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Fungal diseases of temperate annual pasture legumes in southern Queensland

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Summary. Diseases of temperate annual pasture legumes in subtropical southern Queensland were surveyed during 1992 and 1993. The following pathogenic organisms were recorded: *Colletotrichum trifolii*, *Stemphylium vesicarium*, *Oidium* sp., *Uromyces anthyllidis*, *Uromyces striatus* and *Pseudopeziza medicaginis* from annual *Medicago* spp.; *Rhizoctonia solani* and *Colletotrichum destructivum* from *Ornithopus* spp.; and *Oidium* sp. from *Trifolium subterraneum*. Three of these disease interactions had not been previously recorded in Queensland and 5 were new reports for Australia. Rust was the most frequently observed and widespread disease on annual medics (44% of *M. polymorpha* samples). All other diseases of annual medics were found infrequently (2–18% of samples). In contrast, both serradella and subterranean clover were relatively free from any diseases.

The years during which the survey was conducted were dry (as low as 31% of mean March–October rainfall) and the expression of disease may have been restricted. Nevertheless, this improved knowledge of diseases of temperate annual legumes in southern Queensland will assist in the future selection and breeding of suitable cultivars for use in the subtropics.

Introduction

In southern inland Queensland, temperate annual pasture legumes, particularly the annual medics (*Medicago* spp.), subterranean clover (*Trifolium subterraneum* L.) and serradella (*Ornithopus* spp.), are used in pastoral systems and as rotations in cropping systems. Subterranean clover and serradella are adapted to acid, sandy and loamy surfaced soils which have a limited arable potential, where they are sown to provide high protein forage in permanent mixed pasture. The medics, particularly snail [*M. scutellata* (L.) Miller] and barrel (*M. truncatula* Gaertner), are adapted to the alkaline cracking clays and alkaline trend duplex soils that constitute the northern grain belt of Australia. They are used in rotation pastures to sustain soil nitrogen fertility for cropping (Lloyd *et al.* 1991). Additionally, burr medic (*M. polymorpha* L.) is naturalised widely on the cracking clays of the cropping belt. In southern Australia, where these legumes are used mainly in cropping systems (Puckridge and French 1983), the diseases which limit production and persistence have been identified (Barbetti 1983; Murray 1992). These include: phoma blackstem, stagonospora leaf spot and phytophthora root rot of medics; and kabatiella leaf scorch and a number of root rots of subterranean clover (Barbetti and Sivasithamparam 1986; Barbetti *et al.* 1986; Johnstone and Barbetti 1987; Madin 1993). In the development of new cultivars for use in southern Australia, selection for resistance to these important diseases is carried out. For example, Junee subterranean clover is resistant to clover scorch and tolerant to a complex of root rotting fungi (Oram 1990).

The importance and distribution of disease on the persistence and productivity of annual legumes in the subtropics has not been determined. As these legumes are becoming more widely accepted and utilised in subtropical northern Australia, a knowledge of the most important diseases occurring in the local environment is essential for the selection of adapted cultivars. Although there have been no confirmed reports of major epiphytotics, anecdotal evidence suggests that during wet winter seasons, diseases (or syndromes producing

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disease-like symptoms) are often encountered (D. L. Lloyd pers. comm.).

This study aimed to determine the importance of diseases affecting temperate annual pasture legumes in southern Queensland, including both naturalised and commercial strains of burr medic (*M. polymorpha*), snail medic (*M. scutellata*), barrel medic (*M. truncatula*), serradella (*Ornithopus* spp.) and subterranean clover (*Trifolium subterraneum*). The incidence and distribution of diseases on these hosts was assessed by field survey. The relative susceptibilities of annual medics to *Colletotrichum trifolii* Bain and Essary, and *Phytophthora medicaginis* Hansen and Maxwell were determined in glasshouse tests and in field trials.

Materials and methods

Collection of material

During visits to experimental trials, commercial pastures and roadsides throughout southern Queensland during the growing seasons of 1992 and 1993 (March–October), the target species were examined for symptoms of disease. A total of 49 sites throughout southern Queensland were visited during both years. The survey area was bounded by: Wooroolin (26°24'S, 151°48'E), Brisbane (27°30'S, 153°0'E), Stanthorpe (28°42'S, 151°54'E), Cunnamulla (28°03'S, 147°14'E) and Charleville (26°23'S, 146°15'E). The medic species were collected from either alkaline cracking clay soils or brown, dark and red duplex soils with an alkaline pH trend down the soil profile. Samples of serradella and subterranean clover were obtained from acid, yellow duplex soils or acid granitic sands. At some of the survey sites more than one target legume species was assessed for disease. Plants exhibiting abnormal symptoms were collected and isolations were carried out within 48 h. Rainfall during the survey period for Charleville, Cunnamulla, Roma and Toowoomba is recorded in Table 1.

Treatment of samples

Biotrophic pathogens were identified but pathogenicity tests were not conducted, as these organisms cannot be cultured. Plant samples showing signs of common leaf spot [*Pseudopeziza medicaginis* (Lib.) Sacc.], rust (*Uromyces* spp.) or powdery mildew (*Oidium* spp.) were examined microscopically. Plant samples exhibiting symptoms of necrotrophic pathogens were treated by the methods described below.

Isolation procedures for foliar material. Leaves and stems were surface sterilised for 1.5 min in a 2% available chlorine solution (NaOCl). The sections were then blotted dry and cut into pieces of less than 5 mm diameter. These were placed on 2% water agar plates and incubated at 25°C under near-ultraviolet light (NUV).

Isolation procedures for root material. Roots were washed to remove most of the soil and placed under running tap water for 2 h. After washing, the lateral roots and taproots were treated separately. Lateral roots were either placed directly onto water agar plates, or surface sterilised as above and then placed onto water agar plates. Taproots were dipped in 100% ethanol and flamed, and then cut into about 2 mm pieces and plated onto water agar plates. All root isolation plates were incubated in the dark at 25°C.

Identification of isolates

After incubating for 2–3 days, isolation plates from foliar and root material were examined under a stereo-microscope. Fungal growth was subcultured by transferring hyphal tips or a single conidium onto full strength potato dextrose agar (PDA, Difco Laboratories, USA). Isolations from diseased root tissue, suspected of being infected with *Phytophthora* spp., were plated on Ca^{2+} V8 agar media (200 mL Campbell's, Australia, V8 Juice, 2 g $CaCO_3$, 20 g agar, 800 mL deionised water) amended with PVP: 15 g/mL Pimaricin, 100 g/mL Vancomycin and 50 g/mL Penicillin (Eckert and Tsao 1962; Tsao and Ocana 1969). All cultures were incubated under the conditions described previously.

Once pure cultures were obtained, the sexual/asexual reproductive structures were microscopically examined to identify the fungi. All isolates were assigned a University of Queensland Botany Department identification number (UQ) and stored under sterile water (Boeswinkel 1976).

Isolates of some fungi that are commonly found as saprophytes, such as *Colletotrichum* spp. (with curved spores) and *Alternaria* spp., were initially tested for their pathogenicity. When numerous isolates were found to be non-pathogenic, further testing of similar isolates was abandoned.

Pathogenicity tests

A number of techniques were used to test the pathogenicity of isolates. These depended on the type of fungus, and the site and host of the original isolation.

Table 1. Rainfall (mm) at representative locations within the survey area for the sampling period (March–October, 1992 and 1993)

	Charleville			Cunnamulla			Roma			Toowoomba		
	Mean	1992	1993	Mean	1992	1993	Mean	1992	1993	Mean	1992	1993
March	60	1	16	40	0	11	65	14	5	99	38	20
April	32	1	7	27	2	2	33	29	4	66	68	0
May	32	22	0	30	8	15	37	68	6	55	61	56
June	28	1	6	26	3	58	35	1	46	57	8	9
July	28	8	50	23	20	27	37	13	44	54	41	54
August	20	9	50	17	21	26	26	25	18	40	26	19
September	21	14	74	19	6	22	32	19	35	46	46	41
October	35	23	50	25	48	51	51	22	46	72	41	59
Total	256	79	253	208	108	212	315	191	204	491	329	258

Leaf inoculation with Colletotrichum *spp. and* Stemphylium vesicarium. Actively growing plants, 3–6 weeks old, were sprayed to runoff with a suspension of about 1 x 10^6 conidia/mL using an atomiser. Plants were placed in a non-illuminated dew chamber at 20–23°C overnight for approximately 15 h. Alternatively, the plants were covered with a moistened plastic bag secured with a rubber band and incubated in a glasshouse overnight (20–25°C). Plastic bags were removed the next morning. The plants were then grown for a further 10–14 days in a glasshouse growth cabinet under ambient light conditions at 20–25°C, and observed for symptom development. The isolate was considered to be non-pathogenic if plants did not display symptoms or signs of disease after 3 weeks.

Leaf inoculation with Leptosphaerulina trifolii. The inoculation method was adapted from the one used to test the resistance of lucerne genotypes to *Leptosphaerulina* sp. (Leath 1991). Sporulating cultures of *Leptosphaerulina trifolii* (Rostr.) Petr. growing on PDA in Petri dishes were inverted approximately 25 cm above actively growing 3–6-week-old plants in a non-illuminated dew chamber at 23°C and 100% humidity. Cultures were left in that position for a minimum of 10 h after which plants were removed and allowed to dry slowly out of direct sunlight. They were then placed in a glasshouse growth cabinet under ambient light conditions and kept at 23°C for 3 weeks. The plants were subsequently examined for symptoms typical of leptosphaerulina leaf spot. Water agar plates were simultaneously exposed during inoculation beside the test plants to confirm that ascospores had been liberated from the *Leptosphaerulina* cultures.

Damping-off test. The pathogenicity of isolates from roots to seedlings was determined by infesting potting mix (1 : 3 peat : sand by volume) in a 10 cm diameter pot with a slurry of the test fungus. For each pot the slurry consisted of a culture of the isolate grown on half-strength V8 media for 14 days which had been blended in 40 mL sterile water. Control pots were infested with a slurry made from uninoculated plates of media. Twenty seeds were planted in each of the pots and watered twice a day. For fungi such as *Phytophthora medicaginis* that produced motile zoospores, saucers were placed under the pots so that high soil moisture levels could be maintained. The saucers were removed every second day to facilitate free drainage. After 30 days incubation in a controlled-temperature (18–22°C) cabinet under ambient light conditions, the numbers of germinated seedlings in each pot were counted.

Seedling root rot test. Five-week-old seedlings were inoculated with isolates which produced damping-off in the previous test. A slurry of the test fungus was prepared as for the damping-off test, poured onto the surface of the potting mix (1 plate blended in 40 mL water for each pot) and incorporated into the top 2–3 cm of the potting mix. Plants were watered to field capacity twice a day and, for the isolates that produced motile zoospores, saucers were placed under the pots and treated as above. After 20 days the plants were removed and the roots washed to allow inspection of disease. Symptom development was noted.

Re-isolation from experimentally infected material

Isolations from leaf and root lesions were made after pathogenicity testing, to satisfy Koch's postulates (Agrios 1997).

Susceptibility of annual medics to Colletotrichum trifolii

Twenty-one annual medics from *M. orbicularis* (L.) Bartalini, *M. polymorpha, M. scutellata* and *M. truncatula* were tested for resistance to *C. trifolii*. Inoculations were carried out on 5-week-old seedlings as previously described. Plant numbers ranged from 25 to 55 for the different lines. Assessments of disease reaction were made 10 days after inoculation. One-way ANOVA and Tukey pairwise comparisons were conducted using Minitab Release 11 on the arcsine-transformed data for percentage resistant plants (Minitab Statistical Software, USA).

Phytophthora medicaginis *field screening of annual medics*

A field infested with *P. medicaginis* at the Queensland Department of Primary Industries Research Station, Gatton, Queensland, was chosen for this study. Six replicates of 24 cultivars were sown in 3 m rows, 0.5 m apart, with the chickpea (*Cicer arietinum* L.) cv. Tyson (a cultivar which is known to be highly susceptible to root rot; Dale and Irwin 1991) sown every sixth row and at both ends of each replicate. Cultivars were randomised within each block. Replicates were 1 m apart and set out in a 2 by 3 block design. Five weeks after sowing all rows were thinned and the number of seedlings in each row recorded (about 30). From 10–12 weeks after sowing the field was irrigated to saturation on 3 occasions to facilitate infection. Thirteen weeks after sowing the number of remaining plants in each row was recorded. Six weeks later all plants were removed from the soil. The roots were washed and assessed for disease development using a 1–5 scale: 1, no visible symptoms; 2, small tan coloured lesions on lateral roots; 3, numerous lateral root lesions and small lesions on tap root; 4, girdling tap root lesions; 5, plant death (Irwin and Maxwell 1980).

To determine uniformity of soil inoculum, data for the number of surviving *C. arietinum* cv. Tyson plants for each replicate were subject to chi-square analysis to test for agreement between observed and expected ratios (Zar 1984). When subject to analysis, the data did not fit the model of 1:1:1:1:1:1. Results for replicate 2 were then disregarded and the remaining data were re-tested. The data then fitted the model of 1:1:1:1:1 (P = 0.9 – 0.95). Data for replicate 2 were thus omitted from the analysis, leaving 5 replicates.

Due to the discontinuous nature of the disease severity ratings, a non-parametric method was used to analyse the data. The Kruskal-Wallis technique, a 1-way non-parametric analysis of variance (Conover 1980), which approximates the chi-square distribution, was performed on the rankings of disease severity (the higher the number, the more susceptible the cultivar).

Results

Disease-causing agents were not isolated or identified for all specimens that showed symptoms of abnormality. Many fungal isolates that were identified and tested were found to be non-pathogenic under the test conditions.

Fungal pathogens of annual medics in southern Queensland

A number of organisms were isolated from lesions on leaves and stems of the annual medic species sampled. No pathogenic isolates were obtained from annual medic root samples.

Diseases caused by Uromyces *spp.* The 2 rust fungi, *U. striatus* J. Schröt and *U. anthyllidis* (Grev.) Schröt produce the same symptoms on infected leaflets. This disease is characterised by red-brown pustules

Table 2. Summary of disease encountered on temperate annual legumes in southern Queensland, in surveys conducted during the growing seasons of 1992 and 1993

Number of assessments at which disease was present during the surveys (percentages are indicated in parentheses)

Disease or symptom	*M. laciniata*	*M. minima*	*M. polymorpha*	*M. scutellata*	*M. truncatula*	*Medicago* spp.	*Ornithopus* spp.	*T. subterraneum*
Rust		1 (17)	20 (44)	5 (45)	2 (18)			
Anthracnose			3 (7)	1 (9)				
Leaf spot[A]			9 (20)	1 (9)	3 (27)	1 (20)	1 (14)	1 (9)
Root rot			1 (2)	1 (9)		1 (20)		2 (18)
Stemphylium leaf spot			2 (5)	2 (18)	2 (18)			
Rhizoctonia root rot							1 (14)	
Powdery mildew						1 (20)		2 (18)
No disease	7 (100)	5 (83)	16 (35)	3 (27)	4 (37)	2 (40)	5 (72)	6 (55)
Total assessments	7	6	45[B]	11	11	5	7	11

[A] Includes unidentified leaf spots and *Pseudopeziza* sp.
[B] Total number of disease samples within columns is more than 100% for some species due to more than one disease being recorded at a single assessment.

containing urediniospores, which rupture the epidermis of the leaflets and petioles, and also the stems and pods in some samples. The 2 species are easily separated on urediniospore shape, and by germ pore number and position. Urediniospores of *U. striatus* are elliptical and have 4 equatorial germ pores, while those of *U. anthyllidis* possess thick walls, are subglobose, and have 5–8 scattered germ pores (Laundon and Waterston 1965). *Medicago polymorpha* was susceptible to both rusts, and samples were occasionally found with both occurring concurrently on the same leaflet. Hence, for correct species determination it was necessary to make microscopic examinations of urediniospores from single pustules. Rust was the most commonly encountered disease on *M. polymorpha* with 20 (44%) samples infected; *U. anthyllidis* was recorded on 18 (90%) of these samples and *U. striatus* on 4 (20%) samples. Two samples were infected with both rusts. Rust (*U. striatus*) was also the most commonly encountered disease on *M. scutellata* (45% of samples). Rust was observed twice (18%) on *M. truncatula* and only once (17%) on *M. minima* (L.) Bartalini (Table 2).

Disease caused by Stemphylium vesicarium. Stemphylium leaf spot caused by the fungus *S. vesicarium* Wallr. displayed symptoms identical to those previously reported for the cool temperature biotype of *S. botryosum* Wallr. on lucerne in North America (Gilchrist 1990), and for *S. vesicarium* on lucerne in southern Queensland (Irwin 1984). In this study, stemphylium leaf spot was recorded on naturalised *M. polymorpha*, *M. scutellata* cv. Sava and *M. truncatula* cvv. Jemalong and Mogul. The pathogen causes discrete circular lesions with bleached centres and necrotic margins on the leaflets. These lesions often coalesce to produce a generalised blight. Inch *et al.* (1993) found that stemphylium leaf spot was a predominant disease of lucerne in southern Queensland during the cooler months of the year. Stemphylium leaf spot was found on 2 (18%) samples for both *M. scutellata* and *M. truncatula*, and also on 2 samples (5%) for *M. polymorpha* (Table 2).

Disease caused by Colletotrichum trifolii. Anthracnose, caused by the fungus *C. trifolii,* was found on *M. scutellata* and *M. polymorpha*. This fungus causes discrete leaf lesions, predominantly on young leaves, and lesions that girdle petioles and stems causing them to collapse. Under moist conditions, pink-white conidial masses could be seen in stem lesions, and in some samples black bristle-like setae were also seen. Anthracnose was identified on *M. polymorpha* and *M. scutellata* in 3 (7%) and 1 (9%), respectively, of the assessments (Table 2).

Diseases caused by Oidium *sp.* Powdery mildew was found on only one occasion in the field, on *M. lupulina* L. A powdery grey to white mycelial growth covered areas of the upper surface of leaves and less commonly stems,

Table 3. Reaction of annual medics to *C. trifolii*

Means followed by the same letter are not significantly different at $P = 0.05$
Tukey comparison performed on the arcsine-transformed means of percentage resistant data
DSI, disease severity index

Cultivar or line	Species	DSI	Transformed % resistant
Paraggio	*M. truncatula*	0	90.0a
Z769	*M. truncatula*	0.11	90.0a
Cyprus	*M. truncatula*	0.15	90.0a
Z274	*M. truncatula*	0.16	90.0a
Z787	*M. truncatula*	0.25	90.0a
Z788	*M. truncatula*	0.30	90.0a
Sephi	*M. truncatula*	0.35	90.0a
Z783	*M. truncatula*	0.36	90.0a
Mogul	*M. truncatula*	0.52	90.0a
T117	*M. truncatula*	0.23	81.0a
Parabinga	*M. truncatula*	0.60	80.0a
Caliph	*M. truncatula*	0.76	72.9ab
SA8460	*M. orbicularis*	1.93	68.6ab
SA2552	*M. orbicularis*	2.61	52.2bc
Serena	*M. polymorpha*	3.26	28.2cd
SA3110	*M. scutellata*	4.46	10.8d
Kelson	*M. scutellata*	3.74	9.8d
C. Valley	*M. polymorpha*	3.98	3.5d
Sava	*M. scutellata*	4.13	3.3d
Santiago	*M. polymorpha*	4.23	2.4d
SA1868	*M. scutellata*	3.91	0d

accompanied by chlorosis and necrosis in older infections. The disease was also recorded on *M. sphaerocarpos* Bertol. cv. Orion growing in a glasshouse (Table 2).

Disease caused by Pseudopeziza medicaginis. *Pseudopeziza medicaginis* (Lib.) Sacc., the cause of common leaf spot of lucerne, was found at a number of locations on naturalised burr medic (*M. polymorpha*). This pathogen, although not a biotroph, is difficult to culture (K. T. Leath pers. comm.). A culture of the fungus was successfully established, but it was precluded from pathogenicity testing due to its extremely slow growth. The disease was characterised by numerous black spots in which apothecia had ruptured the epidermis. Heavy infections caused defoliation.

Pathogens of serradella in southern Queensland

Two fungi, one isolated from leaves and the other from roots of serradella, were shown to be pathogenic in the study. The diseases caused by these fungi are briefly described.

Disease caused by Colletotrichum destructivum. *Colletotrichum destructivum* O'Gara isolated from *O. compressus* L.*,* causes anthracnose on leaflets and petioles. It is characterised by necrotic lesions with irregular margins that coalesce, girdling the petioles and leading to the collapse of leaves. Masses of orange conidia are produced in acervuli, formed within lesions, during periods of high humidity.

Disease caused by Rhizoctonia solani*. Rhizoctonial solani* Kühn, also isolated from *O. compressus,* causes a rot of the taproot just below the soil surface resulting in chlorosis and wilting of affected plants and eventually death. Brown hyphal strands were observed on the surface of diseased roots that, upon microscopic examination, had branching characteristic of *Rhizoctonia* spp.

One sample each of leaf spot (14% of samples) and rhizoctonia root rot (14%) were noted for serradella. Five (72%) of the serradella assessments were free from disease.

Pathogens of subterranean clover in southern Queensland

Subterranean clover was assessed on 11 occasions, mostly on the southern Darling Downs (10 assessments from 2 sites). Two (18%) of the samples were infected with powdery mildew, while 1 (9%) and 2 (18%) of the samples expressed symptoms of leaf spot and root rot respectively. No disease-causing agents were identified for these samples. More than half (55%) of the samples did not display symptoms of any disease (Table 2). None of the fungi isolated from lesions on subterranean clover leaves and roots were pathogenic.

Disease caused by Oidium *sp.* Powdery mildew caused by the fungus *Oidium* sp. was recorded on subterranean clover at one site. Characteristic growth of powdery white mycelium covered the leaflets; in severe infections chlorosis and necrosis of leaf tissue were observed.

Susceptibility of annual medics to Colletotrichum trifolii

Significant differences in disease reaction occurred within and between different annual medic species (Table 3). Generally *M. truncatula* lines were more resistant than the other species. *Medicago polymorpha* and *M. scutellata* both expressed low to moderate levels of resistance. The 2 lines of *M. orbicularis* exhibited moderate levels of resistance.

Phytophthora medicaginis *field screening of annual medics*

Disease severity ratings for the annual medic cultivars and lines are shown in Table 4. Two naturalised lines of *M. polymorpha* (from Gatton and Roma) germinated poorly owing to excessive hard seededness and were excluded from the analysis. Generally, the annual medic

Table 4. Kruskal-Wallis statistics for disease severity index (DSI) of reaction to phytophthora root rot in the field

Ranks followed by the same letter are not significantly different at $P = 0.05$

Cultivar or line	Species	DSI	Total rank
SA11292	*M. truncatula*	1.05	18.5a
Z508	*M. polymorpha*	1.13	38.5ab
Circle Valley	*M. polymorpha*	1.13	37.5ab
Cyprus	*M. truncatula*	1.46	34.5abc
Borung	*M. truncatula*	1.17	40.0abc
Sair	*M. scutellata*	1.19	43.0abc
Jemalong	*M. truncatula*	1.16	51.5abc
Sava	*M. scutellata*	1.20	54.5abc
Sanza	*M. truncatula*	1.21	49.5abc
Santiago	*M. polymorpha*	1.22	45.5abc
SA1868	*M. scutellata*	1.23	47.0abc
SA4245	*M. polymorpha*	1.26	47.5abc
Serena	*M. polymorpha*	1.29	62.0abc
SA8460	*M. orbicularis*	1.40	79.5abc
Mogul	*M. truncatula*	1.41	73.0abc
Kelson	*M. scutellata*	1.51	73.0abcd
SA3110	*M. scutellata*	1.52	87.0abcd
SA2552	*M. orbicularis*	1.56	85.0abcde
Sephi	*M. truncatula*	1.62	91.0bcde
Parabinga	*M. truncatula*	1.68	92.0cde
Caliph	*M. truncatula*	1.96	108.0de
Paraggio	*M. truncatula*	2.07	113.0e

lines possessed good levels of resistance, with some of the *M. truncatula* lines being the most susceptible.

In the Tyson chickpea check rows, 96.8% of the plants were killed by *P. medicaginis*, indicating that the experimental site and environmental conditions were conducive to disease development.

Discussion

The range and incidence of diseases of temperate annual legumes were limited, probably due to lower-than-average rainfall that was recorded during both growing seasons in most of the areas surveyed. It is not known if the same spectrum of diseases would have been encountered if the survey had been carried out in years of higher rainfall.

Lists of diseases occurring on temperate annual legumes have been compiled for some countries (Chilton *et al.* 1943; Lamprecht and Knox-Davies 1984), and for southern Australia (Barbetti and Sivasithamparam 1986; Murray 1992). Published records were compiled for Queensland by Simmonds (1966), but pathogenicity testing was not performed on the organisms listed in that compilation. This study has revealed diseases not previously reported for Queensland (*S. vesicarium* on *M. truncatula, U. striatus* on *M. minima* and *M. truncatula*), and some that are new for Australia (*C. destructivum* on *O. compressus*, *C. trifolii* on *M. polymorpha* and *M. scutellata*, and *S. vesicarium* on *M. scutellata* and *M. polymorpha*).

The findings of *U. striatus* on *M. minima* and *M. truncatula* are first records for Queensland, although they have been previously recorded elsewhere in Australia (Cook and Dube 1989). In this study *U. anthyllidis* was found only on *M. polymorpha,* but it has previously been reported on a number of other annual medics within Australia (Cook and Dube 1989; J. L. Alcorn unpublished data), and on *M. arabica* (L.) Hudson and *M. lupulina* in New Zealand (Pennycook 1989).

Stemphylium leaf spot has been reported on subterranean clover in Victoria and Western Australia (Woodcock and Clarke 1983; Shivas 1989), on *M. polymorpha* in South Africa and North America (Lamprecht *et al.* 1984; Farr *et al.* 1989), and on *M. truncatula* in South Australia and South Africa (Lamprecht *et al.* 1984; Cook and Dube 1989). Hence the reports of stemphylium leaf spot found in this study are first reports for *M. scutellata* and *M. polymorpha* in Australia and for *M. truncatula* in Queensland. Not all of the isolates of *Stemphylium* sp. were found to be pathogenic. This apparent lack of pathogenicity may have been due to resistance in the test host, as many of the *Stemphylium* sp. isolates were obtained from samples of naturalised burr medic, and these isolates were tested for their pathogenicity on commercial cultivars. Variation in virulence of this pathogen has been shown to occur in southern Queensland on lucerne (Irwin and Bray 1991). All of the pathogenic isolates produced symptoms on their host identical to those produced by the cool temperature biotype on lucerne (Gilchrist 1990). The same symptoms were seen on field-infected specimens. Stemphylium leaf spot, although found on 3 different annual medic species, was never severe, with only a small number of scattered lesions observed on any one plant. Inch *et al.* (1993) suggested that the low disease severity levels that they obtained for *S. vesicarium* on lucerne were due to the lower-than-average rainfall during the winter months when the trial was conducted. The low disease severity levels encountered during this current survey may also have been due to low rainfall.

Leptosphaerulina trifolii, which causes pepper spot, has been recorded previously in Queensland on *M. polymorpha* (J. L. Alcorn unpublished data). In the pathogenicity tests, the number of ascospores which

were liberated was poor, but germination of these spores on water agar plates was observed. All isolates of *L. trifolii* tested during this study were non-pathogenic under the test conditions.

Barbetti (1988) considered rust of annual medics to be of relatively minor importance in the temperate areas of Australia. Conversely, *Phoma medicaginis* Malbr. and Roum., the causal agent of Phoma blackstem and *Stagonospora meliloti* (Lasch) Petr., the causal agent of stagonospora leaf spot were considered the most serious diseases of annual medics. Neither of these fungi have been recorded on annual medics in Queensland. A preliminary survey of foliar diseases of annual medics in South Africa revealed 9 pathogenic fungi occurring in the field (Lamprecht and Knox-Davies 1984) but no attempt was made to determine the relative importance of these pathogens. Nevertheless, these authors considered *Phoma medicaginis* to be the most prevalent pathogen, and noted that one serious epidemic of powdery mildew had occurred during the period of the survey. All of the pathogens they encountered, except *Erysiphe polygoni* DC., had previously been recorded in a survey of foliage diseases of lucerne in South Africa. A survey of lucerne foliage pathogens in southern Queensland (Inch *et al.* 1993) reported the occurrence of 7 stem and foliar pathogens, all of which had been previously recorded. *Leptosphaerulina trifolii*, *U. striatus*, *Pseudopeziza medicaginis* and *Cercospora medicaginis* Ellis and Everh. were found to be the major pathogens. *Leptosphaerulina trifolii* and *S. vesicarium* had peak severities during the cooler months. Both of these cool weather pathogens were encountered on annual medics during the disease surveys.

Colletotrichum trifolii has been shown to cause anthracnose on *M. polymorpha* and *M. lupulina* in North America (Farr *et al.* 1989), *M. polymorpha* and *M. truncatula* in South Africa (Lamprecht and Knox-Davies 1984), and *T. subterraneum* in Western Australia (Shivas 1989). It was also described as being pathogenic on serradella in the original species description (Bain and Essary 1906). This pathogen has not been previously recorded in Australia on *M. polymorpha* or *M. scutellata.* Although the occurrence of anthracnose caused by *C. trifolii* was infrequent, this disease has the potential to spread rapidly given suitable conditions of mild weather, wind and rain. In lucerne, anthracnose can be a serious disease if resistant cultivars are not used (Irwin *et al.* 1980). Variation in disease reaction to different races of this pathogen have been demonstrated for a number of annual medic species (Elgin and Ostazeski 1982).

Colletotrichum trifolii was more virulent on *M. scutellata* than on *M. truncatula*. Lamprecht and Knox-Davies (1984) found that the only *M. scutellata* cultivar they tested (cv. Robinson) was highly susceptible to *C. trifolii*. They also described the reaction of *M. truncatula* cv. Cyprus as resistant but that of cv. Jemalong as being highly susceptible. O'Neill and Bauchan (1996), in assessing the North American annual *Medicago* core collection, found most species to be highly susceptible. This conflicts with our study in which all *M. truncatula* lines, including cv. Jemalong, were resistant. Variability in disease reaction was also demonstrated in 9 *M. polymorpha* lines tested by Lamprecht and Knox-Davies (1984). This variation in susceptibility demonstrates the potential of selection for resistance to this pathogen in the development of new cultivars. Even within the most susceptible species, *M. scutellata*, there appear to be low levels of resistance. In lucerne, substantial improvement in the level of resistance has been made through recurrent cycles of selection (Irwin *et al.* 1980). Field assessments of anthracnose in stands of *M. scutellata* in particular, and of other annual medics, will provide an indication of the potential for selection and breeding of anthracnose-resistant lines.

The disease severity caused by *Phytophthora medicaginis,* under field conditions, was low (DSI range 1.05–2.07). For the *C. arietinum* cv. Tyson check rows, nearly all plants were killed or severely infected. There were only small differences in disease reaction between *Medicago* species but differences in disease severity were encountered among cultivars and lines within a species. Within *M. truncatula,* SA11292 was the most resistant and cv. Paraggio the most susceptible. Cultivars of *M. scutellata* and *M. polymorpha* also varied in their susceptibility, although lines of both species were less variable than those of *M. truncatula*. Field studies by De Haan *et al*. (1996) demonstrated a wide disease reaction (18–100% mortality) of annual *Medicago* stands in a *P. medicaginis* nursery in St Paul, Minnesota. Wide variation in disease reaction to *P. medicaginis*, from resistant to completely susceptible has also been demonstrated for *M. sativa* and other closely related species (Irwin 1974; Irwin and Maxwell 1980).

It is interesting to note that *M. truncatula* cv. Caliph is significantly more susceptible to phytophthora root rot and to anthracnose than is cv. Cyprus. Caliph was derived from Cyprus by backcrossing and selecting for aphid resistance (Lake 1993).

Although *C. destructivum* has been recorded as a

pathogen of subterranean clover in Western Australia (Barbetti 1983) and on *M. polymorpha* in South Africa (Lamprecht and Knox-Davies 1984) this is the first time it has been recorded on serradella in Australia.

Rhizoctonia sp. has previously been reported as causing leaf blight and stem lesions on serradella in Queensland (J. L. Alcorn unpublished data). Serradella is considered to be a relatively drought resistant legume because it has a deep rooting nature. The taproot disease caused by *R. solani* has the potential to be severe because only shallow surface roots are left after infection, resulting in the rapid onset of water stress and death of affected plants. *Rhizoctonia solani* is favoured by conditions of high soil moisture. In pasture situations, serradella is grown on free-draining soils in dry environments and under these conditions, the probability of disease is low, but it is likely to be serious if serradella is irrigated for seed production or after heavy rain.

Subterranean clover is grown over a small area on sandy soils of the Granite Belt and on hardsetting, shallow loamy soils in the Traprock areas of southern Queensland. Therefore the opportunity to assess the important diseases of subterranean clover in the subtropics of Queensland is limited. The most common disease was powdery mildew, although during the 1992 growing season it was neither severe nor widespread.

Powdery mildew of subterranean clover caused by *E. polygoni* and *Oidium* sp. has been recorded in southern and western temperate regions of Australia (Sampson and Walker 1982; Cook and Dube 1989; Shivas 1989) and in Queensland (Simmonds 1966). *Fusarium* spp. and *Pythium* spp. have been previously isolated from rotted roots of subterranean clover in Queensland (Simmonds 1966; J. L. Alcorn unpublished data). In the temperate regions of Australia these fungi form part of a root-rotting complex (Barbetti and Sivasithamparam 1986). There was no direct evidence from this study to link the fungi to root disease of subterranean clover in Queensland as all isolates collected were non-pathogenic. However, more surveys need to be conducted, particularly in wet winters, to determine if these fungi are important in the subtropics.

Phytophthora clandestina Taylor, Pascoe and Greenhalgh, which is considered to be the most significant root rotting pathogen of subterranean clover in New South Wales and Victoria (Murray 1992) was not found during this or other surveys (S. Flett unpublished data). It has not been previously recorded on subterranean clover in Queensland.

Temperate annual pasture legumes in southern Queensland are affected by a number of pathogens, some of which are potentially more serious than others. Under suitable environmental conditions (e.g. mild wet winters), *C. trifolii* has the potential to be a serious pathogen of some annual medics because of its ability to girdle stems, cause the collapse of entire shoots and consequently of whole stands. As there is resistance to the pathogen it is feasible to develop resistant cultivars suitable for a subtropical environment. Because little resistance to anthracnose was found in the North American annual *Medicago* core collection, O'Neill and Bauchan (1996) recommended that resistance should be incorporated into lines intended for use in areas where anthracnose is severe. Phytophthora root rot (*P. medicaginis*) may be an important damping-off disease of annual medics under wet conditions but, it was not found to be serious in mature plant stands during this survey. The dry conditions experienced over the 2 winters of this survey may have contributed to the apparent lack of importance of the disease under natural conditions. Tap root rot of serradella caused by *R. solani* is a potentially serious disease to irrigated seed production stands, as large numbers of plants can be killed very quickly. No potentially serious pathogens of subterranean clover were identified in this environment.

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