 84Y32-42 and 83Y79-20, were totally resistant to Cercospora disease (Table 1). A further 26 genotypes, including var. *yanninicum* cvv. Meteora and Napier, had an incidence of Cercospora <1 and no observable leaf collapse from the disease; 15 genotypes had incidence scores between 1 and <2 but also without any leaf collapse evident; ten genotypes had incidence scores between 2 and <3; eight genotypes had incidence scores between 3 and <5; three genotypes had incidence scores between 5 and <7; eight genotypes had incidence scores between 7 and <9 and 19 genotypes were highly susceptible with incidence scores between 9 and 10. There was excellent overall correlation between Cercospora incidence and leaf collapse across the genotypes tested, with both strong quadratic ($y = -0.17x^2 + 2.50x + 1.33$; $R^2 = 0.89$) and linear ($y = 0.96x + 1.63$; $R^2 = 0.82$) components to this relationship. All genotypes with Cercospora incidence scores <5 had corresponding minimal leaf collapse scores of <1 and, where leaf collapse scores of 5 or more occurred, the incidence scores were 8.5 or greater.

Discussion

The high degree of resistance observed in many of the genotypes to Cercospora highlights the existence of many excellent sources of resistance that could be exploited in cultivar breeding and development programmes to minimise future production losses from this disease. It was interesting that the majority of the crossbred lines and overseas introductions were more resistant to Cercospora than 11 of the 14 comparison cvv. (exceptions being cvv. Meteora, Gosse and Napier), suggesting that the resistance in these genotypes could be utilised to reduce the impact of Cercospora in subterranean clover pastures of southern Australia.

There appeared to be greater levels of resistance to Cercospora in var. *yanninicum* than var. *subterraneum*. Among the 36 genotypes of var. *yanninicum* tested, 81% had Cercospora incidence scores of <2 and a Cercospora leaf collapse score of 0, compared with only 41% of var. *subterraneum* genotypes. Furthermore, no var. *yanninicum* genotypes had leaf collapse scores >0.25. The little or no leaf collapse observed in these genotypes is of significant importance to farmers as it is the level of leaf collapse that most directly relates to reductions in productivity (Barbetti 1987*a*). At the other end of the scale, all highly susceptible genotypes were var. *subterraneum*.

There was some circumstantial evidence of ecogeographical differences for Cercospora resistance among ecotypes collected from different regions. Of the eight new overseas introductions with Cercospora incidence scores of <2 and a Cercospora leaf collapse score of 0, six (EP132Sub-E, EP125Brachy-D, EP142Sub-F, EP145Sub-D, EP158BrachySub-B and EP128Brachy-I) were from Sardinia, whereas the remaining genotypes, CPI 103906F and cv. Meteora, were from Portugal and Greece, respectively. Cultivar Denmark, originally collected from Sardinia, also had a low (2.75) incidence score and no leaf collapse. The remaining 14 overseas introductions had Cercospora incidence scores of 7.75 or greater, with seven originating from Sicily, three from Turkey, two from Sardinia and one each from Libya and Syria. Sardinian ecotypes, therefore, appear to be more resistant to Cercospora than these other ecotypes, particularly compared with Sicilian ecotypes. This raises the possibility that natural selection has previously occurred in Sardinia to favour resistance to this disease, despite the fact that we were not able to locate published records confirming the presence of this disease in Sardinia. However, larger numbers of lines within these ecotype groupings need to be tested before any definite conclusions can be drawn about the importance of geographic origins in relation to resistance to Cercospora.

There was also an association of disease scores and flowering time (flowering times were those recorded in other studies using the same genotypes). Of all 24 genotypes with a Cercospora incidence >8.0, all but three (S3609E, S3623A and 80S42.8.1.1) have a flowering time of less than 105 days after an early May sowing in Perth (P. Nichols, unpublished data). This suggests the possibility of linkage of early flowering genes with genes for Cercospora susceptibility.

Although in general there was excellent correlation between Cercospora incidence and Cercospora leaf collapse, some individual genotypes had more leaf collapse from Cercospora than others for a similar level of incidence. For example, the cvv. Dalkeith and Daliak had the same Cerspospora disease incidence score of 8.5, but Daliak had a much lower Cercospora leaf collapse score of 5.5 compared with Dalkeith with a leaf collapse score of 8.75.

The extent of Cercospora incidence and leaf collapse on susceptible genotypes in this study was very high and likely to approach the maximum levels that occur in paddock situations under natural disease outbreaks. For this reason, it is considered that a single year of data is sufficient to provide comparative results, especially as the relative performance of cultivars was often similar to that reported in an earlier study (Barbetti and Nichols 1994).

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