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Re-emergence of grey leaf spot caused by *Stemphylium botryosum* and its implications for Western Australian lupins

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Abstract Stemphylium grey leaf spot was a damaging disease of *Lupinus angustifolius* crops in Western Australia during the 1970s. It has rarely been reported since the release of resistant cultivars more than 30 years ago. Severe grey leaf spot symptoms, caused by *Stemphylium botryosum*, were observed recently in experimental lupin plots at South Perth, Western Australia. More than a third of *Lupinus angustifolius* advanced breeding lines screened were susceptible, although known resistant cultivars remain effective. These investigations suggest that widespread deployment of susceptible cultivars could result in re-emergence of this disease in Western Australian lupin crops.

Keywords Lupin · *Stemphylium* · Plant breeding · Disease resistance

Grey leaf spot has been described in several countries, including Australia, causing leaf lesions and defoliation as well as stem and pod lesions on narrow-leafed lupin (*Lupinus angustifolius* L.) (Gladstones 1977; Tate 1970; Wells et al. 1961). Significant reductions in seed yield have been shown to occur from heavy disease pressure (Edwardson et al. 1961). *Stemphylium solani* in the USA (Wells et al. 1956) and *Stemphylium botryosum* in New Zealand and the USA (Tate 1970; Wells et al. 1961) have both been reported causing this disease.

Resistance to grey leaf spot, caused by *S. solani*, was first identified as a naturally occurring mutant in a crop of bitter blue forage lupins (*L. angustifolius*) in Gainsford USA (Forbes et al. 1957). This resistance was also found to be effective against *S. botryosum* (Forbes et al. 1961). The resistance was based on a recessive gene pair (gl_1 gl_1) and incorporated into various crosses resulting in the cultivar Rancher (Forbes and Wells 1967). A collaborative breeding program between the USDA and Western Australian Department of Agriculture resulted in the release of the first Australian grey leaf spot resistant variety Marri in 1976.

In the early-mid 1970s in Western Australia (WA), damaging outbreaks of grey leaf spot reportedly caused by *Stemphylium vesicarium*, occurred in susceptible commercial narrow-leafed lupin crops, particularly in wetter seasons with late spring rains (Gladstones 1977). A series of seasons with unfavourable weather conditions coupled with the release of resistant varieties reduced incidence of the disease in WA by the late 1970s (Gladstones 1994).

Grey leaf spot has been almost completely absent from WA lupin crops over the past 30 years and WA lupin breeding lines have not been screened for susceptibility during that time. Symptoms similar to those described as grey leaf spot by Wells et al. (1956), Tate (1970) and Gladstones (1977) were observed on several entries in *L. angustifolius* breeding trials in a shade-house in 2006 and in field plots in 2007, at South Perth, Western Australia. Differences in disease response were evident between breeding lines with some lines exhibiting an absence of any symptoms. Susceptible plants displayed roughly circular lesions on leaflets, or semi-circular lesions at the leaf edge. Immature lesions began as small (1–2 mm) dark brown spots, progressively developing into larger lesions (1–5 mm) with a dark brown margin and light brown interior; some lesions developed a target-type appearance with light and dark zones. With age, some lesions expanded and became ash-grey colour (5 mm or greater). Defoliation of some or all leaflets occurred with very heavy infection.

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On stems, dark brown lesions were present, initially superficial but becoming deeper with age. Lesions were generally 2–5 mm however under severe disease pressure coalesced to form larger brown patches on the stems. Small (1–5 mm) roughly circular sunken lesions were present on pods. Defoliation of lower canopy leaves and associated stem and pod lesions occurred in severely affected plants.

To isolate the causal pathogen, infected leaves were surface sterilised for 30 seconds in 1% sodium hypochlorite, rinsed twice in sterile distilled water and air dried, lesions were excised, plated onto PDA and incubated for 7 days at 20°C under 12 h light/dark cycle. Single spore isolates were obtained and submitted to the Western Australian Culture collection (WAC12986, WAC13136).

To produce conidia, cores taken from the edge of actively growing colonies of the two isolates were placed onto 20% V8 agar plates and incubated at 22±2°C under 12 h light/dark cycle for 14 days. Conidia were scraped from plates and suspended in water and the size and length to width (L/W) ratio was measured for 50 conidia selected at random.

Conidia of WAC12986 and WAC13136 shared similar dimensions and appearance and were consistent with descriptions of *S. botryosum* (Simmons 1967) (Table 1). They were oblong-ovoid in shape, olive-brown in colour, constricted at 1 or more transverse septa but lacking the pointed apex of *S. solani*. While conidial dimensions were within the range published for *S. botryosum* and *S. vesicarium* (Table 1), Simmons (1969) notes that in culture, conidia of *S. vesicarium* have a L/W ratio of 2.5–3.0 compared to *S.botryosum* which have a L/W ratio of about 1.5. Conidial morphology was consistent with that described for *S.botryosum* taken from lupins in the USA (Graham and Zeiders 1960).

To confirm pathogenicity, inoculum of WAC13136 was produced by streaking conidia onto V8P agar (112.5 ml V8 juice, 7.5 g Agar, 7.5 g of Difco Potato Dextrose Agar, 2.25 g $CaCO_3$ and 637.5 ml distilled water) (Kumar 2007) and incubating at 22±2°C under 12 h light/dark cycle for 14 days. A spore suspension was prepared by flooding V8P agar plates with sterile distilled water with 0.1% Tween 20 added and scraping the agar surface with a glass rod. The spore suspension was diluted to 2×10^5 spores per mL. Four replicate pots of 14 day old seedlings of six *L. angustifolius* breeding lines were spray inoculated to run-off with the spore suspension. Lines chosen had exhibited varying levels of susceptibility in initial field observations. Inoculated pots were placed inside an incubation chamber under 90% shade of natural daylight and subjected to intermittent misting for 48 hours before being transferred to the glasshouse bench at 20°C. Disease assessments were carried out 14 days after inoculation and confirmed the pathogenicity of the isolate. Cultivar disease responses confirmed the preliminary field observations.

Using the described inoculum production and glasshouse inoculation techniques, Australian lupin cultivars and selected international lupin cultivars were screened for resistance. Disease severity was assessed on the first two fully expanded leaves on a 0–5 scale (0 = no symptoms, 1 = 1–2 lesions not exceeding 1 mm on each leaflet, 2 = less than 5 lesions of size 1–2 mm on each leaflet, 3 = 1–5 mm lesions, often coalescing with associated chlorosis, 4 = some leaflets completely necrotic or fallen, 5 = all leaflets completely necrotic or complete defoliation). Cultivar effects on disease severity were analysed by analysis of variance, variety means were compared using Fishers protected LSD test. Cultivars scoring less than 1 were considered resistant and were significantly different from those scoring greater than 3, which were considered susceptible. Segregating cultivars contained varying proportions of individual plants that were either resistant or susceptible.

Cultivar resistance responses with the WA isolates reflected historical reports of cultivar responses to grey leaf spot. Rancher was resistant and Borre susceptible to grey leaf spot in the USA (Forbes and Wells 1967) and gave

Table 1 Conidial dimensions of a) *Stemphylium* sp. isolates from narrow leafed lupins at South Perth, Western Australia (WA) in 2006 (WAC12986) and 2007 (WAC13136) and b) published descriptions of *S. botryosum* (Simmons 1967), *S. vesicarium* (Simmons 1969) and *S. solani* (Ellis and Gibson 1975)

Isolate	Dimensions (μm)	L/W ratio (μm)	Shape	Colour
a)				
WAC12986	22.5-40×15-25	1.6±0.2#	oblong-ovoid	olive brown
WAC13136	23-38×13-25	1.7±0.3#	oblong-ovoid	olive brown
b)				
S. botryosum	24-33×15-24	1.2–1.8	oblong-ovoid	olive brown
S. vesicarium	25-42×12-22	1.5–2.7	oblong-broadly oval	olive brown
S. solani	35-55×18-28	2	oblong-pointed apex	golden brown

Mean±SD

corresponding results in our testing. Marri was the first grey leaf spot resistant cultivar released in WA, in 1976, and has the same resistance source as Rancher (Gladstones 1977), it was resistant in our current tests. Uniwhite, Uniharvest and Unicrop were the susceptible cultivars used by WA growers in the early 1970s when grey leaf spot became evident in WA crops (Gladstones 1977) and were correspondingly highly susceptible in our testing. Of the Australian cultivars released following Marri; Belara, Coromup, Chittick, Geebung, Gungurru, Illyarrie, Jindalee, Jenabillup, Mandelup, Merrit, Tanjil, Wandoo, Warrah, Wonga, Yandee and Yorrel were resistant to the current grey leaf spot isolates. Two cultivars, Myallie and Tallerack, were susceptible and four cultivars, Danja, Kalya, Moonah and Quilinock gave segregating responses. The indication from these experiments is that the virulences of current grey leaf spot isolates are similar to those previously found in WA (and in the USA) and that the resistance introduced by Gladstones in the 1960s remains effective.

However, the outbreak of grey leaf spot at South Perth indicated that a significant proportion of the advanced lupin breeding population was susceptible. Severity assessments at the infected 2007 field site (42 lines) and subsequent glasshouse assessments of advanced breeding lines in 2008 (30 lines) and 2009 (74 lines) showed that 35% of tested lines were susceptible. Additionally, 6–7% of lines showed a segregating response. This suggests that the introduction of susceptible parents into the breeding program and the absence of specific screening for this disease have allowed for a gradual erosion of resistance within the breeding population.

Grey leaf spot has been rare in WA lupin crops over the last 30 years, primarily due to the resistance that is evident in most varieties released since the late 1970s. The outbreaks in 2006 and 2007 occurred only in lupin breeding experiments. Testing lupin lines for resistance to grey leaf spot was not an objective of these experiments; however the experimental conditions promoted the disease outbreak through the cultivation of high numbers of susceptible genotypes in the presence of lupin trash (including from susceptible genotypes) and dense plantings which increased canopy humidity. These outbreaks have demonstrated that the pathogen remains present within the WA environment and suggest that in WA cropping regions with favourable environments, widespread deployment of susceptible varieties could once again result in serious disease outbreaks.

Various *Stemphylium* species have previously been associated with grey leaf spot in lupins (Gladstones 1977; Wells et al. 1956, 1961). In this instance, the pathogen involved was *S. botryosum*. Conceivably a number of different *Stemphylium* species could produce similar symptoms on lupins. However, it is apparent from a number of different sources that the resistance associated with gl_1 gl_1 is effective in all cases.

Simple early generation and parental screening, using recent *S. botryosum* isolates, has been introduced into the WA lupin breeding program to eliminate susceptible individuals. Molecular characterisation of isolates and further examination of pathogen diversity, distribution and epidemiology is being conducted to provide better understanding of any ongoing risks associated with this disease for WA lupin production.

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References

Edwardson JR, Wells HD, Forbes I (1961) The effect of *Stemphylium* leaf spot complex on yield of field-planted blue Lupin. Plant Dis Rep 46:958–959

Ellis MB, Gibson IAS (1975) *Stemphylium solani*. [Descriptions of Fungi and Bacteria]. (48):Sheet 472

Forbes I, Wells HD (1967) Registration of rancher blue lupine (Reg. No. 2). Crop Sci 7:278

Forbes I, Wells HD, Edwardson JR (1957) Resistance to the gray leafspot disease in blue lupines. Plant Dis Rep 41:1037–1038

Forbes IJ, Wells HD, Edwardson JR, Ostazeski SA (1961) Inheritance of resistance to gray leaf spot disease and of an antho-cyanin inhibitor in blue lupines, *Lupinus angustifolius* L. Crop Sci 1:184–186

Gladstones JS (1977) The narrow-leafed lupin in Western Australia (*Lupinus angustifolius* L.), Bulletin 3990. Western Australian Department of Agriculture, South Perth

Gladstones JS (1994) An historical review of lupins in Australia. Proceedings of the first Australian Lupin Technical Symposium, Perth, Western Australia, 17-21 October 1994

Graham JH, Zeiders KE (1960) Pathogenicity and morphology of some leguminicolous and related species of *Stemphylium*. Phytopathology 50:757–760

Kumar P (2007) Genetics of resistance to *Stemphylium* leaf blight of lentil (*Lens culinaris*) in the cross barimasur-4× CDC milestone. Master of Science, University of Saskatchewan

Simmons EG (1967) Typification of *Alternaria*, *Stemphylium*, and *Ulocladium*. Mycologia 59:67–92

Simmons EG (1969) Perfect states of *Stemphylium*. Mycologia 61:1–26

Tate KG (1970) A foliage disease of blue lupin caused by *Stemphylium botryosum* Walk. N Z J Agric Res 13:710–716

Wells HD, Forbes I, Webb TE, Edwardson JR (1956) Two *Stemphylium* diseases of blue lupine. Plant Dis Rep 40:803–806

Wells HD, Forbes I, Edwardson JR (1961) The *Stemphylium* leaf spot complex on blue lupins. Plant Dis Rep 45:725–727