IMPROVED METHODOLOGY FOR SCREENING FOR RESISTANCE TO *PLEIOCHAETA SETOSA* ROOT ROT IN *LUPINUS ALBUS*

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ABSTRACT

Pleiochaeta root rot, caused by *Pleiochaeta setosa*, is a world–wide fungal disease in white lupin (*Lupinus albus)* crops. The breeding of resistant genotypes is the preferred control method. An improved screening and scoring method was developed to facilitate selection. A 0–9 lesion severity scale was developed and validated. Seedlings were grown in a controlled environment using potting mix infested with conidiospores raised *in vitro*. 1000 spores g^{-1} of potting mix gave the greatest discrimination between resistant and susceptible host genotypes. There were significant differences in virulence between six isolates of the pathogen, but even the least virulent was able to cause serious disease at high spore concentration (5000 spores g^{-1}). A discriminating, well–sporulating, virulent isolate (PS6) was identified and used for screening for resistance in large numbers of *L. albus* breeding genotypes.

KEYWORDS

broad–leaf lupin, white lupin, pathogen virulence, host resistance, resistance breeding

INTRODUCTION

Pleiochaeta setosa is a pathogen of members of the genus *Lupinus*. It causes the disease brown leaf spot (BLS) and pleiochaeta root rot (PRR) world–wide (Sweetingham, 1984; Jaarsveld, 1985; Paulitz and Atlin, 1992; Bateman, 1997; Pecchia *et al.* 2002; Noffsinger, and Santen, 2005). In south–eastern Australia PRR causes seedling death in newly–emerged commercial crops of *Lupinus albus* (white lupin) leading to reduced plant stands and lower yield potential. BLS is caused by rain–splash, whereas PRR is caused when spores germinate underground and directly attack the roots in the seedling stage, at times leaving the stele intact or severing the root completely (Hill, 1998). BLS symptoms on *L. albus* are rare in south–eastern Australia, however, on the very sandy soils of Western Australia, BLS can be severe on *L. albus* (Sweetingham *et al.* 1994; Yang *et al.* 1996), as well as being very common on *L. angustifolius.*

The methods of PRR control are breeding resistant genotypes (Cowling *et al.* 1997; Wunderlich *et al.* 2008), modifying farming practice (Landers, 1991; Sweetingham, 1991; Szukala *et al.* 1996), crop rotations (Reeves *et al.* 1984; Sweetingham *et al.* 1996), and the use of fungicides (Etheridge and Bateman, 1999).

The aims of this work were: to improve the available screening procedure to test for PRR–resistance in *L. albus*; to define a range of differential host genotypes; to determine the virulence of a number of pathogen isolates across the host differentials; and to investigate the effect of spore concentration on disease levels in screening experiments.

MATERIALS AND METHODS

FUNGAL MATERIALS AND INOCULATION

Eight single–spore isolates of P. setosa were collected from field–grown albus lupins in Wagga Wagga NSW (35°03'S:147°21'E) in 1999 and were freeze–dried in sealed glass ampoules for storage. Ampoules of *P. setosa* were opened for each experiment and grown on 20% V8 media plus 5.4 g $CaCO_3$ and 20 g agar L^{-1}. The plates were maintained with a 12 h photoperiod at 19°C. After 21 days the plates were sub–cultured onto V8 media to produce sufficient inoculum (conidiospores) for each experiment. Spores were washed from Petri dishes with sterile water (including 0.1% Wettasoil®) by dislodging the spores with a flexible rubber spoon scraped over the surface. The concentration of the spore suspension was determined using a haemocytometer, and adjusted to be equivalent to 1000 spores g^{-1} of inoculated soil. In the spore concentration experiment adjustments were made to be equivalent to 250, 500, 1000, 2000, 3000, and 5000 spores g^{-1} of inoculated soil.

The desired spore concentration g^{-1} of soil was achieved by adding the calculated spore number to a measured mix of 80% double–washed river sand and 20% brick sand in a concrete mixer for 90 sec. Sixteen seeds were sown per 175 mm diameter pot in a regular grid. All experiments were conducted in a controlled–environment growth room (16 h photoperiod, 15°C day, 10°C night). Pots were stood in large stainless–steel tubs which enabled the pots to be given extended bottom–

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watering after sowing to ensure uniformity, and encourage good infection. Tubs were drained of water three days after sowing. Pots were watered from above as required.

EXPERIMENTAL DESIGN, SCORING SYSTEM AND DATA ANALYSIS

Experimental designs were generated using DiGGer version 4.01a software (Coombes, 2002) which allowed the pots to be spatially arranged in replicates, rows and columns in an optimal fashion. Data analysis was undertaken using Genstat v8 statistical software (Lawes Agricultural Trust). A scoring system based on a lesion severity scale of 0–5 (Sweetingham, 1989) was redesigned to a 0–9 scale to improve discrimination at the resistant end of the scale (Fig. 1). Each seedling was assessed on the 0–9 scale for each centimetre of root in the top 6 cm. Roots severed in the top 6 cm were scored as 9 for each successive missing cm of root.

Six, well–sporulating *P. setosa* isolates were chosen for inclusion in a race virulence experiment and used at a spore concentration of 1000 spores g^{-1}: PS1, PS2, PS3, PS4, PS6, and PS8. Six differential *L. albus* genotypes were used: Luxor (resistant), Rosetta (moderately–resistant to moderately–susceptible), Ultra (susceptible), Kiev–mutant (very susceptible), K-BC–3A (moderately–resistant) and WK223 (very susceptible). For the spore concentration experiment, two genotypes, Luxor and Kiev–mutant, were tested against a range of spore concentrations using isolates PS3 and PS6.

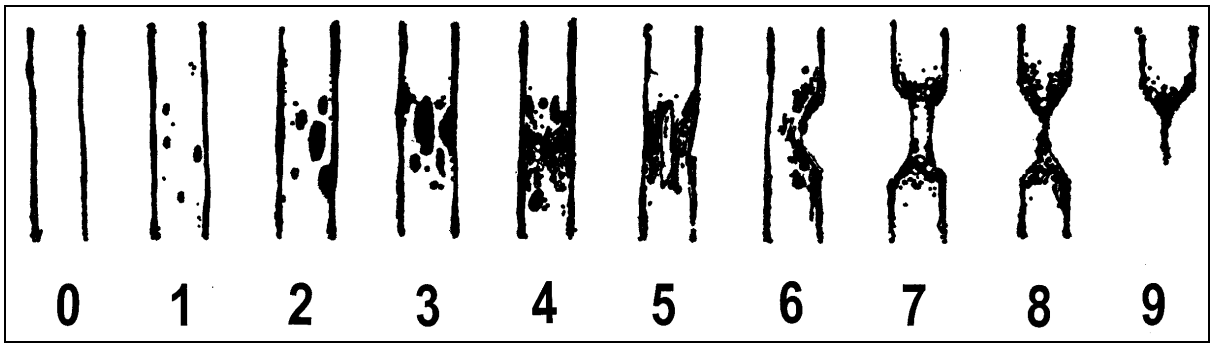

Fig. 1. The 0–9 lesion severity score used to assess Pleiochaeta Root Rot damage to *L. albus* seedling roots.

RESULTS AND DISCUSSION

VIRULENCE EXPERIMENT

The main effects, first, and second–order interactions for the fixed factors (genotype, isolate, and root–position) were highly significant ($P < 0.001$). The effect of isolate was the largest. Of primary interest was the effect of isolate upon genotype (averaged across all root–positions). PS3 was the least virulent isolate, and PS6 the most virulent (Fig. 2). The host genotypes generally responded in a similar way to all isolates, although Ultra and WK223 showed some minor variation in response to PS8 and PS1, respectively (Fig. 2). Averaged over genotype, response to root–position for infection level was approximately parallel for all of the six fungal isolates, with no crossovers. This indicated that all isolates were responding similarly, and there was no evidence to suggest that a mixture of isolates was necessary for effective screening.

SPORE CONCENTRATION EXPERIMENT

The greatest discrimination between the genotypes was achieved in the 1000–3000 spores g^{-1} range (Fig. 3). At high spore concentrations both isolates (PS3 and PS6) were equally virulent and the resistance in LUXOR was overcome. It was confirmed that 1000 spores g^{-1} was a good level for effective screening.

IMPROVEMENTS IN THE SCREENING PROCEDURE

In subsequent experiments it was found that if the screening experiments are conducted in a controlled–environment growth–chamber then replication reduced to two pots (instead of four) was sufficient to screen breeding material for PRR–resistance (data not shown). In addition, a single lesion score per root based on the rating of the most severe lesion anywhere in the top 6 cm of root, on the 0–9 scale (Fig. 1) was sufficient to give discrimination. These improvements have allowed increased and more rapid throughput. Growth room or field disease nursery screening is resource–intensive. Work is underway to identify DNA markers linked to the genes for PRR–resistance in mapping populations using the resistant landraces, P25758 (very resistant) and P27593 (moderately–resistant) crossed with Kiev–Mutant. The future use of marker–assisted selection may permit the pyramiding of multiple resistance genes, resulting in durable resistance in all new *L. albus* cultivars.

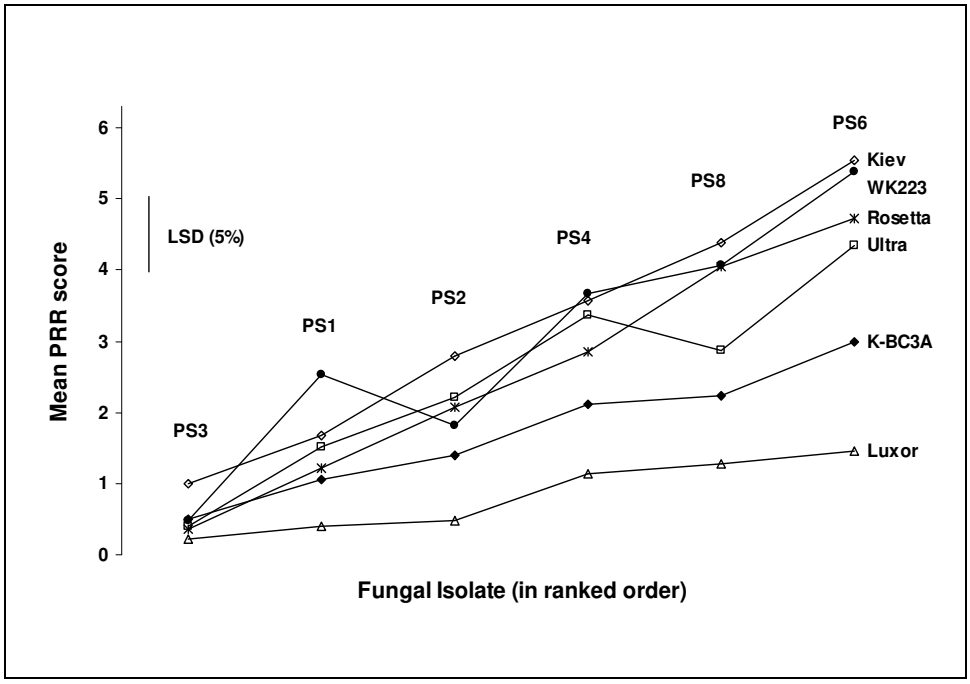

Fig. 2. Mean PRR score (0–9 scale) from a virulence experiment for combinations of genotype (n = 6) x fungal–isolate (n = 6).

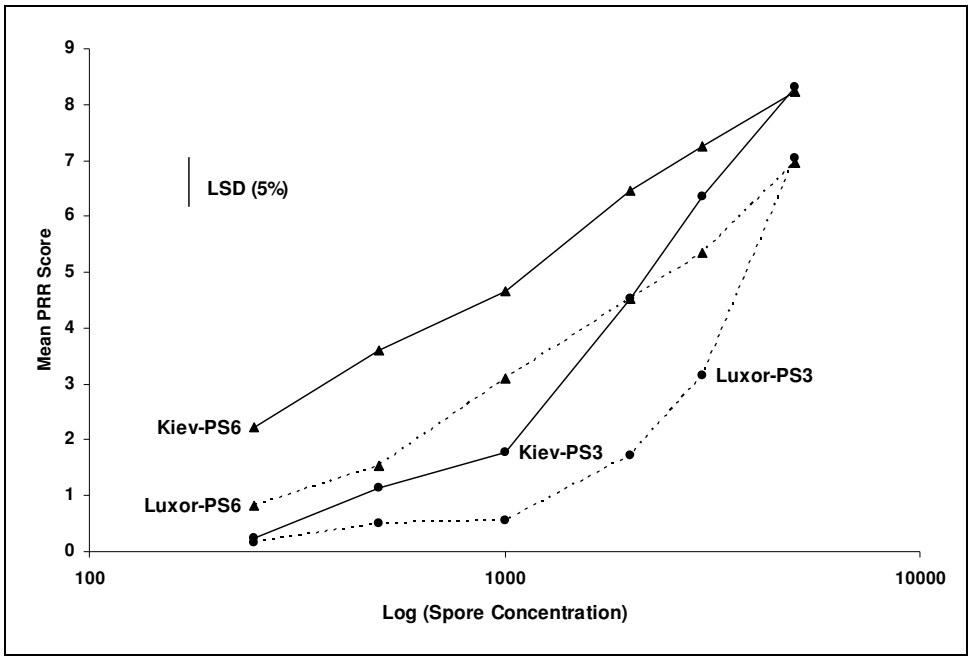

Fig 3. Mean PRR score (0–9 scale) from a spore concentration experiment for combinations of genotype (n = 2) x fungal isolate (n = 2).

LITERATURE CITED

Bateman, G.L. 1997. Pathogenicity of fungi associated with winter loss and injury in white lupin. Plant Pathology 46: 157–167.

Coombes, N.E. 2002. The Reactive Tabu: Search for efficient correlated experimental designs. PhD thesis, John Moores University.

Cowling, W.A., M.W. Sweetingham, D. Diepeveen and B.R. Cullis. 1997. Heritability of resistance to brown spot and root rot of narrow–leafed lupins caused by *Pleiochaeta Setosa* (Kirchn.) Hughes in field experiments. Plant Breeding 116: 341–345.

Etheridge, J.V. and G.L. Bateman. 1999. Fungicidal control of foliar diseases of white lupin (*Lupinus albus*). Crop Protection 18: 349–354.

Hill, D.G. 1998. Diseases of Lupins. *IN* 'The Pathology of Food and Pasture Legumes'. (Eds D.G. Allen and J.M. Lenné). pp. 559–589. (CAB International, UK).

Jaarsveld, A.B. 1985. Fungal pathogens of lupins in South Africa. Phytophylactica 17: 209–213.

Landers, K.F. 1991. Lupin production and research in south–eastern Australia. *IN*. (Technical Bulletin No. 44 – NSW Agriculture & Fisheries.)

Noffsinger, S.L. and E.v. Santen. 2005. Evaluation of *Lupinus albus* L. germplasm for the southeastern USA. Crop Science 45: 1941–1950.

Paulitz, T.C. and G. Atlin. 1992. First report of brown spot of lupines caused by *Pleiochaeta setosa* in Canada. Plant Disease 76: 1185.

Pecchia, S., A. Spurio, M. Forti and M. Ginanni. 2002. Outbreak of brown spot of white lupin caused by *Pleiochaeta setosa* in central Italy. Journal of Plant Pathology 84: 140.

Reeves, T.G., A. Ellington and H.D. Brooke. 1984. Effects of lupin–wheat rotations on soil fertility, crop disease and crop yields. Australian Journal of Experimental Agriculture and Animal Husbandry 24: 595–600.

Sweetingham, M. 1984. *Pleiochaeta setosa* – A Root Pathogen of Lupins. Australasian Plant Pathology 13: 21-2.

Sweetingham, M.W. 1989. Fungi associated with root and hypocotyl diseases of seedling lupins in Western Australia. Australian Journal of Agricultural Research 40: 781–789.

Sweetingham, M.W. 1991. The effect of inoculum distribution and sowing depth on Pleiochaeta root rot of lupins. Australian Journal of Agricultural Research 42: 121–128.

Sweetingham, M.W., W.A. Cowling, B.J. Buirchell and J. Henderson. 1994. Resistance of *Lupinus* spp. to leaf and root diseases caused by *Pleiochaeta setosa*. *IN* 'Advances in lupin research; Proceedings of the 7th International Lupin Conference, Lisbon, Portugal, 1994'. (Eds J. Neves–Martins and M.L. Beriao da Costa) pp. 368–370.

Sweetingham, M.W., R.A.C. Jones and W.A. Cowling. 1996. Advances in lupin disease management in Australia. *IN* 'Proceedings 8th International Lupin Conference, Asilomar, California'. (Ed. D.G. Hill) pp. 466–486.

Szukalam J., D.J. Collins and D.W. Reeves. 1996. Effect of seed treatment and soil phosphorus level on Pleiochaeta root rot of *Lupinus albus*. *IN* 'Proceedings 8th International Lupin Conference, Asilomar, California'. (Ed. D.G. Hill) pp. 535–538.

Wunderlichm N., G.J. Ash, H. J.D.I., R.B. Cowley and D.J. Luckett. 2008. Penetration and symptom development of Pleiochaeta root rot in susceptible and resistant *Lupinus albus* cultivars. Australasian Plant Pathology 37: 387–391.

Yang, H.A., M.W. Sweetingham and W.A. Cowling. 1996. The leaf infection process and resistance to *Pleiochaeta setosa* in three lupin species. Australian Journal of Agricultural Research 47: 787–799.