Root Rot of *Aucuba japonica* Caused by *Phytophthora cinnamomi* and *P. citricola* and Suppressed with Bark Media

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ABSTRACT

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Phytophthora cinnamomi and *P. citricola* were pathogenic to *Aucuba japonica* in laboratory and greenhouse studies. *P. citricola* was isolated more often than *P. cinnamomi* from diseased aucubas in landscape plantings and caused a more severe root rot that often resulted in death of the plant. Root rot was suppressed in well-drained pine bark (pH 4.5) and hardwood bark compost (pH 6.8) but not in a soil-sand medium after 10 days. Suppression was lost if media were kept saturated and the pH of pine bark was raised to 6.5 with lime; however, saturated pine bark at pH 4.5 remained suppressive. An apparently nonpathogenic root rot with symptoms similar to those caused by *Phytophthora* spp. developed when aucuba plants were grown in poorly drained soil amended with cornmeal (1–10% by weight).

Additional key words: Cornaceae, drowning, gold-dust plant, woody ornamental

Aucuba japonica Thunb., the gold-dust plant, is an introduced woody ornamental from Japan commonly used in landscapes in the southeastern United States. In recent years, plants in production and in landscapes have developed a root rot that causes necrosis of the lower stem and wilting of the foliage, followed by death of the plant. *Phytophthora* spp. were isolated from several diseased plants submitted to the North Carolina State University Plant Disease and Insect Clinic; however, pathogenicity was never demonstrated.

Milled hardwood and pine barks are replacing peat in potting media for nursery production of ornamentals (2,10). In addition, hardwood bark compost (7) and bark of Australian radiata pine (4) have been effective in controlling Phytophthora root rot of ornamentals when used as the plant growth medium. However, whether southern pine bark suppresses. Phytophthora root rot has not been demonstrated. In this study, we identified *Phytophthora* spp. isolated from diseased aucubas, tested the pathogenicity of the isolates, and determined whether using bark of pines grown in the southern United States as a growing medium suppresses Phytophthora root rot of aucuba.

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MATERIALS AND METHODS

Identification of *Phytophthora* isolates. Six isolates of *Phytophthora* spp. were obtained from diseased *A. japonica* submitted to the Plant Disease and Insect Clinic. Roots were placed on modified pimaricin-penicillin-polymyxin (PPP) selective medium (1) with 10 mg/L of pimaricin instead of 100 mg/L. Mycelium of each *Phytophthora* isolate was transferred to cornmeal agar to establish a pure culture. One or two 0.7-cm mycelial agar disks from 4-day-old cultures were placed in 25 ml of clarified lima bean extract (50 g of frozen lima beans per liter of water) in petri plates at 25 C in continuous light. The mycelial mats were observed daily for formation of sporangia, oogonia or antheridia, oospores, and chlamydospores. Mycelial mats that had not formed sporangia within 4 days were either rinsed with sterile, distilled water (SDW) and then soaked in SDW for 24 hr, or rinsed in SDW and then soaked in sporangia-stimulating salt solution to induce sporangia formation (11). Sporangia, oospores, and other morphological characteristics were used to identify the fungi with *Phytophthora* spp. keys (8,13).

Pathogenicity studies. Aucubas were rooted from cuttings dipped in a rooting compound and placed in wooden flats containing vermiculite. Plants were kept under intermittent mist for 5–6 wk and then moved to a shaded greenhouse bench, where they were watered daily and fertilized weekly. Plants were 6–18 mo old when used. Each plant was 10–15 cm tall and had six to 12 fleshy roots 0.2–0.3 cm in diameter and 3–10 cm long. Roots usually had two to six lateral branches 1–3 cm long.

A water culture method and an infested soil-sand culture method of inoculation were used. Inoculum was prepared for the water culture method by removing the agar disk from three 5-day-old mycelial mats of each isolate, rinsing the mats in SDW, and fragmenting the mats in 250 ml of SDW in a blender at low speed for 10 sec. The volume of the inoculum mixture of each isolate was brought to 750 ml with SDW. One aucuba plant was placed in each of three 500-ml beakers containing 250 ml of inoculum to cover roots. Three aucubas received water with no inoculum. Roots were observed every 2 days, and incipient lesions were noted. After 1 wk, three to five roots from each plant were surface-sterilized in 0.1% NaOCl and cut into 0.5-cm sections. The pieces were placed sequentially on PPP medium and assayed for the fungus. The test was repeated with the fungi reisolated from the roots.

In the infested soil-sand method, inoculum was prepared as described for the water culture method but was then added to 1,200 cm^3 of a soil-sand medium (1:1, v/v) and thoroughly mixed. Three aucubas were potted individually in 400 cm^3 of the infested medium per aucuba for each isolate. Containers were placed in saucers on a greenhouse bench and watered daily to keep the medium saturated. Seven days after transplanting, plants were removed from the media, and their roots were rated on the following root rot scale: 0 = 0–9% of total root area necrotic; 1 = 10–19% necrotic; 2 = 20–29%; and so on, to 9 = 90–100% of root area necrotic. Roots were surface-sterilized, sectioned, and placed sequentially on PPP medium as described previously. The test was repeated with the fungi reisolated from the roots.

Suppression with bark media. Milled pine bark (PB; pH 4.5), obtained from Coulbourn Lumber Co., Windsor, NC, was compared with hardwood bark compost (HBC; pH 6.8), obtained from H. A. J. Hoitink, Ohio Agricultural Research and Development Center, Wooster. HBC medium has been demonstrated to suppress root rot.

Nutrient and chemical analyses performed by the North Carolina Department of Agriculture (Raleigh)

indicated that PB and HBC have cation-exchange capacities of 10–15 milliequivalents per 100 g, bulk densities of 0.4 g/cm^3, and base saturation percentages of 34 and 95%, respectively. The concentrations of phosphorus, potassium, calcium, magnesium, and manganese were three to five times higher in HBC than in PB; however, nutrients are added to HBC before composting (7).

Seven 5-day-old mycelial mats of *P. cinnamomi*, isolate 137, and *P. citricola*, isolate 611, were rinsed in SDW and fragmented in a blender in 250 ml of SDW as described previously. Inoculum was used to infest 1,200 cm^3 of sand. Infested sand was mixed 1:4 by volume with HBC, pH 6.8; PB, pH 4.5; or soil-sand (1:1 by volume), pH 6.3. Controls for each medium received sterile sand. Each of three aucubas was potted in 400 cm^3 of infested medium, one plant per container. Media were kept saturated. To compare water relations of the media, additional plants were potted in infested and noninfested HBC and PB and placed on upturned saucers to allow for natural drainage. The pH was determined initially and after 7 days. Roots were rated after 7 days, then placed on PPP medium and assayed for *Phytophthora* spp. The experiment was repeated with inoculum mixed directly with the media.

The effect of pine bark pH on suppression of root rot was investigated by raising the pH from 4.5 to 6.5 with agricultural-grade lime. The pH was determined 3 days after lime was added. Four 5-day-old mycelial mats of *P. citricola* were prepared, rinsed in SDW, and blended in 300 ml of SDW as described previously. This inoculum (150 ml) was added to 1,200 cm^3 of PB at either pH 4.5 or pH 6.5. Controls received 150 ml of SDW with no inoculum. Three aucubas were potted individually in 400 cm^3 of medium for each treatment. Media were kept saturated. Roots were rated after 10 days, then placed on PPP medium and assayed for *Phytophthora* spp.

Nonpathogenic root rot of aucuba. The role of high organic matter and water-saturated conditions on the appearance of an apparently nonpathogenic root rot symptom on aucuba was investigated by adding yellow cornmeal to a soil-sand medium (1:1, v/v) at 1:10, 1:100, and 1:1,000 by weight. Soil-sand, peat-soil, PB-soil, and HBC-soil (all 1:1 by volume and not amended with cornmeal) also were prepared. Three aucubas were each potted in 400 cm^3 of medium for each treatment. Media were kept saturated. Roots were rated after 10 days, then placed on PPP medium and assayed for pythiaceous fungi. Media pH values were determined at this time.

RESULTS

***Phytophthora* isolates.** Of the six *Phytophthora* spp. isolated from necrotic roots of aucuba, two were *Phytophthora cinnamomi* Rands and four were *P. citricola* Sawada. Isolates of *P. citricola* produced abundant oogonia and paragynous antheridia within 5 days on cornmeal agar and lima bean extract, and variable-shaped sporangia as described by Zentmyer and Jefferson (15) in lima bean extract. Isolates of *P. cinnamomi* did not produce sporangia on lima bean extract; however, sporangia formed on mycelial mats rinsed and soaked in sporangia-stimulating salt solution for 12–24 hr. No oospores were formed by the *P. cinnamomi* isolates, but chlamydospores were abundant on mycelial mats after 5 days in lima bean extract.

Pathogenicity. Lesions developed on roots in 2–3 days in water culture. No lesions were observed on aucuba roots in water without inoculum. *P. citricola* isolates caused water-soaked root lesions at the tips, middle, and junctions of lateral branches. Lesions grew as incubation was lengthened, and entire roots eventually became necrotic. Necrosis reached the crown area by day 10 and continued into the stem, causing the stem to collapse and the foliage to wilt.

Root lesions caused by *P. cinnamomi* developed mainly at tips and junctions of lateral branches and occasionally on midroot areas. *P. cinnamomi* did not extensively colonize the roots or kill plants as did *P. citricola*; however, both fungi could be isolated from all but the most necrotic tissues and especially from the 0.5–1.5 cm of nonnecrotic roots nearest the edge of necrosis. The recovered fungus was reidentified and used to repeat the experiment, with similar results.

Both fungi caused more extensive root

Table 1. Suppression of Phytophthora root rot of *Aucuba japonica* in infested low-pH or well-drained bark media 7–10 days after transplanting

Test[a]	Medium	Saturation[b]	pH change[c]	Root rot index[d]		
				Control	*P. cinnamomi*	*P. citricola*
1	Soil-sand (1:1, v/v)	+	6.3–6.5	0	2	9
	Hardwood bark compost	+	6.8–6.6	3	4	9
	Hardwood bark compost	–	6.8–7.1	0	0	0
	Pine bark	+	4.4–4.5	0	0	0
	Pine bark	–	4.4–4.5	0	0	0
2	Pine bark	+	4.5–ND[e]	0	...[f]	0
	Pine bark	+	6.5–ND[e]	0	...	9

[a] Roots evaluated after 7 days in test 1 and after 10 days in test 2.
[b] Saturation of media was maintained by setting containers in saucers of water; + = saturated condition; – = drainage not restricted.
[c] pH values of medium initially and 7 days after transplanting.
[d] Mean of three replicates. An index value of 0 = 0–9% of total root area necrotic; 1 = 10–19% necrotic; 2 = 20–29%; and so on, to 9 = 90–100% necrotic.
[e] ND = pH not determined at end of experiment.
[f] ... = Not tested.

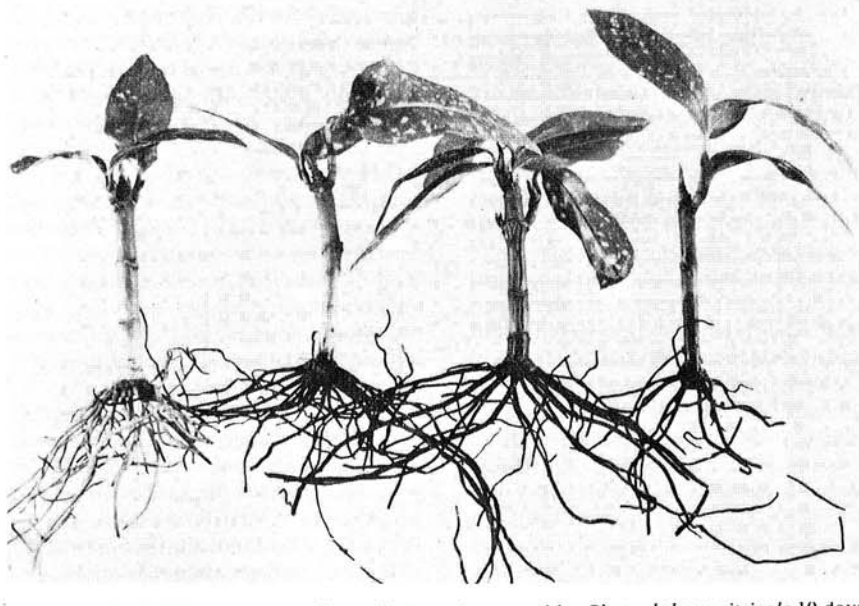

Fig. 1. Root, crown, and stem rot of *Aucuba japonica* caused by *Phytophthora citricola* 10 days after plants were transplanted into infested soil-sand medium that was kept saturated (healthy plant at far left).

Table 2. Effect of high organic matter and water-saturated conditions[a] on development of an apparently nonpathogenic root rot of *Aucuba japonica*

Medium[b]	Cornmeal added to medium (%, by weight)	pH of medium 10 days after transplanting	Root rot index 10 days after transplanting[c]
Soil-sand	10	4.2	9
	1	4.4	9
	0.1	5.2	1
	0	5.4	0
Peat-soil	0	4.1	0
Pine bark-soil	0	4.7	0
Hardwood bark compost-soil	0	5.6	1

[a] Containers were placed in saucers of water to inhibit natural drainage in all media.
[b] All media mixed 1:1 (v/v).
[c] Mean of three replicates. An index value of 0 = 0–9% of total root area necrotic; 1 = 10–19% necrotic; and 9 = 90–100% necrotic.

rot in infested soil-sand culture than in water culture. Seven days after transplanting, all roots of aucubas placed in *P. citricola*-infested soil-sand were 95–100% necrotic; necrosis frequently extended into the crown and stem area by day 10 (Fig. 1). Roots of aucubas placed in *P. cinnamomi*-infested medium were less extensively colonized than those in *P. citricola*-infested medium. Most lateral roots and several main roots were necrotic; total root necrosis was between 40 and 50%. Crown and stem infections were not observed on *P. cinnamomi*-infected plants. Sequential root-piece isolations demonstrated that both fungi could be reisolated from necrotic and nonnecrotic areas, as in the water culture method. The experiment was repeated with the reisolated fungi with similar results.

Suppression with bark media. Unsaturated PB (pH 4.5) and HBC (pH 6.8) and saturated PB (pH 4.5) suppressed root rot caused by *P. cinnamomi* and *P. citricola* on aucuba in 7 days (Table 1). However, all roots of plants placed in saturated, *P. citricola*-infested HBC or soil-sand were 95–100% necrotic. The fungus colonized the crown and stem area as well.

A nonpathogenic root rot of aucuba developed in control treatments of saturated HBC. A decrease in pH and an odor of anaerobic decay were associated with this root rot. No fungi were isolated from these roots on PPP medium; however, roots were not surface-sterilized before being placed on PPP medium, and bacterial growth was heavy. Results were similar when fragmented fungus cultures in water were used as inoculum. However, only slight nonpathogenic root necrosis (less than 10%) was observed on roots of control plants in saturated HBC with no inoculum added.

Raising the pH of PB negated its observed inhibitory property under saturated conditions (Table 1). Total necrosis of all roots was apparent on aucubas placed in saturated, *P. citricola*-infested PB medium at pH 6.5, but less than 10% necrosis was observed on plants in infested medium at pH 4.5.

Nonpathogenic root rot of aucuba. Complete necrosis of all aucuba roots occurred in 10 days in saturated soil-sand medium amended with 1–10% cornmeal (Table 2). Necrosis extended 0.5 and 2 cm upward from the soil line on the stem of all plants in soil-sand amended with 1 and 10% cornmeal, respectively. A decrease in pH and an odor of anaerobic decay were associated with the cornmeal amendment. Roots of plants from soil-sand, peat-soil, and pine bark-soil media without cornmeal amendment were 0–9% necrotic. However, roots of plants from HBC-soil and soil-sand amended with 0.1% cornmeal were 10–19% necrotic. No pythiaceous fungi were isolated on PPP, but bacterial contamination was abundant on assay plates. The experiment was repeated with similar results with only soil-sand amended with cornmeal at the same concentrations.

DISCUSSION

This is the first report of the pathogenicity of *P. cinnamomi* and *P. citricola* on *A. japonica*. *P. cinnamomi* is a cosmopolitan species with a wide host range (14). *P. citricola* causes root rot and/or dieback of rhododendron (6) and *Pieris japonica* (3). Although *P. citricola* was isolated more frequently and caused more severe root rot of aucuba than did *P. cinnamomi*, differences in disease severity may have been caused by differences in infective propagule concentrations, which could not be accurately ascertained. For instance, sporangia that readily released zoospores were dense on the mycelial mats of *P. citricola* used in the pathogenicity and suppression studies, but sporangia were not present on mats of *P. cinnamomi* although chlamydospores were numerous.

Other species of *Phytophthora* may be involved in the aucuba root rot complex, but only *P. cinnamomi* and *P. citricola* were isolated from diseased aucubas and positively identified in this study. Preliminary tests indicate that *P. citricola* also infects foliage and stems of aucuba (S. Spencer, *unpublished*). However, this pathogen was not isolated from aucuba stems with dieback symptoms in nature. Several other diseases (Botryosphaeria dieback, *Sclerotium rolfsii* stem blight) and environmental problems (full sunlight, cold injury, stress) can cause symptoms on aucuba similar to those caused by Phytophthora root rot, so culture identification is essential.

Waterlogged organic soils may cause injury to some plants because of oxygen depletion by bacteria multiplying on the organic matter or the accumulation of toxic substances produced by the microorganisms. Although aucuba roots can remain suspended in water for long periods without developing symptoms of root rot or discoloration, roots placed in a high-nutrient (cornmeal-amended) medium become totally necrotic in a short time under saturated conditions. The root rot observed in noninfested, saturated HBC medium may be related to this high water-organic matter-nutrient injury phenomenon. Insufficiently composted hardwood bark may contain phytotoxic substances (2,5) that may be partly responsible for injury to aucuba roots. Further testing to identify possible nonpythiaceous pathogens is continuing.

HBC (pH 6.8) and PB (pH 4.5) with natural drainage suppress Phytophthora root rot of aucuba more effectively than water-saturated soil-sand medium. Suppression of root rot by PB was not entirely the result of better drainage, however; saturated PB was as suppressive as naturally drained PB at pH 4.5. Suppression of *P. citricola* was overcome by raising PB to pH 6.5 under water-saturated conditions, which suggests that low pH may be the overriding factor. Low pH may inhibit the pathogen or bacteria that stimulate the fungus to produce sporangia, or it may stimulate the growth of antagonistic microorganisms. Pegg (9) found that low pH inhibits Phytophthora root rots. Unfortunately, the inhibitory effect of low pH in PB may be temporary; the pH of PB-sand changed from 4.7 to 5.3 in 20 days with daily watering under greenhouse conditions (S. Spencer, *unpublished*).

Although Hoitink et al (7) suggested that HBC possesses a chemical inhibitor of *P. cinnamomi*, our results indicate that HBC is not as effective in suppressing root rot of aucuba under water-saturated conditions. Further testing of PB and HBC suppression of Phytophthora root rot of hosts that do not develop nonpathogenic root rot in response to high water-organic matter-nutrient conditions may be necessary to eliminate the possibility of predisposition or some other effect. For example, in other experiments with lupine as the host, we found that HBC suppressed root rot more effectively than PB; possible reasons for differences in suppression among media also were investigated (12).

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