

Susceptibility of *Pinus strobus* and *Lupinus* spp. to *Phytophthora cinnamomi*

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ABSTRACT

Two-year-old *Pinus strobus* seedlings were highly susceptible to *Phytophthora cinnamomi* in greenhouse and shadehouse tests. Symptoms include stunting of new growth, chlorosis, necrosis, drooping of necrotic needles, and decay of feeder and small lateral roots. Seedlings were inoculated by dipping roots into a water suspension of inoculum, or by incorporating infested oat grains into the soil. There was no difference in disease development between root-dip and soil inoculations. Seedlings grown in a saturated soil expressed

symptoms earlier and died faster than seedlings grown in a nonsaturated soil. *Lupinus albus* 'Tifton A-10' and *Lupinus angustifolius* 'Rancher' were found to be reliable indicator plants for *P. cinnamomi*. Lupine seeds planted in pots with inoculated *P. strobus* seedlings often failed to emerge. Lupine plants which did emerge developed lesions at the root collar, became partially defoliated, blackened, and collapsed within 40 days after planting.

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Root diseases caused by *Phytophthora cinnamomi* Rands on many diverse species of plants throughout the world (3, 9, 10) have long been a problem confronting foresters, horticulturists, and plant pathologists. The fungus is best known in forests in the southeastern United States for its role in little leaf disease of *Pinus echinata* Mill. (1, 11). *Phytophthora cinnamomi* has also been implicated as one of several pathogens responsible for damping-off of coniferous seedlings in nurseries (4).

Phytophthora cinnamomi is responsible for a severe root rot of *Abies fraseri* Poir. (5, 6, 8), an important Christmas tree and ornamental plant grown in western North Carolina. This disease was most severe on *A. fraseri* planted on wet sites (5).

Symptoms of infection of *A. fraseri* by *P. cinnamomi* include decay of roots (especially small lateral and feeder roots) and a reddish-brown necrosis of the cambial region at the root collar extending several centimeters up the stem and down the larger roots (7). Foliar symptoms include chlorosis, stunting, and eventually a reddish-brown necrosis of the foliage, and in some cases a wilting of the current year's growth. Control has been based on prevention through selection of disease-free planting stock, and planting on well-drained sites is recommended.

There is an increasing trend by Christmas tree and nursery growers to plant *Pinus strobus* L. (eastern white pine) seedlings in areas where *A. fraseri* has been eliminated due to root rot caused by *P. cinnamomi*. Except for reports on damping-off and root rot in nurseries (4), there is little information on the effects of *P. cinnamomi* on older *P. strobus* seedlings.

The objectives of this study were (i) to investigate the susceptibility of 2-year-old *P. strobus* seedlings to *P. cinnamomi* at different soil-moisture regimes under greenhouse and shadehouse conditions, (ii) to evaluate two inoculation techniques, and (iii) to determine the usefulness of two species of *Lupinus* as bioindicator plants as reported by Chee and Newhook (2) in New Zealand.

MATERIALS AND METHODS.—*Phytophthora cinnamomi* (isolate Grand 73-6) isolated from infected *A. fraseri* feeder roots was used in all experiments. Stock cultures were maintained on oatmeal agar at 14 to 16 C.

Two-year-old *P. strobus* seedlings obtained from the Edwards State Forest Tree Nursery, Morganton, North Carolina, were lifted in late March and stored for 7 days at 4 C prior to use.

Seeds of *Lupinus albus* L. 'Tifton A-10' (white lupine) and *Lupinus angustifolius* L. 'Rancher' (blue lupine) were obtained from the Coastal Plain Experiment Station, Tifton, Georgia. Seeds were soaked 24 hours in sterile distilled water prior to use.

Inoculum of *P. cinnamomi* was grown on sterilized oat grains in 1,000-ml Erlenmeyer flasks and on oatmeal agar in 90 × 15-mm plastic petri plates. Cultures were incubated at 25 C until the oat grains were thoroughly infested (19 to 21 days) or abundant mycelium covered the surface of the agar plate (8 to 10 days).

A soil mix of loam and coarse sand (2:1, v/v) was used. The loam and sand were treated with methyl bromide for 74 hours prior to mixing. *Pinus strobus* and *Lupinus* spp. were grown in 15- or 20-cm diameter clay pots.

Inoculation techniques consisted of mixing soil with infested oat grains, or dipping an intact root system into a water suspension of inoculum. Soil inoculation consisted of mixing 40 ml of infested oat grains into the soil in each 15-cm pot and 50 ml of oat grains into each 20-cm pot. Controls received similar amounts of infested oat grains that were autoclaved for 30 minutes at 121 C.

Inoculum used for the root-dip technique was prepared by placing the contents of 12 petri plates into 6,000 ml of sterile distilled water and blending for 30 seconds at low speed. The resulting suspension, consisting of hyphal fragments, sporangia, and chlamydozoospores was placed in a 10-liter plastic bucket. *Pinus strobus* seedlings were inoculated by dipping and swirling the root systems into the inoculum suspension for 5 seconds. Inoculated

seedlings were planted in 15-cm pots. Controls consisted of inoculum similarly prepared and autoclaved for 20 minutes at 121 C. Control seedlings were planted in 20-cm pots.

One *P. strobus* seedling and two *Lupinus* spp. seeds were planted in each pot. Seeds of *Lupinus* spp. were planted 3 cm on either side of the *P. strobus* seedlings and 2 cm deep.

Two conditions of soil moisture were maintained in the pots in the greenhouse. A saturated soil condition was maintained by placing pots in clay or aluminum saucers which were kept filled with water throughout the experiment. A nonsaturated soil was maintained by omission of saucers. All seedlings were watered from the top once daily.

Greenhouse experiments consisted of 15 soil-inoculated and 10 control seedlings, and 15 root-dip inoculated and 10 control seedlings maintained in a saturated soil. The same number of similarly inoculated and control seedlings was maintained in a nonsaturated soil. A total of 200 *Lupinus* spp. were planted in the greenhouse.

Shadehouse experiments consisted of 15 soil-inoculated and 10 control seedlings, and 15 root-dip and 10 control seedlings treated as previously described, planted in 20-cm pots, and maintained in a shadehouse for the duration of the experiment. Inoculated and control seedlings were separated by a wooden barrier covered with plastic tarp to prevent possible spread of *P. cinnamomi* in run-off water. All seedlings received water only as needed from an overhead sprinkler during prolonged drought periods. A total of 100 *Lupinus* spp. seeds were planted in pots maintained in the shadehouse.

RESULTS AND DISCUSSION.—*Phytophthora cinnamomi* was pathogenic on *P. strobus* and *Lupinus* spp. Seventy-eight of 90 inoculated 2-year-old *P. strobus* seedlings were dead or showed advanced symptoms (Table 1) 150 days following inoculation. All of the lupine plants in the greenhouse, and 37 of 60 in the shadehouse placed in pots with inoculated *P. strobus* seedlings failed

to emerge or were dead at the termination of the experiments.

Symptomatology on *Pinus strobus*.—Root symptoms were characteristic of infection by *P. cinnamomi* reported for *A. fraseri* (7, 8) and *Pinus echinata* (1, 11). Feeder roots became soft and flaccid, blackened, and began to decay following necrosis of the root tips. Decay progressed to include small lateral roots. Eventually, most feeder roots and small lateral roots became decayed, resulting in the loss of most of the root system (Fig. 3). There was no distinct discoloration of the cambium region at the root collar or of the large lateral roots as observed on *A. fraseri* (8).

New foliage of infected *P. strobus* seedlings was initially chlorotic and stunted (Figs. 1, 2). Necrosis began at the tips of the needles and progressed toward the base. Necrotic foliage attained a uniform cinnamon or reddish-brown color. Older needles became necrotic and discolored similar to new needles. Drooping of all necrotic needles was characteristic of dead seedlings. Symptom expression was similar for infected seedlings in both the greenhouse and shadehouse.

Symptomatology on *Lupinus* spp.—Both *L. albus* and *L. angustifolius* exhibited similar symptoms. Initially, the roots began to soften and discolor with lesions developing at the root collar. As symptoms progressed, the entire root system became necrotic, resulting in a loss of most of the lateral roots.

Initial foliar symptoms included browning of the leaves and partial defoliation. As symptoms progressed, remaining leaves drooped, and the stem became black at the root collar. Eventually, the entire stem became black, shriveled and collapsed, or dried out and died. Symptom expression was similar for lupine plants in both the greenhouse and shadehouse.

Effect of soil moisture.—*Pinus strobus* seedlings grown in a saturated soil developed symptoms and died faster than those maintained in a nonsaturated soil (Table 1). This agrees with observations by Kuhlman and Wells (8) and Grand and Lapp (5) on the occurrence of *P.*

TABLE 1. Susceptibility of 2-year-old *Pinus strobus* seedlings to *Phytophthora cinnamomi* and effect of soil moisture on disease development in greenhouse and shadehouse conditions

Soil moisture condition	Inoculation technique	Seedlings (no.)	Seedlings showing symptoms ^a (no.)	Time to death [avg. days (range)]
Greenhouse				
Saturated ^b	Root-dip ^c	15	15	95 ^d (77-150)
Saturated	Soil ^e	15	12	87 ^d (77-115)
Nonsaturated	Root-dip	15	12	116 (57-150)
Nonsaturated	Soil	15	12	102 (29-150)
Shadehouse				
Natural ^f	Root-dip	15	12	118 (105-133)
Natural	Soil	15	15	116 (106-123)

^aControls were subjected to root-dip and soil inoculations with autoclaved inoculum. No control plants were diseased 150 days after inoculations.

^bPots were placed in saucers kept filled with water.

^cSeedlings inoculated by dipping root systems into a water suspension of inoculum.

^dChi-square value for saturated vs. nonsaturated soil significant, $P = 0.10$.

^eSeedlings inoculated by incorporation of oat grains infested with *P. cinnamomi* into soil.

^fWater received in form of rain except during extended dry periods.

cinnamomi root rot of *A. fraseri* on wet sites. These results indicate that *P. strobus* seedlings should not be planted on sites known to have a history of root rot

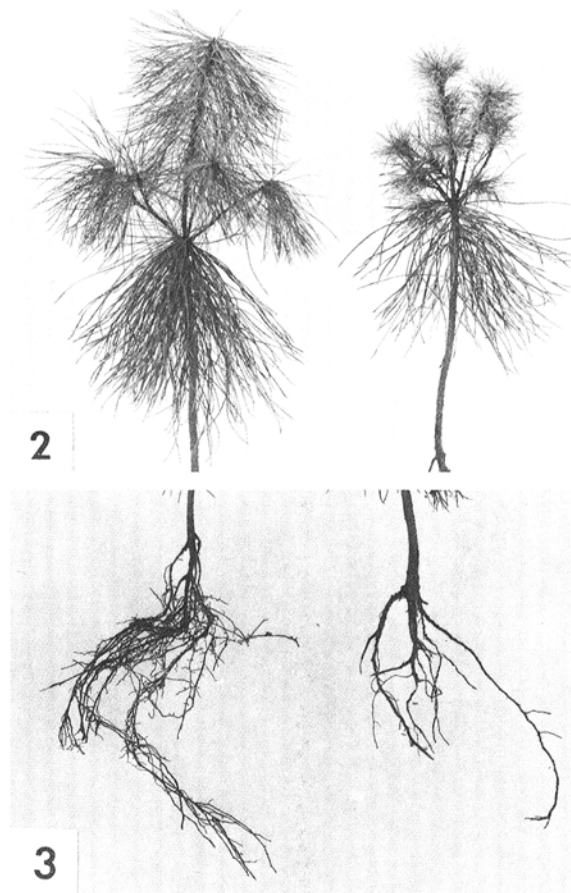
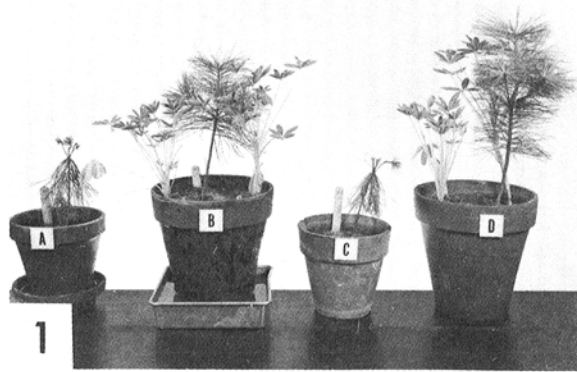


Fig. 1-3. Effects of *Phytophthora cinnamomi* on 2-year-old *Pinus strobus* seedlings and *Lupinus* spp. plants. 1) Inoculated *P. strobus* seedlings (A and C). Note stunted needles and reduced height growth. *Lupinus* spp. plants had collapsed and died by this time, 60 days after inoculation. Controls (B and D). Pots A and B were maintained in a saturated soil. 2) Foliar symptoms on *P. strobus* seedlings. Note stunted needles on new growth of inoculated plant (right) compared to control (left). 3) Root symptoms on *P. strobus*. Note lack of small feeder and lateral roots on inoculated seedlings (right) compared to control (left).

caused by *P. cinnamomi*. This would eliminate the use of *P. strobus* seedlings as an alternate to *Abies fraseri* for Christmas trees or ornamental plants on wet sites.

In contrast, soil moisture did not alter disease development in the lupine plants. Seeds planted in a saturated soil died in 34 days on the average after planting compared to 36 days for those planted in a nonsaturated soil. There was no difference in susceptibility between *L. albus* and *L. angustifolius*.

These results show that *P. cinnamomi* will infect lupine plants under varied soil moisture conditions and that lupine plants can be used to determine the presence of *P. cinnamomi* in soil. This confirms the work of Chee and Newhook (2) who showed that blue lupine (*Lupinus angustifolius* L.) can serve as an indicator plant for certain *Phytophthora* spp. in soil samples in New Zealand.

Effects of inoculation techniques.—Soil and root-dip inoculations were equally effective in the number of infected *P. strobus* seedlings (Table 1). There was no difference between techniques in incubation period, or in days to death for *P. strobus* seedlings or *Lupinus* spp.

The root-dip technique appeared to be the more efficient of the two techniques for *P. strobus* seedling inoculation. Time required for preparation of inoculum is shorter on oatmeal medium (8-10 days) than on sterilized oat grain (19-21 days). The root-dip treatment also allowed rapid and more uniform inoculation of the entire root system prior to planting, and eliminated mixing of oat grain inoculum into the soil. This technique lends itself readily to inoculation of large numbers of seedlings simultaneously.

LITERATURE CITED

- CAMPBELL, W. A., and O. L. COPELAND. 1954. Littleleaf disease of shortleaf and loblolly pines. U.S. Dep. Agric. Circ. 940. 41 p.
- CHEE, K., and F. J. NEWHOOK. 1965. Improved methods for use in studies on *Phytophthora cinnamomi* and other *Phytophthora* species. N.Z.J. Agric. Res. 8:88-95.
- COMMONWEALTH MYCOLOGICAL INSTITUTE. 1968. Distribution maps of plant diseases. Kew, Surrey, England. (Looseleaf n. p.)
- CRANDALL, B. S., G. F. GRAVETT, and M. W. RYAN. 1945. Root disease of *Castanea* species and some coniferous and broad-leaf nursery stocks caused by *Phytophthora cinnamomi*. *Phytopathology* 35:162-180.
- GRAND, L. F., and N. A. LAPP. 1974. *Phytophthora cinnamomi* root rot of fraser fir in North Carolina. *Plant Dis. Rep.* 58:318-320.
- HEPTING, G. H. 1971. Diseases of forest and shade trees of the United States. U.S. Dep. Agric. Handb. 386. 658 p.
- KUHLMAN, E. G., and F. F. HENDRIX, JR. 1963. *Phytophthora* root rot of fraser fir. *Plant Dis. Rep.* 47:552-553.
- KUHLMAN, E. G., and C. G. WELLS. 1971. *Phytophthora* root rot and fertilization damage in Christmas Tree plantations. *Am. Christmas Tree J.* 15:17-20.
- THORN, W. A., and G. A. ZENTMEYER. 1954. Hosts of *Phytophthora cinnamomi*. *Plant Dis. Rep.* 38:47-52.
- WATERHOUSE, G. M., and J. M. WATERMAN. 1966. No. 113 in the Commonwealth Mycological Institute's Descriptions of pathogenic fungi and bacteria. The Eastern Press, London. (Looseleaf, n. p.)
- ZAK, B., and W. A. CAMPBELL. 1958. Susceptibility of southern pines and other species to the littleleaf pathogen in liquid culture. *For. Sci.* 4:156-161.