# Suppression of the Apple Collar Rot Pathogen in Composted Hardwood Bark

D. E. Spring, M. A. Ellis, R. A. Spotts, H. A. J. Hoitink, and A. F. Schmitthenner

Graduate research associate, assistant professor and professors, respectively, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster, and The Ohio State University, Columbus. Present address of third author: Oregon State University, Mid-Columbia Experiment Station, 3005 Experiment Station Drive, Hood River 97031.

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## ABSTRACT

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Percentage kill of 3-wk-old apple seedlings was significantly lower in bark compost container medium than in a peat medium after inoculation with varying concentrations of *Phytophthora cactorum* zoospores and oospores. Sporangium production and zoospore viability was significantly lower in aqueous leachates from bark compost than in leachates from peat.

Collar or crown rot of apple, which is caused by *Phytophthora cactorum* (Leb & Cohn) Schroet., is a serious problem in many Ohio apple orchards. Disease incidence is greater in heavy and poorly drained soils (2,16). The process of infection on apple is not well understood; however, it appears that zoospores serve as the infective propagules (16,20). Oospores appear to be the most important survival structures of the pathogen in soil. Vegetative mycelium rapidly lyses in soil (20).

At present, effective control measures for collar rot are lacking (14,16). Differences in susceptibility of various apple rootstocks are known (19); however, few apple cultivars or commercially used clonal rootstocks are considered to be highly resistant (14,16), and reports of resistance often conflict. Fungicide drenches or sprays applied to soil or infected portions of the tree do not adequately control *P. cactorum* (14). No work has been reported on biological control of this disease.

Suppression of certain soilborne plant pathogens through the use of composted bark has been studied. Hoitink (8,9) reports the suppression of composted hardwood bark to *P. cinnamomi.* When lupine seedlings were grown in an inoculated peat-sand medium, severe root rot developed. Seedlings grown in an inoculated bark compost-sand medium were less affected. Suppression or inhibition of other plant pathogens by composted bark is reported (3,5,10,15,18).

The purpose of this study was to evaluate the suppressive effect of composted hardwood bark on infection of apple seedlings by *P. cactorum.* The effect of aqueous bark compost leachates on zoospore viability and sporangia production also was studied.

## MATERIALS AND METHODS

A peat-perlite-sand medium (2:1:1, v/v), and a bark compost-peat-perlite-sand medium (5:2:2:1, v/v) were used for all studies. The following ingredients were added per liter of peat medium: 1.4 gm treble superphosphate (0-46-0); 0.1 gm fritted trace elements (Brighton By-Products Company, Inc., P.O. Box 23, New Brighton, PA 15066); 10 gm Osmocote slow-release fertilizer (14-14-14) (Sierra Chemical Co., 1001 Yosemite Dr., Milpitas, CA 95035); and hydrated lime to adjust the pH to 6.5. The medium was treated with aerated steam (80 C, 2 hr) before the Osmocote was added. Composted hardwood bark was obtained from Paygro, Inc., So. Charleston, OH 45368. The pH of the bark compost medium was adjusted to 6.5 with hydrated lime and amended with Osmocote only. Composted hardwood bark contained similar levels of available phosphate and trace elements. The air-filled pore space at container capacity of both media was 16–18% (in 10-cm-tall soil columns).

*P. cactorum* used in this study was isolated from an infected apple tree on MM106 rootstock. Stock cultures were maintained on dilute V-8 juice agar (17) in 3.5-ml medicine bottles. Stock cultures were incubated at 24 C for 7 days then refrigerated at 4 C until needed.

To verify and maintain virulence, 3-wk-old McIntosh apple seedlings were inoculated with zoospore suspensions prepared from stock cultures. After 1 wk, root and stem sections from infected seedlings were surface disinfested by soaking in a 0.25% sodium hypochlorite solution for 30 sec, then rinsed in sterile distilled water and plated on PBNC medium (17). *P. cactorum* was reisolated from infected tissues and maintained as previously described. Stratified McIntosh seeds were germinated in vermiculite in the greenhouse. Then two-week-old seedlings were then transferred to 15-cm-diameter (1,000-ml) plastic pots filled with each container medium. Three seedlings were planted per pot and fertilized weekly with 200 ppm N-P-K solution using Peters 20-20-20 fertilizer. Seedlings were maintained in a greenhouse with temperatures ranging from 20 to 30 C.

**Zoospore inoculation of seedlings.** Three 0.5-cm plugs from the edges of 7-day-old V-8 juice agar cultures were added to petri plates containing 15 ml of lima bean broth then incubated in light for 3 days at 24 C. To induce sporangium production, broth cultures were washed with Chen-Zentmyer salt solution (4). The lima bean broth was replaced with salt solution four times at 1-hr intervals. The fourth salt wash solution was left in the plates overnight and abundant sporangia were produced. To induce zoospore production, cultures with sporangia were placed at 4 C for 30 min, then returned to room temperature (24 C). Zoospore discharge was initiated within 25 min. Inoculum was adjusted to various concentrations in $10^{-3}$ M phosphate buffer with a hemocytometer.

Three-wk-old apple seedlings growing in peat mix were inoculated with zoospore concentrations of 0, 200, 400, 800, 1,600, 2,400, 3,200, 4,000, 6,400, and 7,200 zoospores per milliliter. Seedlings growing in the bark compost mix were inoculated with zoospore concentrations of 0, 500, 1,000, 2,000, 3,000, 4,000, 5,000, 7,000, 10,000, and 12,000 zoospores per milliliter. Inoculum (3 ml per seedling) was placed around the base of each seedling with a wide-mouthed pipette. Before inoculation, pots were watered to saturation. Treatments consisted of eight pots (replications) with

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three seedlings per pot. Treatments were arranged in a completely randomized block design. To verify infection by *P. cactorum*, seedlings were removed from pots immediately after symptoms developed, surface disinfested (as previously described), and plated on PBNC agar. Plates were incubated for 3–5 days at 24 C and recoveries of *P. cactorum* were recorded. Isolations were made from infected seedlings of all inoculation tests. The experiment was repeated using only the three highest inoculum levels for each container medium.

**Oospore inoculation of seedlings.** Oospore inoculum was produced in hemp seed broth prepared by autoclaving 30 g of hemp seed in 1 L distilled water for 30 min. Autoclaved broth was filtered through four layers of cheesecloth and autoclaved a second time. A 0.5-cm agar plug from the edge of a 7-day-old culture was added to 125-ml flasks containing 25 ml of hemp seed broth. Flasks were incubated in the dark at 24 C, and oospores were produced in 2–3 wk. Oospores were harvested by grinding cultures in an Omni-mixer for 60 sec at high speed. Ground cultures, which contained mostly oospores, were frozen for 24 hr at −20 C to kill mycelial fragments and sporangia (1). After they were thawed, cultures were reground for 15 sec to suspend the oospores in solution. A hemocytometer was used to count the oospores and concentrations were adjusted by diluting with distilled water.

Three-wk-old apple seedlings in both container media were inoculated with oospore concentrations of 0, 100, 1,000, and 10,000 oospores per milliliter. Pots were watered to saturation before the inoculum was applied. Seedlings were inoculated by pipetting (with a wide-mouthed pipette) 5 ml of oospore suspension around the base of each seedling. Treatments consisted of 10 pots (replications) with three seedlings per pot. Treatments were arranged in a completely randomized block design and the experiment was repeated once.

**Effect of leachates from bark and peat media on zoospore viability and sporangium production.** Plastic pots (15-cm diameter, 1,000 ml) with either bark compost or peat medium were leached with water twice, then watered lightly for 4 days. Thereafter, pots were watered to container capacity and covered with polyethylene. The following morning, leachates were collected from the base of each pot as previously described by Hoitink et al (9). Leachates were sterilized by centrifugation (20 min at 10,000 *g* 10 C) followed by filtration through a 0.45 $\mu$m Millipore filter (9). The pH of leachates from both media was 6.8.

Zoospore viability was measured by pipetting 0.5 ml of a zoospore suspension ($10^5$/ml) into 52-mm-diameter petri plates containing 4.5 ml of either bark compost leachate, peat leachate, or distilled water. Each treatment was replicated three times. Observations on zoospore viability were made with a light microscope, at intervals of 30, 60, and 120 min. After each observation, plates were swirled gently and three 1-ml samples of the suspension were removed from each plate and spread onto PBNC media in 100-mm plates. After 4 hr, germinated cysts were counted in four ×160 fields under the microscope. The experiment was repeated once.

To study the effect of leachates on sporangium production, plugs (0.5 cm) from 7-day-old V-8 agar cultures were placed in 52-mm-diameter petri plates containing 5 ml of each leachate or distilled water. Three plugs were added to each of three petri dishes and incubated in the light for 3 days at 24 C. Sporangia production was observed daily by counting sporangia in four different microscope fields at ×160 on each agar plug. The experiment was repeated once.

TABLE 1. Comparison of mean seedling kill of apple seedlings inoculated with four concentrations of *Phytophthora cactorum* oospores in two container media

| Inoculum level[y] (oospores/ml) | Mean seedling kill | |
|---|---|---|
| | Bark | Peat |
| 0 | 0a[z] | 0 a |
| 100 | 0.1 a | 0.1 a |
| 1,000 | 0.1 a | 0.8 b |
| 10,000 | 0.3 a | 1.4 c |

[y]All seedlings were inoculated by placing 5 ml of oospore inoculum around the base of each seedling.

[z]Mean number seedlings killed per pot based on 10 pots per container medium per inoculum level with three seedlings per pot. Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test. LDS ($P = 0.05$) = 0.3.

## RESULTS

**Zoospore inoculations of seedlings.** Seedling kill increased in both bark compost and peat container media with increased zoospore inoculum levels (Fig. 1). However, seedling kill was always significantly lower in bark compost than in peat, at all inoculum levels. Points indicating percent seedlings killed at each inoculum level for both container media were used to plot regression lines. Slopes for the lines were 0.004 and 0.010 for bark compost and peat, respectively. Slopes were compared by using Student's *t*-test, as described by Steel and Torrie (21), and were significantly different $P = 0.01$. The $ID_{50}$ values in peat and bark compost media were approximately 5,000 and 12,500 zoospores

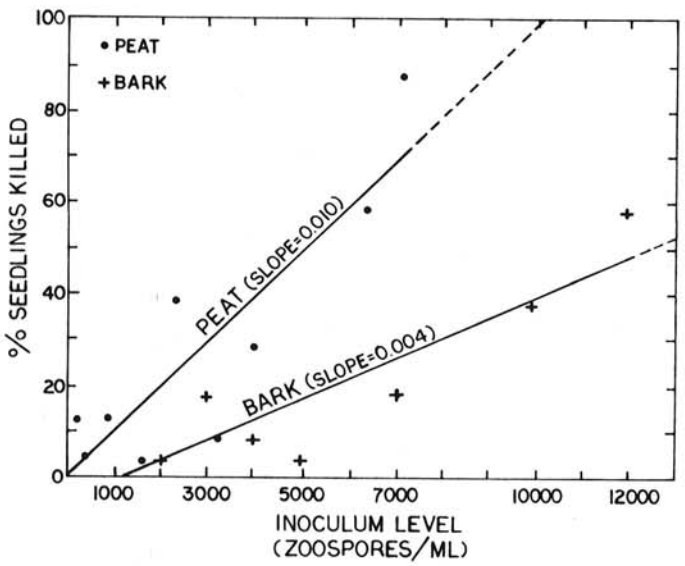


**Fig. 1.** Effect of inoculum level on kill of apple seedlings inoculated with *Phytophthora cactorum* zoospores in two container media ($P = 0.01$).

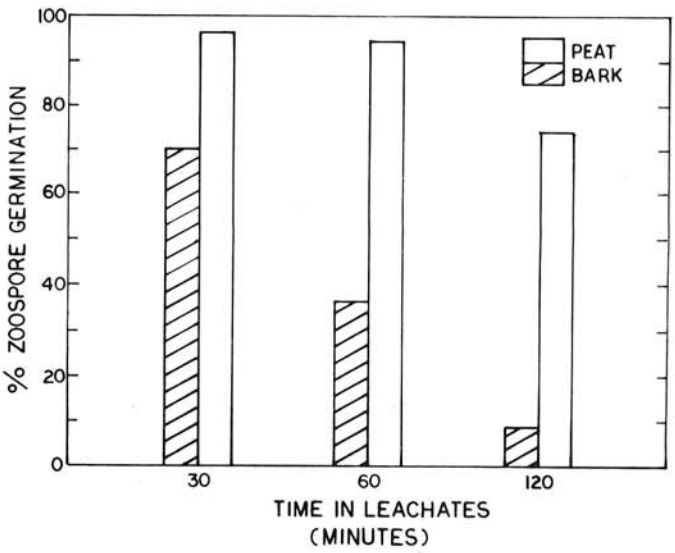


**Fig. 2.** Effect of aqueous bark compost and peat leachates on *Phytophthora cactorum* zoospore viability, as compared to a distilled water control (100%). LSD ($P = 0.01$) after 30 min incubation in leachate = 20.9; after 60 min = 18.9; and after 120 min = 10.7.

per milliliter, respectively.

**Oospore inoculation of seedlings.** There were no significant differences in percent seedling kill between any treatments in bark compost (Table 1). Percentage seedling kill in peat was significantly higher at oospore concentrations of 1,000 and 10,000 per milliliter. Increasing inoculum levels had a significant effect on the number of seedlings killed in peat, but not in bark compost.

**Effect of leachates from container media on zoospore viability and sporangium production.** Significantly fewer zoospores germinated in bark compost leachates than in peat leachates compared to the distilled water control. Zoospore germination decreased with incubation time in both extracts (Fig. 2); however, at all incubation times, zoospore germination was significantly higher ($P = 0.01$) in peat than in bark compost leachates. After 120-min of incubation, zoospore germination was only 8% in bark compost leachate compared with 75% in peat. Zoospores began to encyst in both leachates after 30 min. After 60 min almost all zoospores encysted in both leachates; however, in bark compost leachate, encysted zoospores began to lyse. In the distilled water control, zoospore motility continuted for 120 min, at which time encystment began.

Production of sporangia was always significantly lower ($P = 0.01$) in bark compost than in peat leachates (Fig. 3). After 2 and 3 days, sporangial production in bark compost was less than half that in peat leachate. At 2 days, percent sporangia production was 39% in bark compost and 89% in peat leachate, and at 3 days it was 42% in bark compost and 91% in peat leachate compared to the distilled water control.

## DISCUSSION

Phytophthora collar rot of apple is a serious disease on wet, poorly drained soils (2,16). In this study, seedlings in both container media were watered uniformly and both media drained readily. The reduced seedling kill and suppression of *P. cactorum* in the bark compost medium did not appear to be due to physical factors or drainage of the media. Aqueous leachates of composted hardwood bark inhibit sporangial production, and lyse zoospores of *P. cinnamomi* (9). Similar results are published for raw pine bark extracts (6,7).

Hoitink et al (9) demonstrate that hardwood bark compost is suppressive to *P. cinnamomi* in a lupine seedling assay. They also report that inoculum concentration influenced the level of seedling kill for the different container media used. Low inoculum levels did not kill a significant number of seedlings except in a peat-sand medium. High inoculum levels kill significantly more seedlings in peat media than in composted bark-sand medium. The suppression of *P. cactorum* observed in this study appears to be related to inhibitory substances in bark compost which had a direct adverse effect on the pathogen. Inhibitory substances could be indigenous to composted hardwood bark itself and/or produced by antagonistic micoorganisms (11) that recolonized the bark compost after heating.

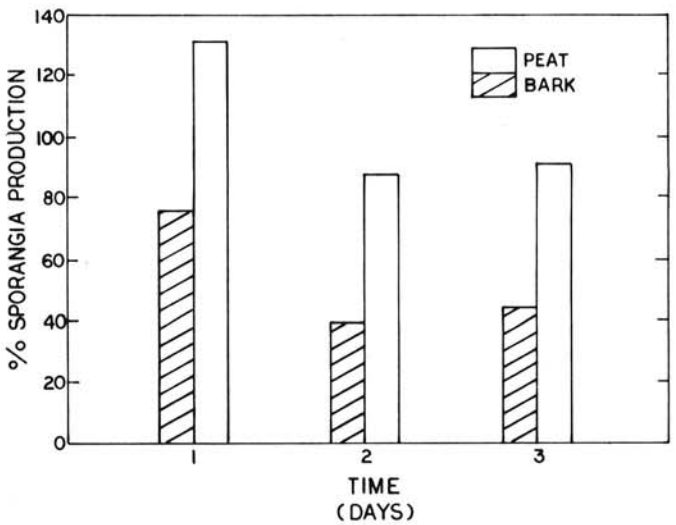


**Fig. 3.** Percentage *Phytophthora cactorum* sporangium production in aqueous bark compost and peat leachates, as compared to a distilled water control (100%). LSD ($P = 0.01$) after 1 day of incubation in leachate = 35.5; after 2 days = 14.7; and after 3 days = 11.1.

Inhibition of sporangium production and lysis of zoospores should be an effective means of reducing the number of infective propagules of *P. cactorum*. Since zoospores appear to be the most important propagule involved in the infection of apple (16,20), the use of composted hardwood bark could have a beneficial effect in control of collar rot under field conditions. Houck (12) reports that ammoniated Douglas-fir bark incorporated into *P. fragariae*-infested soils at rates of 36,288 to 90,720 kilograms per hectare (40 to 100 tons per acre) reduced the incidence and severity of red stele disease of strawberry under field conditions.

Satisfactory control measures for collar rot of apple have not been developed or proven entirely effective (14,16). The possibility of biological control through the use of composted hardwood bark needs to be examined further. In orchards, bark compost could be applied as mulch around the base of trees and/or mixed with soil to fill in auger holes when trees are planted. In nurseries, where clonal rootstock may initially become infected (13), bark compost could be used as a mulch in which the rootstock is propagated. Before the use of composted hardwood bark can be recommended as a control measure for collar rot, field studies must be conducted.

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