Suppression of Phytophthora cinnamomi in a Composted Hardwood Bark Potting Medium

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ABSTRACT

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Concentrations of *Phytophthora cinnamomi* propagules that caused severe root rot of lupine seedlings in peat-sand potting media produced little damage to similar seedlings growing in hardwood bark-sand compost. The low disease level in the suppressive bark mix was not related to drainage since all media drained rapidly and the air-filled pore space at various negative pressures (7.5, 22.5, 50, and 100 millibars) was lower in bark compost than in the peat media. Mycelium of *P. cinnamomi* did not grow through any of the media, but a significant inhibition of sporangium production occurred in the bark compost. Inhibitors in leachates from fresh bark composts lysed zoospores and cysts. These inhibitors were not detected in leachates from bark compost in which rhododendrons had been grown for 2 yr. Therefore, the suppressive effect of the bark compost probably is due to chemical and biological rather than physical factors.

Additional key words: lupine, peat, conducive media.

Production of woody ornamentals in containers has increased during the past two decades. Artificial potting media composed of combinations of peat, perlite, vermiculite, and other substrates have been widely used. Pythium and Phytophthora root rots frequently develop on plants produced in media that contain peat (6, 8, 18). Various types of hammermilled tree bark recently have been substituted for peat (5, 11, 21). Rhododendrons produced in composted hardwood bark without the use of soil fungicides or fumigants, are free of Phytophthora root rot (9). We reported previously that absence of root rot could be due to death of plant pathogens during the composting process, resulting in a "pathogen-free" mix (7). Furthermore, hardwood bark compost was shown to be suppressive to *Phytophthora* spp. (6) and also some plant pathogenic nematodes (14). This research was designed to examine the effects of some physical, chemical, and biological properties of two peat media and a composted hardwood bark medium on infection of lupine by *Phytophthora cinnamomi* Rands.

MATERIALS AND METHODS

Potting media.—A peat-sand medium (P_1) was prepared by mixing Canadian peat and sharp, coarse silica sand (3:2, v/v). The pH was adjusted to 6.4 with dolomitic lime. Super phosphate (26% P_2O_5) and fritted trace elements (Brighton By-Products Company, Inc., P. O. Box 23, New Brighton, PA 15066) were added at 2.5 kg and 0.5 kg/m^3 of medium, respectively. A peat-perlite-sand mix (35:22:3, v/v) (P_2) was prepared (pH 6.4) with the same additives. Batches of hardwood bark-sand compost (C_1 and C_2) were prepared in windrows (pH 6.4) under aerobic conditions as described previously (7). Roots of *Rhododendron catawbiense* 'Roseum Elegans' were removed from similar composted hardwood bark-sand and peat-sand media in which these plants had been growing from cuttings over a 2-yr period. Care was taken to remove as much of the root system from the media as possible. These media, C_3 and P_3, respectively, were adjusted to pH 6.4 with dolomitic lime.

Internal drainage of potting media.—Pore space of each potting medium was measured during desorption of cores on a Leamer and Shaw suction apparatus (13). This apparatus consists of a fine-porosity tension plate to which a water column is attached. The height of the water column (cm) between the middle of a soil core on top of the tension plate and the surface of the water reservoir is adjusted to give the desired negative pressure expressed in millibars (mb). Cylindrical aluminum cores (7 cm inside diameter and 15 cm long) were packed loosely with each of three potting media (C_2, P_1, and P_2) in duplicate. Cores were watered daily for 3 wk to allow settling similar to that in commercial potting procedures. Cores were then brought to saturation by standing them in water, which entered through the bottom. Saturated cores were weighed after each had come to equilibrium on a tension plate at 7.5, 22.5, 50, and 100 mb average negative pressure. Air-filled pore space was calculated for each negative pressure from the total volume of the core, wet

weights, and assuming the density of water to be 1.0 gm cm^{-3}

Pathogen suppression test.—Millet seed colonized by *P. cinnamomi* was used as inoculum (R. D. Raabe, Dept. of Plant Pathology, Univ. of Calif., Berkeley, Calif., *unpublished*). Seed (50 ml in 40 ml water/250-ml Erlenmeyer flask) were autoclaved 20 min on 3 successive days, inoculated with isolate 248 of *P. cinnamomi* (A_2 mating type, isolated from rhododendron) and incubated 3 wk at 25 C. Colonized seed were incorporated into the three potting media C_2, P_1, and P_2 with a P-K Twin-Shell Lab intensifier blender (Patterson-Kelly Company, East Stroudsburg, PA 18301) for 5 min at rates of 0, 10, 100, and 1,000 colonized seed/liter. Media were placed in 15-cm diameter polyvinyl pots (1,000 ml) and seeded with 20 lupine cultivar Sweet Frost (*Lupinus angustifolius* L.) seed/pot (four replications/treatment). Pots were maintained in a growth chamber at night and day (16 hr, 26 Klux) temperatures of 20 ± 0.5 C and 26 ± 0.5 C, respectively, and watered daily with a 20-20-20 fertilizer solution (200 μg N/ml). Surviving seedlings were counted at 7, 12, 19, 25, and 32 days after seeding. Damped-off seedlings were plated on a selective medium (19) for verification of *P. cinnamomi* infection.

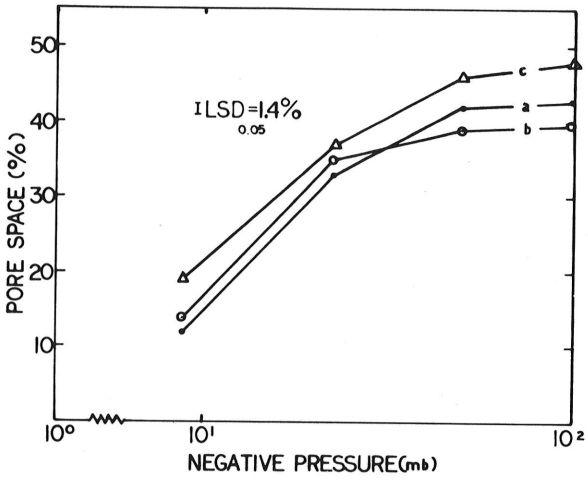

Fig. 1. Percentage air-filled pore space at various negative pressures (mb) in three potting media: (a) hardwood bark-sand compost, (b) peat-sand, and (c) peat-perlite-sand potting media. Measurements were made during desorption of cores (7 cm inside diameter and 15 cm long) on a Leamer and Shaw suction apparatus. The pore space at 7.5 mb represents conditions in 1-liter pots (15 cm tall) immediately after draining of excess water after daily flooding.

Production of sporangia in leachates.—Leachates were prepared from all media 3 wk after seeding with lupines. Pots were flooded in the evening and leachates were collected the following morning by suction through Whatman No. 1 filter paper from the base of each pot. Agar disks (5-mm diameter) from 4-day-old *P. cinnamomi* cultures (24 C) on dilute V-8 juice agar supplemented with cholesterol (19, 20) were placed first in sterile distilled water (4 hr, 10 ml/disk) to leach out nutrients and next in 52-mm diameter disposable petri dishes (one disk/dish, three/treatment) and flooded with 10 ml of leachate/dish. Leachates were replaced after 24 and 48 hr with fresh leachate from the same pot. Dishes were incubated for 3 days at 25 C, next for 30 min at 4 C, and then at 24 C for induction of zoospore release. Large numbers of spherical protozoa similar to *Phytophthora* cysts in size and shape occurred in some compost batches. For this reason the number of zoospores released in compost could not be determined accurately. To obtain some measure of zoospore release the total number of sporangia and the percentage of sporangia releasing zoospores per 3.14 mm^2 disk area (three reps/disk) were counted. After release began, observations were made on lysis of zoospores and germination and lysis of cysts.

To check whether lack of sporangial production was due to the absence of bacteria necessary for their production (15) the following experiment was performed. Leachates were centrifuged 20 min at 10,000 *g* (10 C), and supernatant fluids were passed through 0.45 μm (pore

TABLE 1. Effect of three inoculum levels of *Phytophthora cinnamomi* on the survival of lupine seedlings in three potting media

Potting medium	Inoculum level[a]	Surviving seedlings out of 20/pot (mean no.)				
		7 days	12 days	19 days	25 days	32 days
Bark-sand compost	0	15	15	13	12	12
	10	14	14	13	13	13
	100	16	15	10	10	10
	1,000	15	10	6	6	5
Peat-sand	0	15	15	13	12	10
	10	16	15	11	9	6
	100	12	8	4	2	1
	1,000	0	5	1	1	0
Peat-perlite-sand	0	12	12	10	9	8
	10	13	13	10	8	7
	100	10	5	1	1	1
	1,000	13	0	0	0	0
LSD ($P = 0.05$)		2.2	2.7	2.3	2.2	2.0

[a]Number of *P. cinnamomi*-colonized millet seed mixed in 1 liter of potting medium.

size) Millipore filters. The pellets were resuspended (i) in the filtered leachate supernatant fluids from which they were prepared, (ii) in the filtered supernatant fluids from other potting media, and (iii) in a standard solution for *Phytophthora* sporangia production. Zoospore production was studied as described above.

Survival structures in potting media.—To detect survival structures of *P. cinnamomi*, mycelial mats cultured on nylon fabric were buried in composted bark-sand (C_2) and in the peat-sand medium (P_1) (17). Inoculum was prepared by taking small pieces of mycelial inoculum from margins of 4-day-old cultures (25 C) on dilute V-8 juice-cholesterol agar, transferring them to 25 ml dilute V-8 juice broth in a 250-ml flask and incubating them for 48 hr (25 C). Then mycelium from these cultures was comminuted in a Sorvall Omni-Mixer (1 sec at half speed) and placed on nylon fabric squares (20-mm diameter and approximately 0.1-mm mesh). Squares were incubated 40 hr in dilute V-8 juice broth, dipped in ten successive changes of distilled water (100 ml) and buried vertically 7.5 cm deep measured from the center of the squares, in composted bark-sand or peat medium (four replications/pot). During the preceding 3 wk lupines had been grown in these same pots, as described for the lupine assay. Lupine roots were removed before burial of mats. After 1, 2, 4, 7, and 14 days, the squares were recovered, floated on 10 ml of leachate obtained by suction from pots in which the squares had been buried, and incubated (as described before) for induction of zoospore release. Observations were made with Zeiss Nomarski interference contrast microscopy without staining. Then, squares were plated on the selective medium and examined 18 hr later to check for survival of *P. cinnamomi*.

TABLE 2. Sporangium and zoospore production by *Phytophthora cinnamomi* and zoospore lysis in leachates from potting media

Potting medium[a]	Sporangia[b] (mean no.)	Sporangia that released zoospores (%)	Zoospore lysis[c]
Bark-sand compost C_1	0		+
Bark-sand compost C_2	4		+
Bark-sand compost C_3	27	52	−
Peat-sand P_1	11	82	−
Peat-sand P_3	40	60	−
LSD ($P = 0.05$)	8		

[a]Bark-sand composts C_1 and C_2 were prepared as separate batches in compost windrows. Peat-sand (P_1) contained Canadian peat and sharp, coarse silica sand (3:2, v/v). Compost C_3 and peat medium P_3 were similar to media C_1 and P_1 in composition, but were removed with care from roots of 2-year-old rhododendron plants.

[b]Sporangia (nine reps/treatment) produced per 3.4 mm^2 agar disk area after 72 hr of incubation in leachates of potting media (leachates were replaced each day).

[c]The symbol (±) indicates lysis of zoospores within sporangia before release, lysis after release, or lysis of cysts before germination.

RESULTS

Saturated conductivity of all media was in excess of 10 cm $hour^{-1}$ in cores used to measure air-filled pore space. Exact values were not determined since this rate is sufficient to rapidly drain standing water after rainfall or irrigation. The three potting media had similar but significantly different water desorption curves ($P = 0.05$) indicating different pore size distributions (Fig. 1). At the average negative pressure of 7.5 mb used in the inoculum study (Table 1) the percentages of air-filled pore space for the bark-sand compost (C_2), the peat-sand (P_1), and the peat-perlite-sand (P_2) media were 12, 14, and 19%, respectively.

Pathogen suppression test.—Lupine seedlings emerged 12 days after seeding. Some seedlings that developed from poor quality seed collapsed at various times after seeding independent of treatment throughout four experiments. The low inoculum level (10 colonized millet seed/pot) did not kill a significant number of seedlings ($P = 0.05$), except in the peat-sand medium (Table 1). The higher inoculum levels (100 and 1,000 seed/pot) killed significantly more seedlings in the peat media than in the composted bark-sand medium.

Production of sporangia in leachates.—The pH of all leachates ranged from 6.0 to 6.7. More sporangia were produced on disks in peat-sand (P_1) leachates removed from pots 3 weeks after seeding with lupines than on those in the composted bark-sand (C_1 and C_2) leachates (Table 2). Leachates from compost batch C_1 completely inhibited sporangium production.

Pellets and sterile filtrates were prepared from leachates of the media P_1 and C_1. No sporangia were produced in filtered leachates without addition of microorganisms. However, sporangia did not develop on disks incubated in filtered bark leachate (C_1) into which microorganisms from the peat leachate pellet (P_1) had been resuspended. Sporangia were produced on disks incubated in filtered peat leachate (P_1) into which the pellet from the bark compost (C_1) leachate had been resuspended. Sporangia also developed on disks incubated in salt solutions into which pellets containing microorganisms from this bark compost leachate or peat leachate were added. Significantly more sporangia developed in leachates prepared from 2-yr-old nursery rhododendron plants in similar peat-sand (P_3) than in a hardwood bark-sand compost medium (C_3), although the numbers were within the same order of magnitude.

Zoospores released from sporangia in fresh composted bark (C_2) lysed before release or after encystment. Cysts did not germinate in this compost leachate or in leachates prepared from other fresh compost batches collected from various nurseries in Ohio. However, zoospores encysted and germinated when released into the peat leachates from fresh and old potting media (P_1, P_2, and P_3) and the leachate from 2-yr-old bark compost (C_3).

Survival structures in compost.—Mycelium on nylon fabric buried in compost completely lysed within 48 hr. Bacteria and various protozoa were observed in contact with mycelium. A layer of bacteria frequently engulfed mycelium and sporangia before and during lysis. Mycelium lysed after 4 days in the peat-sand medium (P_1), but was not engulfed by bacteria as in compost. Some mycelium was present at 7 days, but not after 10

days. Sporangia developed during the first 24 hr in both media. After 4 days, however, none was found in the bark compost, whereas some were present on mycelium in peat.

After incubation at 4 C, zoospores were released from sporangia on nylon squares in leachates from the peat medium. They encysted and germinated. In compost leachates, zoospores lysed during cleavage, at the time of release, or after encystment. Chlamydospores were detected first at 2 days and were the only structures observed after 14 days in both potting media. It was difficult to trace the origin of colonies developing on fabric squares in the selective agar due to the presence of unidentified bacteria, nematodes, other worms, and protozoa. However where colony origin could be traced, chlamydospores were the surviving structures. No oospores were detected.

DISCUSSION

The lupine seedling assay demonstrated clearly that hardwood bark compost is suppressive to *P. cinnamomi*. Several factors may contribute to this effect. Under field conditions losses in shortleaf and loblolly pine (4, 23), and radiata pine (10) were associated and with poorly-drained soils. The low disease level in our composted bark mix, however, was not associated with drainage, since all three potting media drained rapidly. The air-filled pore space was lowest in compost.

Mycelium of *P. cinnamomi* did not grow through the suppressive compost nor through the conducive peat-sand medium. This is supported by previous reports on lysis of *Phytophthora* mycelium in soil in the absence of an adequate food base (1, 16, 17). It differs from a report on suppressive and conducive avocado field soils in Australia, where mycelium grew through untreated conducive, but not through suppressive, soils (2). Survival in compost as well as in peat appeared to depend on formation of chlamydospores. This is supported by previous reports on survival of *P. cinnamomi* in soils (16, 17).

In leachates from freshly prepared compost as well as from compost in which rhododendrons had been grown for two growing seasons, a significant inhibition of sporangium formation occurred as compared to peat leachates. Although freshly prepared composts contained substances that lysed zoospores and cysts before germination, leachates from 2-yr-old compost did not contain similar substances. In Ohio nurseries, rhododendrons were free of Phytophthora root rot for a period of at least 2 yr. On the other hand, rhododendrons produced in peat media in these same nurseries became infected. The suppressive effect of hardwood bark compost 2 yr after potting, therefore, may not be due to inhibitors, but to antagonistic microorganisms, as reported for the avocado soil (3). Various microbial antagonists have been isolated from hardwood bark compost and their role in suppression is under investigation.

Batches of hardwood bark compost vary in suppressive effect. The hardwood bark we used was removed from a mixture of tree species (5). Inhibitors of plant growth and of various microorganisms have been found in bark from various woody plants (12, 22). Chemical identity of these inhibitors has rarely been determined (12). Composting (30 days) of silver maple, hackberry, sycamore, and cottonwood bark reduced or removed phenolic inhibitors of mung bean cuttings and cucumber seedlings (22). Composting of spruce bark also removed inhibitors of plant growth and various enzyme systems of microorganisms (12). Hardwood bark used in this study was composted for 3 mo. Several plants have been grown successfully in this hardwood bark compost without detrimental effects (5, 11, 21). Zoospores, therefore, may be more sensitive than higher plants to the compounds present in bark.

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