The Expression of Resistance to Latent Stem Infection by *Diaporthe toxica* in Narrow-Leafed Lupin

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ABSTRACT

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Resistance to latent infection by *Diaporthe toxica* was examined in resistant and susceptible cultivars and breeding lines of *Lupinus angustifolius* (narrow-leafed lupin or blue lupine). Conidial germination (91 ± 1% at 3 days after inoculation) or penetration of the cuticle (14 ± 1% at 3 days and 24 ± 1% at 5 days) by *D. toxica* were not affected by host resistance. After 5 days, the relative size and number of subcuticular coralloid infection hyphae differed on resistant and susceptible hosts. On susceptible hosts, the majority of coralloid hyphae were large (>100 µm long at 21 days). Resistant hosts had the same density of coralloid hyphae (approximately 300 hyphae per cm^2), but the majority were small (<100 µm long). Breeding line CE2:435, which has intermediate resistance, had an equal density (approximately 150 hyphae per cm^2) of both types of coralloid hyphae. In excised stems, saprophytic growth from subcuticular latent infections was faster from large coralloid hyphae in susceptible hosts than from small coralloid hyphae in resistant hosts. Small coralloid hyphae often failed to produce saprophytic mycelia and were apparently nonviable. Resistance to latent infection by *D. toxica* in narrow-leafed lupin is expressed as a reduction in the frequency of large coralloid hyphae, an increase in the frequency of smaller and apparently nonviable coralloid hyphae, and slower saprophytic colonization of host tissue. The type of resistance described, in which the host appears to actively suppress the establishment of "saprophytically competent" latent infection structures, is a new phenomenon in plant disease resistance.

Diaporthe toxica P.M. Will., A.S. Highet, W. Gams, & K. Sivasith. is the cause of Phomopsis stem blight of lupin (lupine) (*Lupinus* spp.) (13) and is responsible for the mycotoxicosis of sheep known as lupinosis (1,17). The anamorph of *D. toxica* was formerly known as *Phomopsis leptostromiformis* (Kühn) Bubák var. *leptostromiformis* (12), but the specific epithet is in doubt after inspection of the neotype of *P. leptostromiformis* by Williamson et al. (13). *D. toxica* produces subcuticular coralloid hyphae upon latent infection of stem tissue of *L. angustifolius* L. (narrow-leafed lupin or blue lupine) (15). Saprophytic invasion of stems and formation of stem blight symptoms occur after mycelia grow from coralloid hyphae during stem senescence at plant maturity (15) or earlier if tissue is stressed or treated with paraquat herbicide (8). A similar macroscopic disease syndrome occurs in Phomopsis stem blight of soybean when treated with paraquat herbicide (3).

During saprophytic colonization of stems, *D. toxica* produces the mycotoxin phomopsin (9), which is responsible for lupinosis in animals grazing lupin stubble (1,17). Phomopsin also has been detected during the latent stage of stem infection of susceptible, but not resistant, cultivars of *L. angustifolius* (16).

Measurement of resistance to *D. toxica* in *L. angustifolius* in field plots showed that resistance is relatively stable over sites and years in Australia (6) and is expressed in stems, pods, and seeds (7). Resistance is of immense practical value because it lowers the toxicity of plant residues to grazing animals (5). Williamson et al. (15) found that both the size and number of subcuticular coralloid hyphae were reduced on stems of a highly resistant *L. angustifolius* breeding line compared to a susceptible cultivar. We examined the infection process by *D. toxica* on *L. angustifolius* cultivars and breeding lines with a wide range of resistance and susceptibility to better understand this new type of resistance to latent infection and to develop a rapid and nondestructive bioassay for resistance for use in lupin-breeding programs.

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MATERIALS AND METHODS

Plant growth and inoculation of stems. Six *L. angustifolius* cultivars and breeding lines (hereafter collectively referred to as lines) were used: cvs. Unicrop (very susceptible), Yandee (very susceptible), Danja (susceptible), and Gungurru (resistant) and breeding lines CE2:435 (intermediately resistant) and 75A:258 (very resistant). Typical field ratings of Phomopsis stem blight severity in this group of lines are shown in Table 1. Plants were grown in a greenhouse (average daily maximum and minimum temperatures of 25 and 15°C) in 180-mm-diameter plastic pots containing 4 kg of coarse river sand (unsterilized). Seeds were treated with a suspension of *Bradyrhizobium lupini* (commercial lupin inoculant group G) before sowing. Pots were fertilized with 2 g of a fertilizer mix (480 g of single superphosphate, 320 g of KCl, 140 g of $MgSO_4$, 3 g of $MnSO_4$, and 39 g of Borax) at the beginning of every experiment.

Conditions for producing inocula of *D. toxica* and application of inocula through an artist's airbrush was done according to Williamson et al. (15), except for the following modifications. Twenty-one days after sowing, stems were sprayed to run-off with a conidial suspension (10^7 conidia per ml) of *D. toxica* (isolate WAC 8771, culture collection, Agriculture Western Australia, South Perth) applied to the cotyledonary internode (between the cotyledonary node and the first leaf node) of the main stem. High humidity in the pots was created by watering pots just before in-

oculation and covering plants with a clear plastic bag that was sealed around the top of the pot with a rubber band for 72 h after inoculation. Before covering pots, the plastic bag was misted internally with water. Pots were shaded from direct sunlight during the period of high humidity. Sterile distilled water was applied to plants of cv. Yandee in each experiment as a control.

Experimental design and statistical analysis. In standard experiments, six seeds were sown in each pot, and six pots of each line were placed in a completely randomized design on the greenhouse bench. Assessments were generally made on six plants of each line per treatment or sampling time (one plant chosen randomly from each replicate pot). Sampling error within pots was not measured. Each experiment was repeated at least twice, and data from a representative experiment are presented. Analysis of variance was computed by Genstat5 (Genstat5 Release Reference Manual, Oxford University Press, Oxford). Treatment means are reported plus/minus x, where x represents the standard error of the mean. Comparisons between means are based on the standard error of the difference (SED) between means. Data were transformed where necessary to normalize residuals, in which case SED applies only to transformed data. Transformed means were back-transformed where necessary for presentation of normal means in the results.

Conidial attachment, germination, and cuticle penetration. The following modifications were made to the histological procedures for light microscopy described by Williamson et al. (15). Stem segments (1 cm) from the inoculated region were excised 3, 5, 10, 14, and 21 days after inoculation and frozen for 12 h at –20°C. The epidermal layer was carefully peeled from the entire circumference of the stem, cleared with 3% KOH for 10 min at 60°C, stained in lactoglycerol aniline blue (0.02 g of aniline blue, 20 ml of glycerol, 10 ml of lactic acid, and 10 ml of distilled water) for 2 h at 60°C, and left in the stain for 12 h at room temperature. The stained epidermal tissue was mounted on a glass microscope slide in lactoglycerol aniline blue.

Conidial attachment, germination and penetration, and development of subcuticular coralloid hyphae were observed in the six lines under a light microscope according to the standard experimental procedure. The number of conidia remaining on the stem surface after staining was calculated for each peel. A random sample of 100 conidia was assessed for germination on stained epidermal tissue from six plants of each cultivar 3 days after inoculation. The proportion of conidia that had penetrated the cuticle was assessed 3 and 5 days after inoculation with a random sample of 100 conidia on stained epidermal tissue from six plants of each cultivar.

The density of coralloid hyphae per square centimeter of epidermal tissue was assessed 3, 5, 10, 14, and 21 days after inoculation by counting and classifying all coralloid hyphae on epidermal peels from six plants per line at each sampling time, according to the standard experimental procedure. Two groups of coralloid hyphae were observed: large branching types and small restricted types with a dark halo around them (Figs. 1 and 2). The difference between large and small groups was particularly pronounced on resistant plants, and the dividing line for separation into large and small types at each assessment time was based on their appearance on resistant plants. The dividing line between large and small coralloid hyphae at each sampling time was greater than or less than 30 µm (5 days), 50 µm (10 days), 80 µm (14 days), and 100 µm (21 days). Comparisons among lines, therefore, are meaningful only at one particular sampling time, and no attempt was made to compare the numbers in each size category between different sampling times. The purpose of this exercise was to determine whether resistant lines could be distinguished from susceptible lines based on two size categories at each sampling time. At 21 days, the distribution of sizes of coralloid hyphae on the six lines was examined further by classifying hyphae into five groups on the basis of length: 10 to 25, 25 to 50, 50 to 100, 100 to 250, and 250 to 600 µm.

Saprophytic growth of *D. toxica* from subcuticular coralloid hyphae. Plants were grown, and the cotyledonary internode was inoculated as described above. Twenty-one days after inoculation, the cotyledonary internode was excised (cut immediately above

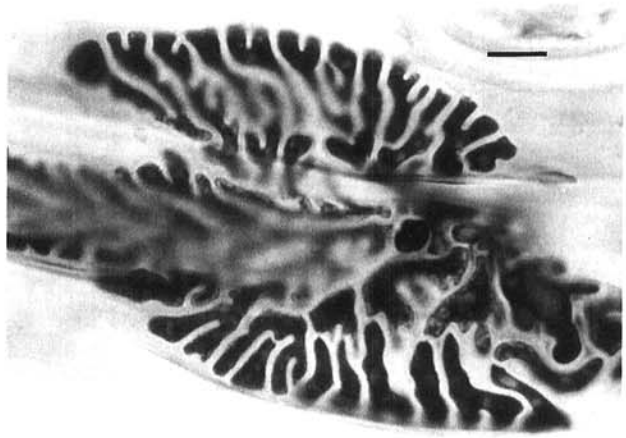

Fig. 1. Portion of a large subcuticular coralloid infection hypha of *Diaporthe toxica* in susceptible *Lupinus angustifolius* cv. Yandee at 10 days after inoculation. Bar = 10 µm.

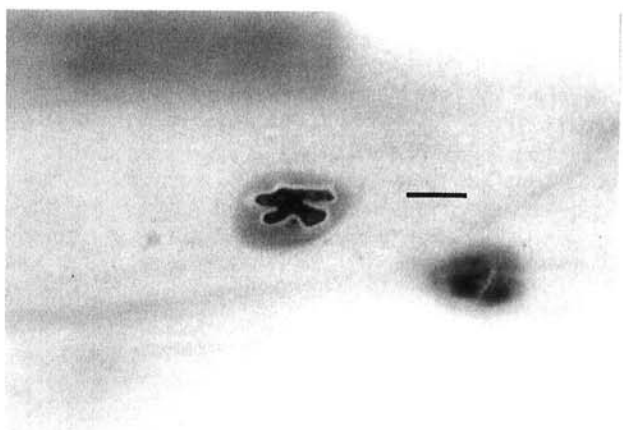

Fig. 2. A small subcuticular coralloid infection hypha of *Diaporthe toxica* in very resistant *Lupinus angustifolius* breeding line 75A:258 at 10 days after inoculation. Bar = 10 µm.

TABLE 1. Phomopsis stem ratings (PSR, measured as the percent stem area affected by symptoms of Phomospsis stem blight) and resistance categories of *Lupinus angustifolius* breeding lines and cultivars used in the experiments in field trials across southern Australia

Breeding line or cultivar	WA[a,b]	EA[a,b]	Wandering, WA, 1987[b]	Category
Unicrop	...	34 (32)	...	Very susceptible
Yandee	37 (36)	34 (32)	44 (48)	Very susceptible
Danja	...	...	28 (22)	Susceptible
CE2:435	...	...	20 (12)	Intermediate
Gungurru (75A61-3)	12 (4)	11 (4)	9 (3)	Resistant
75A:258 (75A14-10)	5 (1)	7 (2)	...	Very resistant
SED[b]	2	2	4	

[a] WA = Western Australia; EA = Eastern Australia. Average of four sites in each region in 1983. Data adapted from Cowling and Wood (7).

[b] PSR data were arcsine-transformed for analysis of variance and estimation of the standard error of the difference between means (SED); arcsine-transformed averages in degrees are followed by back-transformed percent averages in parentheses.

TABLE 2. Frequency (hyphae per square centimeter) of large and small subcuticular coralloid hyphae of *Diaporthe toxica* in stems of various cultivars and breeding lines of *Lupinus angustifolius* at different times after inoculation

Cultivar or breeding line	Days after inoculation								
	5	10	14	21	3[a]	5	10	14	21
	Large coralloid hyphae (length, μm)[b]				Small coralloid hyphae (length, μm)[b]				
	30–50	50–200	80–400	100–600	5–30	5–30	5–50	10–80	10–100
Unicrop	5.8 (34)	17.5 (306)	16.9 (286)	17.2 (296)	17.6 (310)	16.4 (269)	3.4 (12)	2.8 (8)	3.7 (14)
Yandee	6.0 (36)	17.1 (292)	16.1 (259)	16.6 (276)	17.0 (289)	16.2 (262)	3.4 (12)	3.2 (10)	4.4 (19)
Danja	5.5 (30)	16.7 (279)	16.5 (272)	15.3 (234)	16.9 (286)	15.9 (253)	3.8 (14)	3.4 (12)	6.4 (41)
CE2:435	5.1 (26)	11.7 (137)	12.8 (164)	12.1 (146)	16.5 (272)	16.3 (266)	11.2 (125)	9.5 (91)	12.5 (156)
Gungurru	4.7 (22)	5.3 (28)	4.3 (19)	5.3 (28)	17.3 (299)	16.1 (259)	17.2 (296)	16.5 (272)	16.8 (282)
75A:258	2.0 (4)	3.1 (10)	3.4 (12)	1.9 (4)	17.6 (310)	17.0 (289)	17.6 (310)	16.8 (282)	18.4 (338)
SED	0.4	0.5	0.5	0.7	n.s.[c]	0.6	0.5	0.5	0.5

[a] All coralloid hyphae at 3 days were <30 μm in length.
[b] Frequency data were square root-transformed for analysis of variance and estimation of the standard error of the difference between means (SED); square-root averages are followed by back-transformed averages (hyphae per square centimeter) in parentheses.
[c] n.s. = not significant.

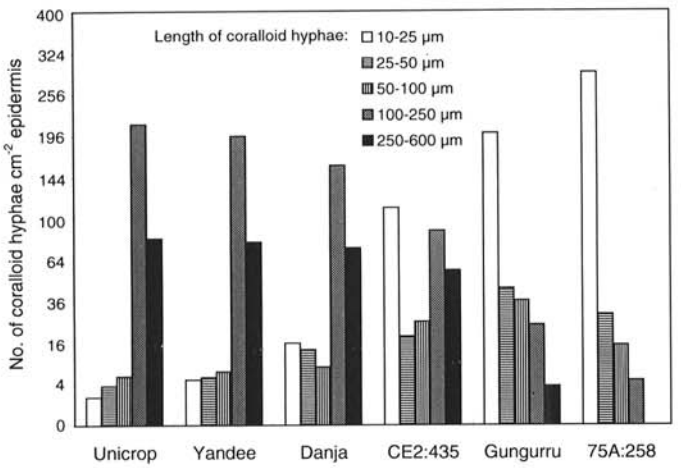

Fig. 3. Frequency distribution of length (micrometers) of subcuticular coralloid hyphae of *Diaporthe toxica* in stems of various cultivars and breeding lines of *Lupinus angustifolius* at 21 days after inoculation. Data were square-root transformed before analysis of variance. Standard errors of the difference between lines for frequency of hyphae in length categories 10 to 25, 25 to 50, 50 to 100, 100 to 250, and 250 to 600 μm were 0.6, 0.4, 0.7, 0.6, and 0.8 μm, respectively.

the cotyledons and above the first and second leaf nodes), soaked in 0.24% NaOCl for 2 to 3 min, rinsed three times with sterile distilled water, and incubated in sterile petri dishes lined with sterile, moist filter paper at 20°C. At various times after excision (12 h and 1, 2, 3, and 4 days), six excised cotyledonary internodes from each line were frozen, and the epidermal tissue was peeled and stained as described above. The length of filamentous mycelia growing away from the coralloid hyphae was measured on six viable coralloid hyphae in the central portion of each peel for each incubation period per line. The apparent viability of coralloid hyphae (percent showing saprophytic growth) also was measured 4 days after excision. Also, at 21 days after inoculation, six cotyledonary internodes per line were excised and incubated in sterile petri dishes for 6, 9, or 12 days, and observations were made on lesion progression and formation of pycnidial stromata of *D. toxica*. Lesion progression and formation of pycnidia on stems of trimmed plants remaining in pots on the greenhouse bench also was observed at 10, 14, and 18 days after trimming.

RESULTS

Conidial attachment, germination, and cuticle penetration. The average number of conidia remaining on the stem surface after staining was 1,887 ± 82 hyphae per cm², with no significant differences among lines. Germination was observed as a swelling at one end of a conidium, and penetration was deemed to have occurred when a small subcuticular infection hypha was observed under the conidium. An average of 91 ± 1% of attached conidia germinated 3 days after inoculation of stems, with no significant difference in germination among lines. Penetration was observed in an average of 14 ± 1% of the attached conidia at 3 days, increasing to an average of 24 ± 1% at 5 days, with no significant differences among lines at either date.

The size and density of large and small coralloid hyphae varied among lines at various times after inoculation, with the exception of 3 days, when there were between 270 and 310 coralloid hyphae per cm² of epidermal tissue (dimensions 5 to 30 × 5 μm), with no significant differences among lines (Table 2). At 5 days after inoculation, the very resistant line, 75A:258, had significantly fewer large coralloid hyphae per square centimeter (>30 μm long) than all other lines based on the SED of transformed means (Table 2). The resistant and very resistant lines were clearly distinguished from susceptible lines 10 days after inoculation. Large coralloid hyphae 50 to 200 μm long and 5 to 70 μm wide (Fig. 1) were observed in susceptible cvs. Unicrop, Yandee, and Danja at a frequency of about 300 hyphae per cm² at 10 days (Table 2). Small coralloid hyphae 5 to 50 μm long and 5 to 10 μm wide (Fig. 2) were observed mostly in the resistant cv. Gungurru and the very resistant line 75A:258 at a frequency of about 300 hyphae per cm² at 10 days. Often haloes that stained deep blue were observed around the small coralloid hyphae (Fig. 2). Line CE2:435, intermediately resistant, had between 130 and 160 of both large and small types of coralloid hyphae per cm². The total number of latent infections (approximately 300 per cm²) was about the same in all lines and did not increase noticeably from 5 to 21 days after inoculation.

The categories of "large" and "small" coralloid hyphae were useful to distinguish susceptible, intermediate, and resistant cultivars at 14 and 21 days after inoculation (Table 2). However, the distribution of sizes of coralloid hyphae was more closely examined at 21 days on each line to judge the importance of size of coralloid hyphae as an expression of resistance.

At 21 days, the distribution of coralloid hyphae in five size categories (Fig. 3) was clearly related to the ranking of lines for resistance in the field (Table 1). The frequency of small coralloid hyphae (5 to 50 μm) was highest in cultivars with the greatest field resistance, and the frequency of large coralloid hyphae (100 to 600 μm) was highest in cultivars with greater field susceptibility (Fig. 3).

Saprophytic growth of *D. toxica* from subcuticular coralloid hyphae. Saprophytic growth began as early as 12 h after excision of the infected cotyledonary internode from plants. Filamentous mycelia were observed growing away from coralloid hyphae into

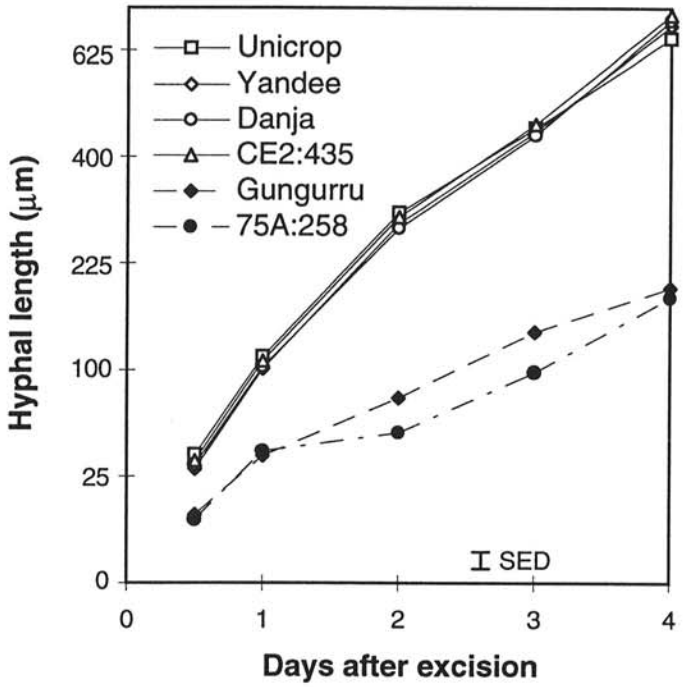

Fig. 4. Growth of saprophytic hyphae (micrometers) of *Diaporthe toxica* from subcuticular coralloid hyphae in excised stems of various cultivars and breeding lines of *Lupinus angustifolius*. Data were square-root transformed before analysis of variance and estimation of the standard error of the difference (SED) between means.

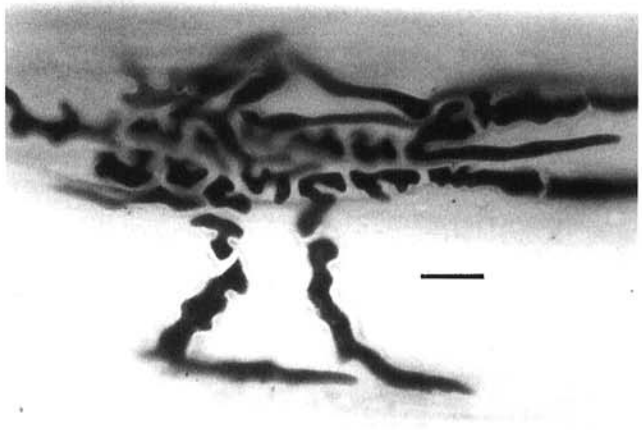

Fig. 5. Saprophytic mycelium growing from a large subcuticular coralloid infection hypha of *Diaporthe toxica* in susceptible *Lupinus angustifolius* cv. Yandee 12 h after excision of stem from the plant. Bar = 10 µm.

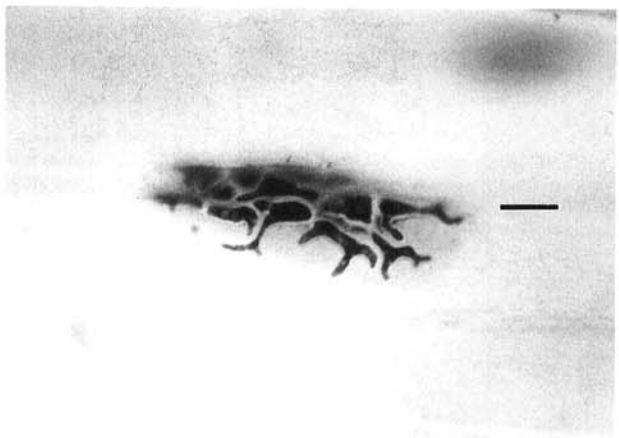

Fig. 6. Saprophytic mycelium growing from a small subcuticular coralloid infection hypha of *Diaporthe toxica* in very resistant *Lupinus angustifolius* breeding line 75A:258 12 h after excision of stem from the plant. Bar = 10 µm.

subepidermal spaces and epidermal cells. The length of saprophytic mycelia growing from coralloid hyphae in susceptible and intermediate lines was about four times greater than the length in resistant and very resistant lines 1 day after excision and six times greater at 4 days after excision (Fig. 4). The two categories of resistance also could be distinguished under a microscope 12 h after excision (Figs. 5 and 6). The apparent viability of coralloid hyphae 4 days after excision also was greater in susceptible lines than in resistant lines, i.e., no growth was observed from many coralloid hyphae on resistant lines. Line CE2:435 had intermediate viability of coralloid hyphae (Table 3).

Browning of excised stems in petri dishes was observed in susceptible cvs. Unicrop, Yandee, and Danja and in intermediate line CE2:435 after 6 days, and a progressive increase was observed in browning in all lines with time after excision, although in the very resistant line 75A:258 there was little browning of tissue 12 days after excision. At 12 days, pycnidia formed on excised stems of the susceptible cultivars and line CE2:435 (Fig. 7). On the trimmed stems remaining in the pots after excision of cotyledonary internodes, browning was observed in susceptible cvs. Unicrop, Yandee, and Danja and in line CE2:435 10 days after excision, beginning near the point of excision and gradually progressing downward. Formation of pycnidia in the susceptible cultivars occurred at 14 days and in line CE2:435 at 18 days. Stems of Gungurru and 75A:258 remained green throughout, and young axillary shoots grew in the cotyledonary nodes, whereas little regeneration occurred in susceptible or intermediately resistant lines.

DISCUSSION

Resistance to Phomopsis stem blight of narrow-leafed lupin caused by *D. toxica* was expressed in the latent infection phase as a reduction in the establishment of large subcuticular infection hyphae and was accompanied by lower apparent viability of latent infections and slower saprophytic colonization upon stem senescence than were observed in susceptible lines. Resistant lines had predominantly small infection hyphae (<100 µm long) 21 days after inoculation, from which saprophytic mycelia colonized host tissue slowly or not at all, whereas susceptible lines had predominantly large viable infection hyphae (>100 µm long) that rapidly colonized host tissue upon senescence. A line with intermediate resistance, CE2:435, had intermediate numbers of both types of infection hyphae but colonized host tissue rapidly upon senescence as in susceptible types.

The existence of two types of coralloid hyphae (large and viable versus small and apparently nonviable) in all lines, the constant total frequency of coralloid hyphae in all lines, and the relative change in frequency of small and large coralloid hyphae in resistant and susceptible lines are new discoveries that help explain the mechanisms of resistance to this disease. Conidial germination and penetration of the cuticle are not affected by host resistance, and resistance appears to be the result of preexisting or induced resistance responses in epidermal cells below the infection site. Resistance actively delays or prevents the establishment of large subcuticular coralloid hyphae during the latent phase of this disease and, possibly as a direct consequence of this, delays or prevents the colonization of host tissue during the saprophytic phase.

We believe this is one of the few reports describing resistance to a latent infection in plants. Resistance is normally thought of as a decrease in visible disease symptoms on living plants. In the case of Phomopsis stem blight of lupin, symptoms normally appear

on senescing or dead stems. Resistance begins operating very soon after fungal penetration of the cuticle and well before the latent infection develops into macroscopic symptoms.

The subcuticular coralloid hyphae are difficult to detect in whole-tissue mounts due to interference from subepidermal tissue. We improved the previous technique (15) of peeling and staining the epidermis, first by freezing tissue at –20°C, which allowed the epidermal layer to peel free of subepidermal tissue, and then by immersing tissue in hot KOH to clear the epidermal cells. This increased the contrast between stained coralloid hyphae and cleared epidermal cells.

The density of large coralloid hyphae in cv. Yandee in our study (250 to 300 per cm) was higher than that reported by Williamson et al. (15) who used 10^6 conidia per ml and a 48-h dew period but was of the same order of magnitude as the results reported by Williamson and Sivasithamparam (14) at 10^7 conidia per ml and 72-h dew periods. It was necessary to use the higher conidial concentration and longer period of humidity to clearly distinguish resistant from intermediate and susceptible lines. The number of conidia attached to the epidermis after staining in this study was less than that reported previously (15), possibly because more conidia were dislodged during staining.

The techniques developed here will be useful for detecting resistance in living plants in the greenhouse. The possibility of doing this nondestructively will greatly improve the efficiency of breeding for resistance to the disease. It should be possible to assess resistance of early generation progeny by inoculating one lateral branch and harvesting seed from another branch of the same plant. In the past, resistance could only be assessed in later generations in replicated field trials, and studies of the genetics of resistance were severely hampered by the lack of a greenhouse test for resistance (4).

Previous studies showed that the mycotoxin phomopsin was detected at higher levels in epidermal peels from susceptible than resistant lines latently infected with *D. toxica* (16). Based on our results, it is possible that resistance-screening tests could be developed using the enzyme-linked immunosorbent assay test for phomopsin. Alternatively, a colorimetric assay of total fungal chitin after allowing a longer colonization period of epidermal peels or inoculated stem tissue may distinguish resistance. A simple but effective test would involve observations of the rate of formation of pycnidia in excised stems of inoculated plants in petri dishes.

Our observations indicate that the apparent viability of infection hyphae is lower, stem colonization during senescence by saprophytic hyphae is slower, and formation of pycnidia is delayed in resistant compared to susceptible lines. This helps explain certain difficulties in understanding the impact of resistance on stem colonization and toxicity (5). The speed of formation and maturity of pycnidia on stems of resistant lines was delayed compared to susceptible lines, but in many cases, the fungus was isolated with equal frequency from stems of both groups (5). In the field, there are most likely enough infection sites in stems of all but the very resistant lines to produce a high frequency of isolation in resistant and susceptible lines, despite the slower colonization and (most likely) lower density of saprophytic mycelia. In fact, very resistant line 75A:258 (previously identified as 75A14-10) had a significantly lower frequency of isolation than susceptible lines at 0 or 14 weeks after harvest, whereas resistant cv. Yorrel (previously identified as 75A45-10) had a frequency of isolation similar to susceptible lines (5). Yorrel has a level of field resistance similar to Gungurru, previously identified as line 75A61-3 (5). The delayed colonization of resistant lines correlates well with their reduced toxicity, which increases with time after harvest (5).

The results reported here also explain why a period of 20 to 30 days after inoculation was required to achieve extensive stromatal development on herbicide-treated plants of cv. Yandee (8). Little stem colonization or stromatal development occurred if plants were treated with herbicide 2 to 3 days after inoculation (W. A. Cowling, *unpublished data*). Coralloid hyphae expand for at least 21 days after inoculation (Table 2) and may need to reach a certain size to achieve "saprophytic competence." The frequency of very large (>250 μm) coralloid infection hyphae at 21 days (Fig. 3) is a strong indicator of host resistance or susceptibility (Table 1) and the potential toxicity of infected lupin stubble (7). Any host resistance mechanism that prevents coralloid infection hyphae from achieving saprophytic competence may be very valuable. Further research is needed to help define saprophytic competence and the viability of various sizes of coralloid hyphae.

There are two levels of resistance operating in this disease: first, a new form of resistance that reduces the frequency of establishment of saprophytically competent latent infections, and second, a type of resistance that keeps the fungus in the latent phase and prevents parasitic hyphae from invading the host while it is healthy. It is not clear what biochemical or physical changes occur when stems are excised from the plant, but almost immediately after excision, "normal" mycelia are observed growing away from latent coralloid hyphae (Fig. 4). The pathogen recognizes and responds quickly to the changes that occur after excision. In healthy plants in the field, these infections may remain latent for 3 to 6 months before natural plant senescence takes place and stem blight symptoms appear.

There are several reports of sucuticular and latent infections among plant pathogens, such as *Venturia inaequalis* (11) and *Rhyncosporium secalis* (2). However, infection of lupin by *D. toxica* seems to be the first report of host resistance actively preventing

TABLE 3. Frequency of apparently viable coralloid hyphae of *Diaporthe toxica* in excised stems of various cultivars and breeding lines of *Lupinus angustifolius*

Cultivar or breeding line	Frequency of apparently viable coralloid hyphae (arcsine degrees [%])[a]
Unicrop	74.8 (93.1)
Yandee	71.0 (89.4)
Danja	71.1 (89.5)
CE2:435	50.0 (58.7)
Gungurru	26.2 (19.5)
75A:258	18.5 (10.1)
SED	2.5

[a] Frequency data were arcsine-transformed for analysis of variance and estimation of the standard error of the difference between means (SED); arcsine-transformed averages in degrees are followed by back-transformed percent averages in parentheses.

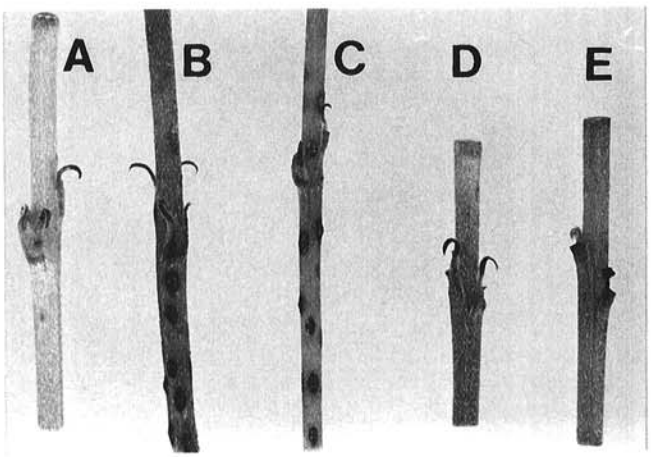

Fig. 7. Browning and formation of pycnidia on stems of various cultivars and breeding lines of *Lupinus angustifolius* previously inoculated with *Diaporthe toxica* excised from plants 21 days after inoculation and incubated for 12 days in a moist petri dish: **A,** control (water inoculation only), **B,** susceptible cv. Yandee, **C,** intermediately resistant breeding line CE2:435, **D,** resistant cv. Gungurru, and **E,** very resistant breeding line 75A:258.

the establishment of latent infection. *Phomopsis sojae* remains semi-dormant and close to the point of inoculation until soybean plants begin to mature, and the proliferation of the fungus results from multiple localized infections (10). Given this similarity in the epidemiology of Phomopsis stem blight of soybean and lupin, it is possible that resistance to latent infection by *P. sojae* in soybean will be expressed in a manner similar to resistance to *D. toxica* in lupin.

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