USING DETACHED LEAVES AND PODS TO SCREEN FOR RESISTANCE TO PHOMOPSIS (*DIAPORTHE TOXICA*) IN *LUPINUS ALBUS*

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ABSTRACT

Phomopsis (*Diaporthe toxica*, anamorph *Phomopsis leptostromiformis*) is a world-wide pathogen of lupin species. In Australia, commercially-grown albus lupin cultivars have been generally regarded as resistant to phomopsis. Recently in south-eastern Australia the number and severity of albus crops infected with phomopsis has increased, and lead to more research on this disease. We have developed/refined screening assays using detached leaves and pods to screen for resistance. There were significant differences between genotypes for both assays ($P < 0.001$). The correlation between the two assays was only moderate ($r = 0.36$) but significant ($P < 0.05$), however, only accounted for 13% of the variability. Genotypes that were resistant in one assay were not always resistant in the other. Therefore we postulate that there may be different phases of the disease in albus lupins, with resistance to each phase under independent genetic control.

KEYWORDS

white lupins, phomopsis, disease screening, multi-phase design, disease resistance

INTRODUCTION

Phomopsis infection of lupin crops has been a ubiquitous issue in many cropping areas around the world (Van Warmelo and Marasas, 1972). Extensive research has been directed towards understanding the interaction between phomopsis and narrow-leafed lupin (*Lupinus angustifolius*) (Wood, 1986; Williamson *et al,.* 1994), finding genetic resistance (Shankar *et al.* 2002) and limiting the impact of lupinosis (stock poisoning through eating contaminated stubble or seed) (Allen, 1986; Cowling *et al.* 1988). Considerably less studied is the disease in *L. albus*, which has generally been regarded as resistant (Wood and Allen, 1980). Only recently in Eastern Australia has the pathogen emerged as being a potentially serious threat to *L. albus* crops. Research is now being conducted to determine why the pathogen appears to have increased its prevalence on *L. albus*, with efforts directed towards determining the extent of genetic resistance to this pathogen in the existing cultivars and the available germplasm.

The study of disease response to phomopsis in *albus* lupin reported here was undertaken by screening detached leaves and pods. Similar studies have been reported in several legume crops, including soybean, chickpea and fieldpea (Balducchi and McGee, 1987; Dolar *et al.* 1994; Onfroy *et al.* 2007). A detached pod or leaf assay conducted *in vitro* is only of benefit to the plant breeder if it correlates well to field responses (Irwin *et al.* 2003). Detached plant-part screening assays are useful because they enable assessments to be made under highly-controlled conditions, serve as rapid screening techniques that can be adopted by breeding programs and can assist our understanding of host-pathogen interactions (Huang *et al.* 2005; Bradley *et al.* 2006; Ergon and Tronsmo, 2006).

In *albus* lupins phomopsis seed decay can greatly reduce yield and may result in the seed-lot being rejected. While this is not a common occurrence, it is worth assessing germplasm and breeding lines for their response to this pathogen. The pod assay allows for greater control over experimental conditions and ensures that all pods being tested are at a similar physiological age. In contrast with pods, phomopsis infection in leaves is not considered to be an agronomically important character in albus lupins, but may play a role in epidemics (Brown, 1984). This method of assay has been undertaken to ascertain if foliar symptoms correlate with either pod or stem resistance. Assessing the leaves rather than pods has appeal in that it is enables rapid screening of genotypes, without the need to grow plants to maturity. This is the first report of resistance to phomopsis in albus lupins being assessed using detached leaf and detached pod assays.

MATERIALS AND METHODS

FUNGAL ISOLATE

The single-spore isolate used in this study was sourced from an infected commercial seed-lot of cv. Kiev Mutant grown at Tarcutta NSW, Australia, in 2004.

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EXPERIMENTAL DESIGN AND SOURCE OF PLANT MATERIAL

Assay experiments were setup as multi-phase designs with extra replication in the laboratory phase to enable the estimation of error variance in Phase 2 (Smith *et al.* 2006). Phase 1 of the experiments was the screen-house grown trial to produce leaves and pods, and Phase 2 was the laboratory component to introduce the disease and assess damage. The trial consisted of 40 genotypes (11 cultivars, 21 breeding lines and 8 germplasm accessions) grown in 1 m-long rows in a randomised complete block with three replicates. The spatial arrangements of Phase 1 and 2 were optimised by using DiGGer software (Coombes, 2002). The genotypes were classified as being late-, mid- or early-flowering. Genotypes in each category were sown at two week intervals to maximise the probability of all genotypes flowering and subsequently podding at the same time. Three leaves were randomly selected and removed from each row on 24 October 2007, about 17 weeks after sowing. They were all the first fully-expanded leaf below a lateral flowering inflorescence. Four pods were cut from each row of plants at green ripening, described as growth stage 4.4 by Dracup and Kirby (1996), about 20 weeks after sowing.

The three leaves from each row were carefully arranged on sterilised paper towel and the upper surface thoroughly sprayed with a 5×10^6 spore solution. Each leaf was placed on a TAKA medium (technical agar 10 g, kinetin 10 mg and aueromycin 0.1g L^{-1}) in 9 cm-diameter Petri dishes, and left unsealed. The addition of the hormone kinetin delayed leaf senescence thereby allowing decay symptoms to be attributed to the pathogen (Twizeyimana *et al.* 2007). The Petri dishes were arranged in a 10 × 6 grid on 6 shelves, randomised in three dimensions, in a culture room maintained at 20°C with 12 hour light. Disease expression (discolouration and necrosis) was scored as percentage-leaf-area-affected (LAA) at 3, 5 and 7 days after inoculation. The number of sporulating pycnidia produced on each leaflet was counted at 7-10 days, along with the leaflet width (mm) and length (mm). These data were used to calculate the number of pycnidia produced per cm^2 per leaflet per leaf.

After surface-sterilisation in hydrolysed water (pH 2.71) for 20 mins, the pods were inoculated by immersion in a conidiospore suspension at 5×10^6 spores/mL for 15 seconds. They were incubated at 20°C under 12 hr light in a culture room in closed, plastic, take-away food containers which contained 3 mL of sterile water in the bottom of the container to maintain high humidity. The pods were placed on rigid plastic mesh supports to avoid direct contact with the water. The lids were removed after 17 days and each pod was assessed for disease symptoms on 0, 3, 5, and 7 days later, using a 0-9 scale (0 = no symptoms, 9 = total pod rot).

DATA ANALYSIS

All data was analysed using ASREML (Gilmour *et al.* 2006). As both the pod and the leaf experiments were designed to contain multi-phases, error terms for each phase of the experiment were incorporated into the analysis (Smith *et al.* 2006), along with main effects of genotype and day-of-scoring plus the spatial effects in Phase 1 (field) and Phase 2 (laboratory). Correlations were performed in GENSTAT v9 using Pearson's correlation coefficient analysis. The pycnidia per cm^2 data were square-root transformed prior to analysis.

RESULTS AND DISCUSSION

LEAF ASSAY

In the detached leaf assay foliar lesions were evident from 3 days after inoculation. The laboratory error was greater than the field error in this experiment. All genotypes trended upward in a tightly parallel manner through the duration of the experiment.

Three days after inoculation, leaf decay (discolouration) was evident in some genotypes. By seven days, actively-sporulating pycnidia had been formed on many of the leaves. All terms in the fitted model were highly significant ($P < 0.001$) for both LAA and pycnidia per cm^2. The correlation between both scoring methods was high ($r = 0.87$) indicating that either assessment was adequate at determining resistant and susceptible responses.

Leaf resistance was identified in P27593 (a *Pleiochaeta* root rot resistant wildtype), WK320, Rosetta, WK264 and Magna (LAA means = 3.59, 7.60, 8.02, 8.74 and 8.84 respectively). The same genotypes also had the lowest number of pycnidia produced per cm^2 (back transformed means = 8.91, 18.92, 24.28, 33.86, and 16.45 respectively). Kiev Mutant was again the most susceptible genotype (LAA mean = 21.13 and 69.47 pycnidia per cm^2).

POD ASSAY

In the pod experiment all terms in the fitted model were highly significant ($P < 0.001$). The Phase 1 error was larger than the Phase 2 error. All genotypes trended upwards over the three scoring days. Consequently, genotype means are presented averaged across days.

A number of genotypes with pod resistance were detected. The Ethiopian landrace P27174 (an Anthracnose resistant genotype) was found to be resistant to phomopsis in pods (mean = 2.80). The breeding lines WALAB2008 and 98B001-5-5, both derived from P27174, have inherited some of the resistance (means = 2.23 and 3.56 respectively). The cultivar Rosetta and breeding lines WK278 and WK302 were not significantly different ($P < 0.05$) to P27174 with means of 4.00, 4.32, 4.33 respectively. The *Pleiochaeta setosa* resistant landraces P25758 and P28096 were moderately resistant (means = 4.55 and 5.07). In this assay Kiev Mutant was the most susceptible (mean = 8.54).

CORRELATIONS

The pycnidia number per cm^2 and the percentage-leaf-area-affected data were moderately correlated with the detached pod score for the 40 genotypes (r = 0.374 and 0.360 respectively). Some genotypes displayed useful resistance in the leaf assay but susceptibility in the pod assay (e.g. WK320, P27593), or visa versa (e.g. WALAB2008, 98B001-5-5). Some genotypes were resistant in both assays (e.g. Rosetta, P25758, WK302) or susceptible in both assays (e.g. Kiev Mutant, Astra, P29029). This work suggests that tissue-specific resistance to phomopsis exists in albus, particularly with genotypes P27174 and P27593 (Fig. 1). Further work is required to fine-tune these assays, to correlate these findings with field data, to determine the genetic mechanism of resistance, and to find molecular markers for resistance genes using mapping populations.

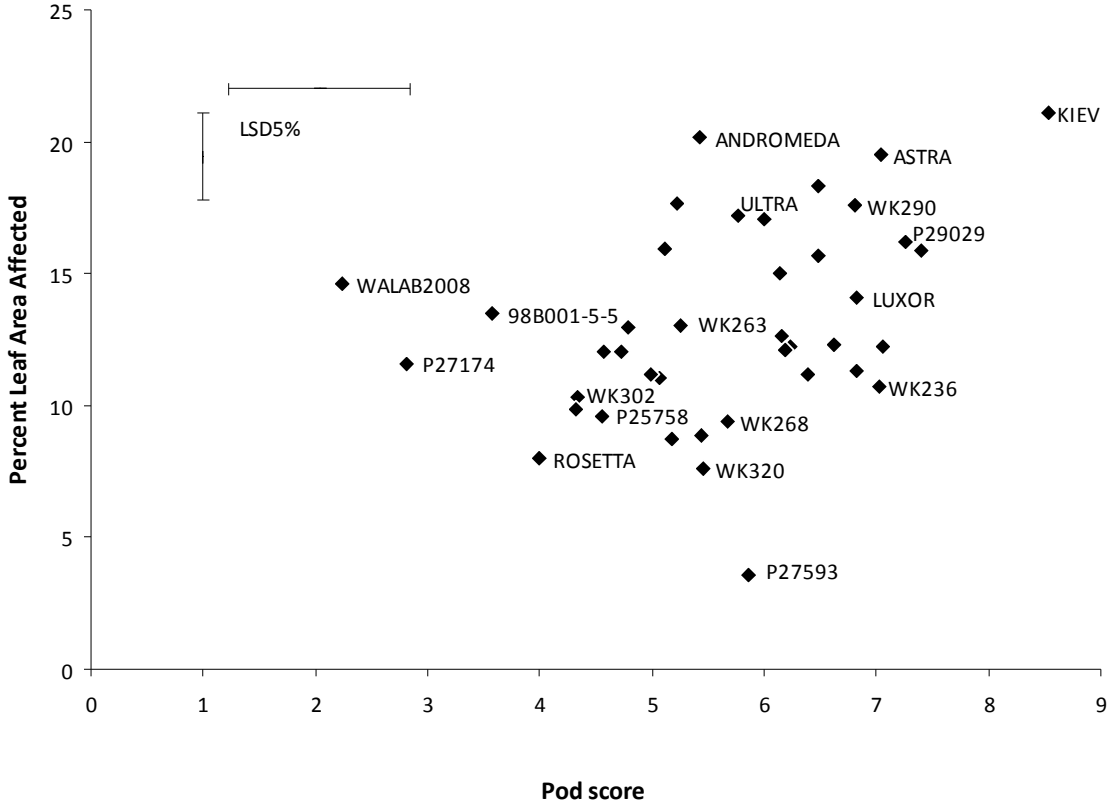

Fig. 1. Detached pod and leaf resistance to phomopsis infection. Pod score rated on a 0-9 scale, leaf score % leaf area affected by visual observation in 40 *L. albus* genotypes.

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