

Survival of Some Plant Pathogens During Composting of Hardwood Tree Bark

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ABSTRACT

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Rhododendron crowns and roots (with residual adhering soil) infected with *Phytophthora cinnamomi* and *Pythium irregulare*, sugarbeets infected with *Rhizoctonia solani*, geranium tissues infected with *Botrytis cinerea*, and chrysanthemum cuttings infected with *Erwinia carotovora* var. *chrysanthemi* were buried in a hardwood bark compost stack, and a "noncompost" stack (without added nitrogen).

Additional key words: container media.

None of the pathogens could be recovered by direct isolation on selective media or baiting with seedlings from the samples retrieved after incubation in the compost stack (internal temperature 40-60 C for 10-12 weeks). From samples stored in a "noncompost" stack, to which nitrogen had not been added (25 C maximum), *P. cinnamomi*, *P. irregulare*, and *R. solani* could be reisolated.

Pot mixes for production of greenhouse and nursery crops traditionally have been steam-sterilized or fumigated to kill plant pathogens. Aerated steam treatments were introduced to selectively destroy pathogens and reduce subsequent recolonization (1). During the last decade, composted pot mixes have been used without sterilization for production of ornamental plants (14, 26). In the past, composts were too variable for use as potting mixes (21), but considerable information has since become available on the composting process, resulting in composts highly suitable for agricultural use (3, 7, 14, 16, 18, 25, 26).

Ornamentals and bedding plants produced in peat-sand mixes commonly become infected with various root pathogens. Plants produced in composted bark media appear free of root diseases even when no fungicides are used (14). This absence of root rot might be due to (i) eradication of pathogens during composting resulting in a "pathogen-free" potting mix and (ii) a suppressive nature of the finished compost itself. This paper describes the eradication of several plant pathogens from a hardwood bark potting mix through composting.

MATERIALS AND METHODS

Compost preparation and survival of pathogens.—Ground hardwood bark was obtained from the Paygro Division of The Mead Paper Group, First National Plaza, Dayton, Ohio 45402. The particle size distribution and tree species composition of the bark was as described previously (7). In the first trial, ground bark

and sharp coarse silica sand (2:1, v/v) were mixed with the following ingredients: 5 kg ammonium nitrate, 2.5 kg super phosphate (0-20-0), 0.5 kg elemental soil sulfur, and 0.5 kg $\text{FeSO}_4 \cdot 4 \text{H}_2\text{O}$ per m^3 of bark. Moisture content of this mix was adjusted to approximately 85% (dry weight basis).

Survival of pathogens in diseased rhododendron and sugarbeet plant parts was followed in outdoor, exposed compost stacks (2.5 m high, 4 m wide) from September until the end of December 1975. Stacks were turned once, after 4 weeks. A stack of bark-sand mix with the same additives, except for ammonium nitrate, was used to follow survival of pathogens in a "noncompost" mix. In addition, samples were buried in a sand pile in which no heating occurred.

In another composting trial during March-June 1975, samples of infected rhododendron, geranium, and chrysanthemum plant parts were buried in similar compost and "noncompost" stacks (four replicates for each diseased tissue type). Samples were recovered from these stacks after 13 weeks, which was before heating had subsided. Total carbon for C/N ratios of composts was determined by chromic acid oxidation of samples, followed by titration of carbon (17).

Infested residues and methods of isolation.—Crowns and roots with adhering soil of field-grown *Rhododendron catawbiense* cv. 'Roseum Elegans', infested with *Phytophthora cinnamomi* Rands, *Pythium irregulare* Buism., and an unidentified sphaerosporangial *Pythium* sp. were cut into 5-mm pieces and mixed for 30 minutes in a concrete mixer. This inoculum (40% moisture by weight) for the 1974 composting trial was stored at 4 C in polyethylene bags. Rhododendron residue (400 ml) was mixed with 1,600 ml of bark-sand

mix and tied in a nylon fabric bag at the time of preparation of the compost stack. Samples (four replicates per treatment) were placed at various locations during September 1974 in: (i) the compost stack; (ii) the "noncompost" stack; (iii) a sand pile; and (iv) a cold room (4 C). Temperatures were recorded daily at various locations in each stack. At the end of composting, after heating had subsided, pathogen samples were retrieved and assayed for survival. Attempts were made to isolate *P. cinnamomi* and the *Pythium* spp. from rhododendron crown tissue (15 replicates per sample) by direct plating on a *Phytophthora* selective agar (24), as described previously (13), and by baiting from soil samples with lupine seedlings (4). Lupine seedlings with 3- to 4-cm radicles germinated in vermiculite in a greenhouse (25-30 C), were suspended in distilled water (500 ml) in jars that contained plant residue samples tied in nylon fabric bags (100 gm/sample). Jars (four replicates per sample, four seedlings per jar) were incubated at 25 C and a 16-hour photoperiod (22,000 lux) for 3 days. Radicles were rinsed with deionized water, surface-sterilized for 10 seconds in 1% NaClO, and 1 cm-long sections were plated on the *Phytophthora*-selective agar.

Portions of field-grown sugarbeets diseased with *Rhizoctonia* crown rot (*Rhizoctonia solani* Kühn) were sliced into 5-cm³ pieces. Survival in 400-ml batches of this residue was tested three ways: (i) beet pieces alone bagged in nylon fabric; (ii) beet pieces mixed uniformly with 1,600 ml of bark-sand mix per bag; and (iii) beet pieces placed only in the center of 1,600 ml of this mix per bag. The bags (four replicates per location) were then located as described for rhododendron samples.

Upon completion of composting, beet pieces were removed from each bag and assayed for *R. solani* survival by a sugarbeet seedling disease assay. The beet pieces (400 ml originally) in each treatment were diced into approximate 6-mm cubes. These cubes were distributed equally among eight 450-ml styrofoam cups in a layer in sterile peat-perlite-soil mix (1:1:1, v/v). Pots were filled in the following order: 50 ml of mix, 50 ml of diced beet cubes, 250 ml of mix, and 25 beet seeds (cultivar USH20) covered with 50 ml of soil. In addition to these cubes, bark-sand mix surrounding beet pieces in the fabric bags was assayed to establish whether it had been colonized by *R. solani*. It was divided among four styrofoam cups and planted with beet seed as follows: 350 ml of this test bark-sand mix, 25 beet seed, and 50 ml of test bark-sand mix as cover. Final seedling counts were taken 37 days after planting and seedling losses were calculated. Germination controls were ascertained from emergence in sterile peat-perlite-soil (1:1:1, v/v) mix for the diced beet assay and from emergence in compost for the bagged bark-sand mix assays.

In the second composting experiment, which was conducted in the spring of 1975, chrysanthemum cuttings Iceberg from Yoder Bros., Barberton, Ohio, were inoculated with *Erwinia chrysanthemi* isolate EC12 and incubated, as described previously (11). Infected cuttings (40 per sample) were mixed with bark-sand mix and buried in nylon fabric bags (four replicates per treatment) as described for the rhododendron samples. Infected cuttings were also stored at 4 C. Rotted chrysanthemum cuttings were removed from samples in the stacks after 12 weeks, homogenized in 0.02 M, pH 6.9 potassium

phosphate buffer (10 replicates per sample), then plated and incubated at 25 C on two selective media for *Erwinia* spp. (5, 23). Suspect colonies were inoculated into "pathogen-free" Iceberg cuttings by stabbing stems with a needle to verify pathogenicity (11).

Geranium stem and leaf tissue infected with *Botrytis cinerea* Pers. ex. Fr. was collected from commercial greenhouses and stored in the dark at 4 C in polyethylene bags to increase inoculum. Samples (400 g per replicate) were mixed with the bark-sand mix, tied in nylon fabric bags, and buried as described for rhododendron samples. After recovery of samples, small tissue pieces (20 pieces per replicate) were surface-sterilized (0.5% NaClO, 1 minute), plated on PDA or a *Botrytis*-selective medium (6), and incubated in the dark at 24 C.

Effect of incubation temperature on survival.—Geranium, chrysanthemum, and rhododendron plant and/or soil samples infected with *B. cinerea*, *E. chrysanthemi*, *P. cinnamomi*, *P. irregulare*, and the *Pythium* sp., respectively, were stored in incubators at 4, 40, and 50 C in polyethylene bags (three replicates per treatment). In addition, barley seed (100 g per polyethylene bag) colonized with three virulent isolates of *R. solani* of sugarbeet was incubated at each temperature. Each *R. solani* isolate was cultured separately (3 weeks, 25 C), ground in a Wiley mill (1-mm screen), and then mixed in equal quantities by weight. At weekly intervals, attempts were made to isolate each pathogen from the infested samples with techniques described before, except for *R. solani*-colonized barley seed that was plated on water agar and PDA and incubated at 25 C for 7 days.

RESULTS

Survival in compost stacks.—Temperatures of the compost stack prepared in the fall of 1974 did not exceed 50 C (Fig. 1), whereas temperatures during composting of the stack prepared in April 1975 ranged from 40-60 C over

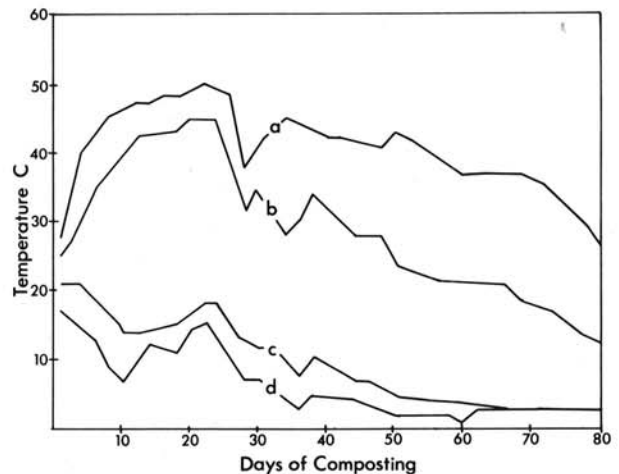


Fig. 1. Temperatures during fall composting: (a) in the center of a compost stack; (b) 8 cm below the surface of the compost stack; (c) in the center of a "noncompost" stack; and (d) in a sand pile at various days after preparation. Stacks were turned at 28 days.

a 3-month period. In the "noncompost" stack the temperature did not exceed 25 C in either batch. The C/N ratio of both compost mixes ranged from 1:35 to 1:41, whereas that of the "noncompost" mixes ranged from 1:90 to 1:137. *Phytophthora cinnamomi*, *P. irregulare*, and a *Pythium* sp. all were recovered by baiting from all rhododendron crowns, from the adhering soil, and from lupine seedling samples that were buried in (i) the sand pile, (ii) the "noncompost" stack, and (iii) from the samples stored at 4 C. They were not isolated from any samples buried in either of the compost stacks. To determine whether the lack of isolation of *P. cinnamomi* by baiting from composted samples was due to the absence of bacteria necessary for sporangium formation (22) or due to the presence of inhibitors in compost, the following tests were performed: (i) 0.1 g mycelium (blotted dry) from 2-week-old *P. cinnamomi* hemp broth cultures (13) was added to 100 g compost in lupine baiting jars; and (ii) various amounts of infested rhododendron soil (0.125, 0.25, and 0.5 g) were added to 100 g of compost. *Phytophthora cinnamomi* was recovered by baiting from all samples. Under these conditions lack of isolation of *P. cinnamomi* from compost, therefore, probably was not due to the presence of inhibitors in compost or the absence of bacteria for sporangium formation.

None of the *R. solani*-colonized beet samples recovered from the compost stack caused damping-off in the beet seedling assays. In contrast, samples retrieved from the "noncompost" stack and sand pile, resulted in 8-25% and 25-36% beet seedling mortality, respectively. The inoculum control (beet pieces stored at 4 C) caused a beet seedling kill of 44%. Bark-sand mix taken from immediately around infected beet pieces inside the nylon fabric bag in samples recovered from (i) the "noncompost" stack and (ii) the sand pile, caused a 40 and 26% seedling loss, respectively. On the other hand, the bark-sand mix surrounding the beet pieces in the compost pile after composting did not cause seedling losses. Apparently *R. solani* colonized the bark-sand mix surrounding infected beet pieces in the "noncompost" stack and sand pile but not in the compost stack.

Botrytis sporulated profusely on infected geranium stems maintained in the dark at 4 C, but was not isolated on PDA or on Ellerbrock's *Botrytis* medium from tissue pieces recovered from the compost-stack or the "noncompost" stack. From geranium samples in the "noncompost" stack, a *Trichoderma* sp. was recovered consistently at both 15 and 24 C. No fungi were isolated from geranium samples recovered from the compost pile and incubated on PDA or Ellerbrock's medium at 15 or 24 C (the temperature of the stack was 60 C at the time of sample retrieval). After 1 week, these plates were moved to an incubator at 40 C and after 4 days an unidentified, thermophilic *Humicola* sp. was isolated. Several other observed colonies of thermophilic fungi were not identified.

Chrysanthemum cuttings recovered from both the compost and the "noncompost" stack were decomposed except for the vascular tissues which were still recognizable. No colonies resembling those of *E. chrysanthemi* were detected on Miller's medium. However, on Kelman's medium one strongly pectolytic bacterial isolate was obtained but it was not virulent to

chrysanthemum. In the "noncomposted" sample, high populations (10^6 cells in 0.25 gm of tissue) of pectolytic bacteria occurred but they were atypical of the *Erwinia* spp. that cause soft rot. None of 40 pectolytic isolates picked at random was virulent to chrysanthemum.

Survival at 4, 40, and 50 C in incubators.—*Erwinia chrysanthemi* was not isolated from samples stored for 1 week at 40 and 50 C, but it was isolated from samples stored at 4 C. *Botrytis* was isolated from geranium stem tissue stored at 40 C after 2 weeks, but not after 3 weeks. It was not isolated from samples stored at 50 C for 1 week, but sporulated profusely on samples after 12 weeks at 4 C.

Rhizoctonia solani could not be isolated from colonized-barley seed samples stored for 1 week at 50 C, but it was isolated after 6 weeks from samples stored at 40 C. After 7 weeks, no isolates were obtained from samples stored at 40 C. All samples stored at 4 C were positive for *R. solani*.

Phytophthora cinnamomi, *P. irregulare*, and the *Pythium* sp. were isolated by baiting and direct plating on the selective *Phytophthora* agar from rhododendron samples stored at 4 C after 12 weeks. They were not isolated from samples incubated at 40 or 50 C for 1 week or longer.

DISCUSSION

Failure to isolate pathogens from samples incubated in the compost stack indicates that they probably did not survive composting. Furthermore, the survival of *P. cinnamomi*, *P. irregulare*, the unidentified *Pythium* sp., and *R. solani* in the "noncompost" stacks, to which ammonium nitrate had not been added, and in which some heating did occur, supports composting survival data reported for various pathogens and parasites of man; these organisms can survive composting in the absence of high temperatures or adequate mixing (8, 19, 27). A minimum composting temperature of 60 C and adequate mixing has been recommended for "thermal kill" of common pathogens in sewage sludge (8).

Little information has been published on eradication of plant pathogens from plant residue through composting. Pathogens of tobacco seedlings, including *Thielaviopsis basicola* (Berk.) Ferraris, *Rhizoctonia*, and tobacco mosaic virus, apparently, were killed in parts of compost heaps where temperatures did not exceed 49 C (9). These authors also reported that chlamydospores of *T. basicola* did not germinate after being heated to 40, 45, 50, 55, 60, 65, 70, and 75 C for 115, 48, 24, 6, 5, 4, and 1.5 hours, respectively. Also, sclerotia of *Rhizoctonia* no longer germinated after exposure to 45, 50, 55, and 60 C for 48, 10, 8, and 0.5 hours, respectively (10). The apparent lack of survival during composting in our study could be explained on the basis of temperature exposure alone since all pathogens were killed in crop residues after incubation at 40 C for less than 1 week, except *R. solani* which could be isolated up to 6 weeks. This is supported by other reports on low-temperature survival (2).

In municipal composts, some pathogens apparently are killed by a combination of temperature exposure and the presence of inhibitors or through competition with other microorganisms. Cultures of *Salmonella paratyphi* and *S. cairo* incubated at 50 C and 50% moisture in compost died after 7 days, but survived in an incubator under

similar conditions. It was shown that aqueous extracts of the compost were toxic (19). Inhibitors and antagonists also may be involved in killing of plant pathogens during composting since hardwood bark compost is suppressive to plant pathogenic nematodes (20), *Phytophthora* spp. (12), *Corticium rolfsii* (15), and *Fusarium oxysporum* f. sp. *chrysanthemi* (Hoitink, unpublished).

Nurserymen in Ohio and other states, and to a lesser extent florists, have relied on composting as the principal or sole method of root disease control. The absence of root rots on plants produced in these composts compared to their occurrence on similar plants produced in peat media merits further investigation. It appears that plant pathogens, except perhaps for some viruses, may be eradicated from potting mixes through composting. Adequate mixing of compost piles throughout the composting process, can improve the uniformity of kill, especially of organisms that otherwise survive in the outer low-temperature layer of a nonturned compost stack. The high composting temperatures reported in this study during a fall and spring trial, also occur in winter compost stacks. Under low outdoor-temperature winter conditions, however, larger stacks are essential for generation of adequate heat to ensure composting at 40 C or higher. Survival of viruses, including tobacco mosaic virus, during composting is presently under investigation.

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