Field Evaluation of Seed, Pod, and Stem Rot in White Lupine Germ Plasm

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ABSTRACT

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Disease symptoms were evaluated on 92 accessions of white lupine (*Lupinus albus*) grown in replicated field trials in Québec, New Brunswick, and Nova Scotia. Pod, stem, and seed rot symptoms caused by *Phoma* sp. were more severe at the Maritime sites than at the Québec site. At all sites, there were significant differences among accessions. Pod lesions were more severe on early-maturing than on late-maturing accessions. Pod lesion ratings taken in the field were not correlated with severity of discoloration on harvested seed. Accession means for ratings of seed discoloration from each site were positively correlated with those from the other sites, with more similarity between the two Maritime sites than between the Québec and Maritime sites. The incidence of infection with *Phoma* sp. and *Pleiochaeta setosa* was evaluated on random samples of seed for a subset of nine accessions. *Phoma* sp. was common in both regions, whereas *P. setosa* was common in the Maritime region but rare in Québec. Among these nine accessions pooled over all sites, there was no significant correlation between the incidence of infection by the two pathogens and severity of seed discoloration, even though inoculation with *Phoma* sp. at the Québec site appeared to induce more seed discoloration. At the Québec site, however, 80% of the samples that had low levels of seed discoloration also had low levels of *Phoma* infection. Thus, it may be possible to use seed discoloration to screen white lupine germ plasm for resistance to *Phoma* sp. and other seedborne pathogens, at least in environments where conditions do not favor colonization of pods and seeds by other saprophytes that may discolor seeds. Severity of pod symptoms may reflect the physiological age of the plant at the time of assessment rather than genetic resistance and may not be as useful as seed discoloration in evalution of resistance in the field.

White lupine (*Lupinus albus* L.) was only recently introduced to northern areas of North America (i.e., Minnesota, Maine, Ontario, Québec, and the Maritime Provinces of Canada), and there is as yet little information on diseases of lupine in northeastern North America. This crop is a nitrogen-fixing legume that can be used as a high-protein grain or as a forage or green manure in crop rotations. As a grain legume, lupine is a potential substitute for soybean meal or heat-treated whole soybean in animal feeds. Lupine can be grown in areas too cool for soybean production, and it can be utilized easily on-farm as livestock feed.

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Lupine has been grown as a grain legume in Europe, Australia, and the southern United States for over 40 yr following the development of sweet, low-alkaloid cultivars. A number of seedborne stem and pod diseases are economically important on lupine worldwide, including *Phomopsis leptostromiformis* (Kühn) Bubák in Kab. & Bubák on *L. angustifolius* L. (1), *Pleiochaeta setosa* (Kirchn.) S.J. Hughes (3–5,12), and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. (2,6,13). Root and foot rot pathogens, such as *P. setosa, Pythium* spp., *Fusarium* spp., and *Rhizoctonia solani* Kühn, can also attack lupine (9,12).

The climatic conditions of northeastern North America (warm temperatures and high rainfall in summer) favor the development of stem and pod diseases. The brown spot pathogen, *P. setosa*, previously thought to be confined to the southern United States, was observed in Minnesota in 1989 (8). The pathogens *Ascochyta* sp., *Fusarium avenaceum* (Fr.:Fr.) Sacc., and *F. oxysporum* Schlechtend.:Fr. have also been isolated from white lupine in north-central Minnesota (7). Paulitz and Atlin (10) reported *P. setosa* from New Brunswick and Nova Scotia, the first report of brown spot of lupine in Canada. Paulitz and Coté (11) described an unknown species of *Phoma* as a stem and pod pathogen on white lupine in Québec. This pathogen is identical to the one referred

to as *Ascochyta* by other workers in Canada (R. Martin, *personal communication*). Both *P. setosa* and *Phoma* sp. are seedborne. We have also isolated *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore and *C. gloeosporioides* from senescent stems in the field, but these fungi were not pathogenic when tested under greenhouse conditions. A collection of white lupine accessions obtained from the University of Maine, Orono, was grown at three sites in eastern Canada in 1991 for evaluation of agronomic characteristics. In these experiments, the severity of disease symptoms on stems, pods, and seeds was rated to identify accessions with resistance or tolerance to prevalent pathogens and to determine whether disease reaction was positively or negatively associated with important agronomic characteristics.

MATERIALS AND METHODS

Experimental design. In 1991, 99 white lupine germ plasm accessions of diverse origin (designated LG86 through LG166) were obtained from the University of Maine for evaluation at three sites: a fertile sandy loam at Ste-Anne-de-Bellevue, Québec, and sandy loams at Sussex, New Brunswick, and Truro, Nova Scotia.

At the Québec site, experiments were seeded on 16 May in two adjacent randomized complete block designs with two replicates each. At the Maritime sites, the experiments were grown in split-plot designs with two replicates, with seeding dates (early or late) as main plots and accessions as subplots. The seeding dates were 30 April and 16 May at the New Brunswick site and 6 and 17 May at the Nova Scotia site. All seed was inoculated with *Rhizobium lupini* (Schroeter) Eckhardt et al. At all sites, each plot consisted of a single row 2 m long, with 40 cm between rows. Seeding was done mechanically at a rate of 50 seeds per 2-m row at the Québec site and by hand at a rate of 20 seeds per 2-m row at the Maritime sites. Throughout the growing season, plots were hand-weeded as needed.

Inoculation. At the Québec site, plots in one of the two experiments were inoculated with *Phoma* sp. on 5 July by applying 25 ml of a spore suspension to pods on plants within 0.5 m of one end of each row. Inoculum was prepared by culturing *Phoma* sp. (isolated from diseased lupines in 1990) on potato-dextrose agar at 25 C under 12 hr light/12 hr dark fluorescent lighting for approximately 4 wk, when pycnidia developed. Then, 10 ml of sterile water was poured on each plate, and the agar was scraped with a sterile glass rod to remove conidia. The suspension was filtered to remove mycelial fragments, and the filtrate was diluted with sterile water to obtain the required volume of inoculum. The inoculum contained 5×10^4 conidia per milliliter as determined by counts with a hemacytometer. On the day after inoculation, pods were moistened by spraying with tap water twice at 6-hr intervals.

Disease assessment. The severity of lesions on stems and pods was scored at the New Brunswick site on 29 August and at the Nova Scotia site on 30 September. At the Québec site, severity of lesions on pods was scored on 26 July.

Pods were hand-harvested from the center 1 m of each row at the Québec site in August and from entire rows at the Maritime sites in October. Harvested pods were subsequently threshed. Agronomic data recorded at the Québec site included percent emergence, number of days from seeding to the beginning of flowering, number of days from seeding to 50% pod maturity, and plant height. At the Maritime sites, emergence was visually rated, as were maturity on 29 August at the New Brunswick site and on 23 September at the Nova Scotia site and severity of lodging. At the Nova Scotia site only, the number of days from seeding to 10% flowering and the number of days from seeding to 90% pod maturity were also recorded. At all sites, seed yield per square meter and average seed weight were determined.

The seed harvested in the Maritime sites was examined and discoloration was scored on a scale from 1 (no discolored seed) to 10 (all seed discolored). Representative samples of 100 seeds from the Québec site were examined, and the percentage of seeds with discoloration on 20% or more of the testa surface area was recorded.

Of the 99 accessions included in the experiments, seven had missing disease information or other data for one or more replicates or sites. Analysis of variance and calculation of correlation coefficients were conducted only for the 92 accessions with complete data.

Assessment of seed infection. Nine accessions, chosen to represent a range of symptom expression and relative maturity, were assayed for pathogenic fungi. For each of these accessions, a sample of 100 seeds was drawn from a bulk sample from the Maritime sites and a sample of 25 seeds was drawn from each of the four replicates from the Québec site. Seeds were surface-disinfested for 3 min in a solution of 0.5% sodium hypochlorite and 0.5% ethanol. Seeds were then rinsed four times, each time for 5 min, in sterile distilled water. Seeds were blotted on sterilized paper towels and placed on half-strength V8 medium (75 ml of V8 juice, 1.5 g of $CaCO_3$, 20 g of Difco agar, and 100 mg of chloramphenicol in 1 L of distilled water). Five seeds were placed on each plate, for a total of 20 plates per accession. The plates were incubated at 26 C for 1–2 wk under a combination of cool-white fluorescent and long-wave UV black light, 16 hr/day. All plates were examined under a dissecting microscope. The presence of brown pycnidia indicated infection by *Phoma* sp., which was confirmed by examination of pycnidiospores. Colonies with gray backgrounds containing black phragmospores with apical appendages indicated *P. setosa*.

RESULTS AND DISCUSSION

Brown lesions were visible on pods in plots treated with *Phoma* sp. about 3–4 wk after inoculation. These lesions enlarged during pod filling, covering most of the pod surface at maturity. Toward the end of the season, pycnidia were present in the lesions, radiating out from the center in a targetlike pattern. Brown lesions were also visible on lower stems, originating from the area of leaf scars left by senescent leaves. Although no lesions were observed on leaves, inoculated plants became senescent sooner than noninoculated plants. Similar but less severe symptoms were visible in the central 1-m section of plots not inoculated with *Phoma* sp. and also in noninoculated plots. At maturity, seed from all plots exhibited light brown discoloration. At the Maritime sites, dark brown lesions were visible on lower stems of most accessions by the middle of flowering. These lesions grew to cover most of the main stem below the primary inflorescence by the time lower pods were ripe. Brown lesions on pods, which were observed on all accessions, expanded and developed pycnidia as pods senesced. Some pod lesions developed an orange tinge. Seed discoloration ranging from moderate to severe was observed in all plots.

Even though no artificial inoculation was carried out at the Maritime sites, disease symptoms were severe enough to allow assessment of lesion severity on

Table 1. Correlation coefficients for disease severity and agronomic traits of white lupine germ plasm evaluated at Ste-Anne-de-Bellevue, Québec

Agronomic traits	Incidence of seed discoloration[a]	Disease severity on pods[b]
Emergence	ns[c]	ns[c]
Days to flowering	0.48**	−0.31**
Days to maturity	0.59**	−0.37**
Plant height	0.48**	−0.33**
Seed yield	−0.22*	ns
Seed weight	ns	ns
Disease severity on pods	ns	...

[a] Discoloration on more than 20% of the testa surface area.

[b] Based on a 0–3 rating scale, where 0 = no lesions, 1 = lesions covering 1–10% of pod surface, 2 = lesions covering 11–25% of pod surface, and 3 = lesions covering >25% of pod surface.

[c] ns = Not significant at the 0.05 level of probability; * = significant at the 0.05 and ** = significant at the 0.01 level of probability.

both pods and stems. At the Québec site, disease symptoms were severe enough to warrant assessment only on pods in the inoculated replicates. For pod lesion rating, there was significant variation among accessions ($P < 0.05$ at Québec, $P < 0.01$ at the Maritime sites), but accession means were not significantly correlated with seed discoloration ratings taken after harvest (Table 1). At the Maritime sites, stem lesion ratings varied significantly among accessions ($P < 0.01$) and were weakly correlated with pod lesion rating at the New Brunswick site and with seed discoloration and pod lesion rating at the Nova Scotia site (Table 2).

At the Québec site, all accessions in the experiment matured early enough to be harvested in August. Under the cooler conditions prevalent at the Maritime sites, the experiment could not be harvested until October, and only the earliest-maturing accessions were considered to have agronomic potential. Pod lesions tended to be more severe in early-maturing accessions (Tables 1 and 2), suggesting that early-maturing cultivars are more susceptible than late-maturing ones. However, another plausible explanation is that the pod lesions on the late-maturing accessions had not fully developed at the time of assessment. Symptom expression on pods may be influenced by physiological age of the plant, since lesion size was larger on pods approaching senescence. Seed discoloration tended to be most severe in late-maturing accessions (Tables 1 and 2). At two of the three sites there was a weak but statistically significant negative association between seed discoloration and seed yield.

Table 2. Correlation coefficients for disease severity and agronomic traits of white lupine germ plasm evaluated at Sussex, New Brunswick, and Truro, Nova Scotia

	Disease severity[a]		
Agronomic traits	**Seeds**	**Pods**	**Stems**
Sussex, New Brunswick			
Emergence rating	ns[b]	ns	ns
Maturity rating	0.36**	−0.42**	ns
Lodging rating	0.41**	ns	ns
Seed yield	−0.38**	ns	ns
Seed weight	0.22*	ns	−0.30**
Disease severity on pods	ns	...	...
Disease severity on stems	ns	0.34**	...
Truro, Nova Scotia			
Emergence rating	−0.22*	ns	ns
Days to flowering	0.49**	−0.22*	0.50**
Maturity rating	0.57**	−0.34**	ns
Days to maturity	0.62**	−0.44**	ns
Lodging rating	0.23*	ns	ns
Seed yield	ns	ns	ns
Seed weight	ns	ns	−0.33**
Disease severity on pods	ns	...	...
Disease severity on stems	0.27**	0.22*	...

[a]Severity of seed discoloration rated on 1–10 scale, where 1 = no discoloration and 10 = all seeds discolored; severity of pod and stem lesions rated on a 1–5 scale, where 1 = no lesions and 5 = severe lesions.
[b]ns = Not significant at the 0.05 level of probability; * = significant at the 0.05 and ** = significant at the 0.01 level of probability.

At all three locations, seed discoloration ratings varied significantly ($P < 0.01$) among replicates and accessions. At the Québec site, the mean percentage of discolored seeds was 9 and 12.2% in the noninoculated and inoculated plots, respectively; accession means ranged from 0.25% for LG92 to 39.5% for LG69. At the New Brunswick site, the mean seed discoloration rating was 4.9 and 7.5 in early-seeded and late-seeded plots, respectively. At the Nova Scotia site, this rating averaged 7.5 in early-seeded and 8.4 in late-seeded entries. Accession means ranged from 3.3 (LG97) to 9.3 (LG86) at the New Brunswick site and from 5.3 (LG97) to 10.0 (LG86 and LG164) at the Nova Scotia site. Seed discoloration at the Maritime sites was considerably more severe than at the Québec site (a rating of 10 on the scale used at the Maritime sites would correspond to 100% on the scale used at the Québec site).

The accession means for seed discoloration from each location were positively correlated with means from other locations, with more similarity between the two Maritime sites ($r = 0.60$, $P < 0.01$) than between the Québec and Maritime sites ($r = 0.50$, $P < 0.01$ for Québec–New Brunswick and $r = 0.42$, $P < 0.01$ for Québec–Nova Scotia). Figure 1 shows the accession means for Québec plotted against those for the Maritime sites. Several accessions had relatively little seed discoloration in both the Québec and the Maritime regions; these accessions are being investigated as possible useful sources of resistance or tolerance. Others were badly discolored in both regions.

In the accessions for which pathogen identification was conducted, *P. setosa* was consistently found at a low frequency (2–10%) in the Maritime samples and was rarely found (1% infection in two of the 10 accessions) at the Québec site (Table 3). *Phoma* sp. was more common, with frequencies from 17 to 47% in the Maritime samples and averaging 1–33% at the Québec site. As expected, the frequency of *Phoma* sp. was generally higher in inoculated plots. More than 30% of the seeds were also colonized by

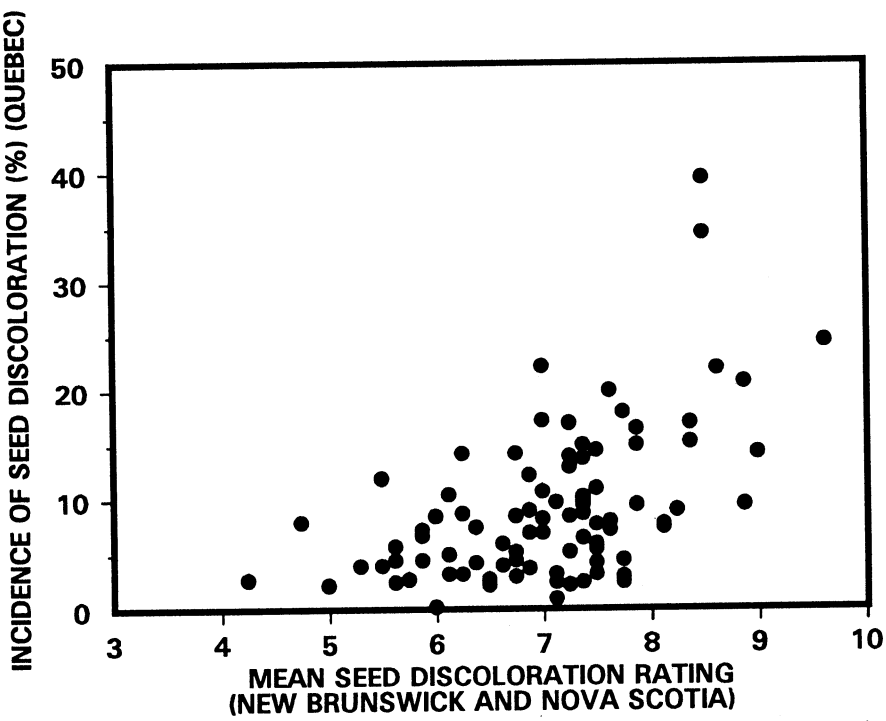

Fig. 1. Percent incidence of discolored seeds in 92 accessions of white lupine evaluated in Québec compared with average seed discoloration rating (1 = no discolored seeds to 10 = all seeds discolored) of the same accessions grown in trials at New Brunswick and Nova Scotia.

Table 3. Severity of seed discoloration and frequency of detection of *Pleiochaeta setosa* and *Phoma* sp. on seeds of nine lupine accessions at three locations in Canada: Ste-Anne-de-Bellevue, Québec (QC); Sussex, New Brunswick (NB); and Truro, Nova Scotia (NS)

				Incidence of seed infection (%)			
	Seed discoloration[a]			*P. setosa*		*Phoma* sp.	
Accessions	QC (%)	NB (1-10)	NS	NB/NS[b]	QC	NB/NS[b]	QC
LG69, Multalupa	46.0 (33.0)[c]	7.50	9.50	3	0	17	34 (26)[c]
LG101, ICARDA IFLU-29	38.5 (30.5)	8.00	9.00	2	0	37	8 (42)
LG84, Bethlehem Best	8.5 (20.0)	8.75	9.25	9	0	29	22 (44)
LG116, L1027N	7.0 (13.0)	6.50	8.25	4	0	29	10 (18)
LG128, 46-10	4.5 (4.5)	4.50	6.75	6	0	21	6 (14)
LG159, Ultra	3.0 (41.5)	6.75	7.25	10	1	35	0 (2)
LG90, WTD 180	2.0 (16.0)	6.00	7.75	3	0	47	14 (20)
LG102, TR2	2.0 (7.0)	4.50	7.25	9	0	27	6 (10)
LG157, Kiev mutant	1.0 (1.0)	6.25	8.00	9	1	44	2 (26)

[a]In Quebéc, rated as percent incidence of seeds with discoloration on more than 20% of the testa surface area; in New Brunswick and Nova Scotia, rated on a 1–10 scale, where 1 = no discoloration and 10 = all seeds discolored.
[b]Seeds from the two sites were pooled.
[c]Data from inoculated replicates in parentheses.

Alternaria spp., but there were no apparent differences between accessions (*unpublished*).

When pathogen identification and disease symptom data for the same accessions were compared, there was no significant correlation between severity of seed discoloration and presence of *P. setosa*. At the Québec site, badly discolored seed tended to have a high frequency of *Phoma* sp., but at the Maritime sites, highly discolored seed had a lower frequency of *Phoma* sp. Even though both *P. setosa* and *Phoma* sp. were confirmed to be present in both regions, it is not clear whether the severe symptoms observed at the Maritime sites were caused by the same organism that induced mild symptoms at the Québec site. Four of the 36 samples from Québec showed a high level of seed infection with *Phoma* sp. but a low level of seed discoloration, indicating a latent symptomless infection. Conversely, the high level of seed discoloration in the wetter climate at the Maritime sites may have been caused by saprophytic fungi such as *Alternaria*, colonizing senescent pods and discoloring seed. Thus, it is difficult to determine what fungus is causing the seed discoloration. In general, however, most seed samples from the Québec site that had low incidence (<20%) of seed discoloration also had low incidence (<20%) of seed infection by *Phoma* sp. (17 of 21 samples). Of the accessions included in sampling for pathogen identification, probably only LG157 (Kiev mutant) should be considered as a possible source of tolerance to seed pathogens, and this might only be useful for the Québec environment (Table 3). *Phoma* sp. was detected on only 2% of the seeds of this accession when it was grown without inoculation in Québec. With inoculation, the incidence of seed infection with *Phoma* sp. rose to 26%, yet the percentage of discolored seeds remained at only 1%. In contrast, LG157 did not appear to possess useful resistance or tolerance at the Maritime sites.

In conclusion, differences in disease severity and seed infection by two seedborne pathogens of white lupine were detected among 92 accessions grown in three locations in eastern Canada. Selection for resistance in white lupine to these two pathogens may be possible. Disease severity and incidence of seed infection were greater in the wetter climate of the two Maritime sites. The incidence of seed infection by *Phoma* sp. was much higher than that of infection by *P. setosa* at both sites. *P. setosa* was rarely detected in Québec, even though the crop was grown from the same seed lots as those at the Maritime sites. Pod symptoms were more severe on early-maturing cultivars, yet these cultivars had the least seed discoloration. Thus, disease symptoms on the pod appear to be related more to physiological age of the plant than to disease resistance. Rating disease severity on pods may not be useful in selecting for resistance, particularly among accessions at different physiological ages. However, rating seed discoloration may be of some use in selecting for resistance to *Phoma* sp., since most seed samples with low levels of discoloration also had low levels of infection by *Phoma* sp. in the Québec site. This relationship was much more variable in the Maritimes, possibly because of the confounding effect of other unknown seed-discoloring fungi. The relationship between seed infection by *P. setosa* and seed discoloration was not significant in either site, possibly because of the low incidence at the Québec site and coinfection by *Phoma* sp. in the Maritimes.

Further work is needed to clarify the epidemiology, disease cycle, and control of these two pathogens of white lupine in eastern Canada.

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