Factors Affecting Suppression of *Rhizoctonia solani* in Container Media

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ABSTRACT

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Container media containing high levels of composted hardwood bark (CHB) suppressed Rhizoctonia damping-off on Celosia and radish. Container media containing pine bark were only mildly suppressive and media containing high levels of peat were conducive. Increasing seedling density from 245–735 seedlings per square meter did not increase damping-off in either CHB or peat media. Substitution of 0, 28, 50, 72, and 100% of the CHB with peat resulted in a dilution of suppression. The suppressive effect of CHB was not detectable regardless of propagule size when hosts were exposed to inoculum densities of >20–25 propagules per gram.

Tree bark is widely used as an organic component of container media for the production of greenhouse and nursery crops (10). Composted hardwood bark media, commonly utilized in the Midwest, are suppressive to diseases caused by *Rhizoctonia solani* Kühn whereas media composed largely of peat are conducive (6,17). For example, Rhizoctonia crown rot of Poinsettia (*Euphorbia mulcherrima*) and damping-off of Celosia (*Celosia argenta* 'Red Fox') were suppressed in composted hardwood bark media but not in peat media (6,17). The level of suppression of Poinsettia crown rot is equivalent to that obtained in a steam-treated peat medium drenched twice with PCNB (6).

Several factors affecting the activity of *R. solani* in media composed of tree bark and/or peat were examined to better define the limits of suppressiveness of bark composts. Although studies of Rhizoctonia-suppressive soils indicated that suppressiveness is dependent upon the size and density of Rhizoctonia propagules (19), pH and moisture levels (5,15), concentration of suppressive soil (19), and soil type (9), there have been no comparable studies of disease suppression in bark media. Effects of bark type, bark-peat ratio, inoculum density and seedling density on damping-off are examined here to characterize properties of suppressive bark media more thoroughly.

MATERIALS AND METHODS

Preparation of container media. Organic components used in container media were Canadian peat (CP), composted hardwood bark (CHB), raw pine bark (RPB), and composted pine bark (CPB). The term "raw bark" refers to bark removed from trees less than 2 wk before and not subjected to thermophilic decomposition. The CHB used in most experiments was prepared from a mixture of tree species (~75% oak, *Quercus rubra, Q. alba;* ~15% *Fraxinus, Acer*, and *Carya* spp.; ~15% miscellaneous hardwoods) and was received from Paygro, Inc., South Charleston, OH 45368. Some composted samples were obtained from Ohio nurserymen (largely oak bark). Percentage of readily decomposable carbon (carbon evolved as CO_2 in soil over 60 days) in bark proper from these species ranges from 9.6 to 42.0% (1). The hardwood bark mixtures contained 70–80% bark and 20–30% woodchips. All particles were <2.0 cm in diameter. The pH of the CHB samples ranged from 5.5 to 7.5.

Raw pine bark (pH 5.4), a mixture of mostly loblolly (*Pinus taeda*), and smaller amounts of slash (*P. elliottii* Englm. var. *elliotti*), shortleaf (*P. echinata*), and longleaf pines (*P. palustris*), was received from Kamlar Corp., Rocky Mount, NC 27801. Percentage of readily decomposable carbon in the bark proper from these species ranges from 3.5 to 9.3% (1). Small amounts of similar pine bark were also obtained from one source each in Alabama and Arkansas. Pine bark obtained from all sources contained ~90% bark and 10% woodchips. All particles were <2.0 cm in diameter.

The pine bark was composted according to the following procedure: 1.2 g urea (45-0-0) and 0.6 g treble superphosphate (0-46-0) were added per liter fresh bark. The moisture level was adjusted to 50–55% (w/w). The mixture was placed in 200-L insulated drums, incubated in a heated (40 C) room and turned weekly. Temperatures in the compost ranged from 45 to 55 C. Water was added to maintain 50–55% moisture. The compost ceased to heat above room temperature (40 C) after 3 wk. It was incubated further at 25 C for another 3 wk without turning. The CPB was maintained thereafter at 4 C. The pH of the CPB ranged from 5.4 to 6.6. Factors affecting composting of tree bark have been detailed elsewhere (11).

Neutral aggregates used were coarse perlite (W. R. Grace Co., Cambridge, MA 02140) and coarse and fine grades of sharp silica sand (Sidley Sand Co., Thompson, OH 44086). All container media consisted of mixtures of one or more of the above described organic components, perlite, and sharp silica sand (5:3:2, v/v). Since bark particles were coarser than peat particles, fine silica sand was added to media containing high levels of bark. Coarser sand or a mixture of the two grades of sand, was added to media containing high levels of peat. This provided an air-filled pore space (at container capacity) of 15–20% (in a 10-cm-tall column) in all media. In some experiments where mixtures of bark and peat were used, the CHB, CPB, or RPB was replaced with 0, 28, 50, 72, or 100% CP (v/v). These bark-peat media were designated as 50-0, 36-14, 25-25, 14-36, and 0-50, respectively.

Container media with RPB or CP in the organic component were amended with 10 g treble superphosphate (0-46-0) and 0.1 g of fritted trace elements (Brighton By-Products Co., Inc., New Brighton, PA 15066) per liter. CHB and CPB contained adequate levels of available phosphate and trace elements (H. A. Poole, *personal communication*). All media were adjusted to pH 6.4 with a 1:1 mixture of hydrated lime and dolomitic limestone and amended with 10 g Osmocote (14-14-14) per liter (Sierra Chemical Co., Milpitas, CA 95053). All components were mixed 3 min in a

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concrete mixer. Tap water was added to a level that insured uniform mixing of individual components. Container media were used immediately after preparation or stored for less than 1 wk in polyethylene bags at 4 C until use. Individual components or media were not sterilized before use.

Preparation of inoculum. A culture of *R. solani* (isolate R-19) isolated from Salvia (*Salvia splendens*) (17) was used throughout this study. It was maintained on potato-dextrose agar (PDA) at 26 C. In some experiments, inoculum consisted of 11-mm-diameter colonized agar disks taken from 72-hr PDA cultures of *R. solani*. In experiments requiring uniform distribution of inoculum throughout a container medium, an air-dried chopped potato-soil mix (CPS) described by Ko and Hora was used (13). It was ground in a mortar and pestle and sieved through a 2.0-mm screen. Different propagule sizes were obtained by passing inoculum through a 590-μm sieve. The residue that remained on the sieve was assumed to contain large propagules while that which passed through the sieve contained small propagules (7). Container media were infested by mixing CPS with the media in a polyethylene bag. Propagule density was determined by dilution plating (2).

Assays for suppressiveness. Two methods were used to determine suppressiveness of container media. In most experiments, the Celosia suppression assay (14 seedlings per cm of row length) was used (17). In experiments designed to test the effects of seedling density on suppression, the actual density was determined by seed weight (mean wt 300 seeds = 0.216 g). A standard weight of seed, which corresponded to the desired seedling density, was placed in a small copper tube that was cut in half longitudinally. Seeds were spaced approximately equidistant. The tube was then held over a 17-cm furrow (two furrows per flat) and rotated 180 degrees, dropping the seed into the furrow. Seeds were then covered with 1.0-cm container medium. Flats were placed in individual polyethylene bags to prevent moisture loss. Four 0.5-cm holes were punched in each bag to keep the air-moisture levels in the bag below saturation to avoid webbing by the pathogen. Flats were incubated in a growth chamber as described by Stephens et al (17). After seedlings emerged (3 days), one 11-mm-diameter agar disk was placed 1.0 cm below the surface and adjacent to the first seedling in each row. The mean damped-off row length after 8 days determined the suppressiveness of the container medium. Damped-off seedlings were plated on PDA routinely to verify identity of the pathogen.

In experiments with a variety of inoculum densities, a radish seedling assay was used (8). Pots (10-cm standard) were filled with the infested container media and seeded. Thirty-two radish seeds (*Raphanus sativus* 'Early Scarlet Globe') were placed randomly in each pot and covered with 1.0 cm container medium. Pots were incubated in a growth chamber at 26 C under continuous illumination (7,500 lux). After 7 days, the number of healthy seedlings in each pot (five pots per treatment) was recorded and the percent disease determined. The cause of damping-off was checked routinely by plating diseased seedlings on PDA.

All experiments were repeated at least once. Results were analyzed with a two-way analysis of variance or regression analysis where appropriate. Means were separated by using the LSD test and the Duncan's new multiple range test.

RESULTS

Comparison of damping-off in hardwood bark, pine bark, and peat container media. Differences in damped-off row lengths (Celosia assay) were not observed between media containing CPB and those containing RPB. Significantly more damping-off was observed in the medium containing 100% CP in the organic component (0-50) than in media containing either CPB or RPB as the sole organic component. Substitution of half of the CPB with CP (25-25) did not cause a significant reduction in the mean damped-off row length, whereas replacement of RPB with CP (25-25) did. For example, substituting RPB with 0, 50, and 100% CP resulted in mean lengths of row, which was damped-off of 6.6, 8.4, and 10.5 cm, respectively. Similar results were obtained in other trials with pine bark from Arkansas (raw and composted) and Alabama (raw).

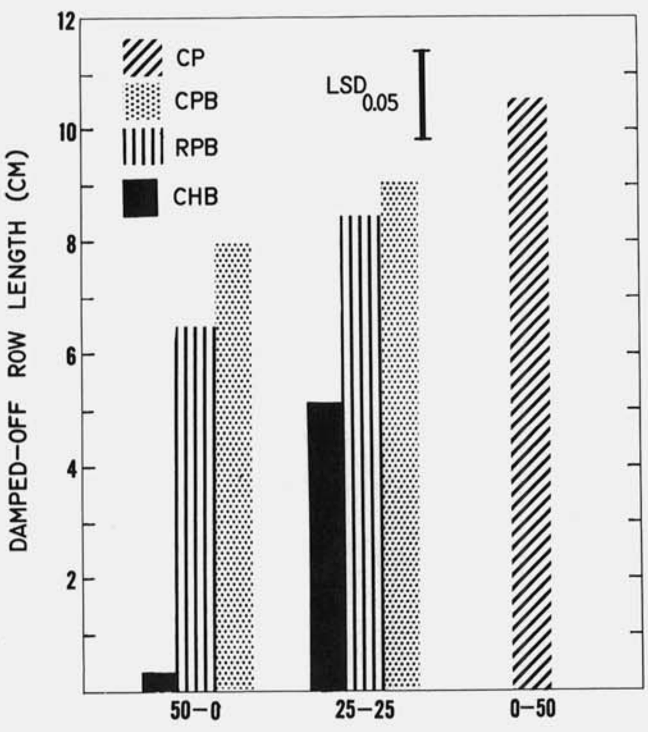

Fig. 1. Comparison of Celosia damping-off caused by *Rhizoctonia solani* in composted hardwood bark (CHB), composted pine bark (CPB), raw pine bark (RPB), and Canadian peat (CP) container media. (50-0 = Only bark in the organic component; 25-25 = equal volumes of bark and peat in the organic component; 0-50 = only peat in the organic component). LSD (P = 0.05) = 1.6.

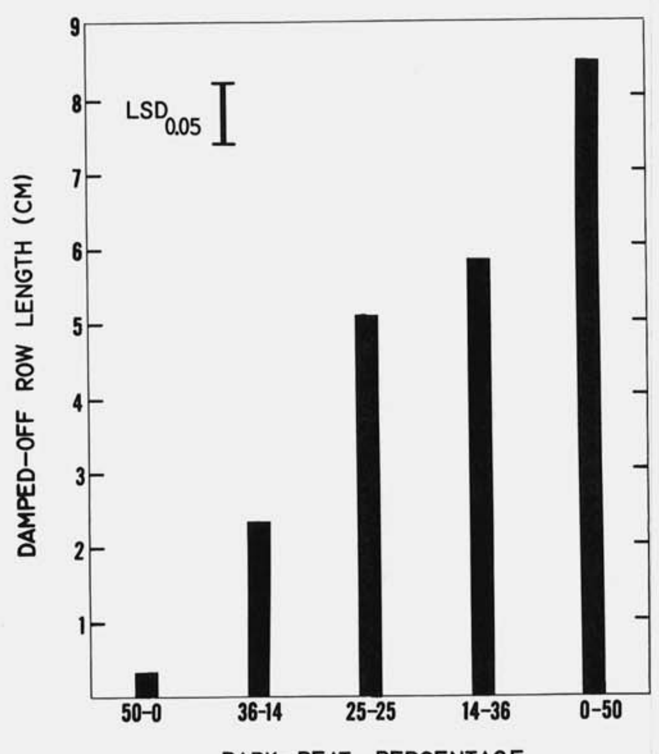

Fig. 2. Effect of the ratio of composted hardwood bark (CHB) to Canadian peat (CP) in the organic component on Celosia damping-off caused by *Rhizoctonia solani*. LSD (P = 0.05)=1.3.

The differences in damping-off among media containing CHB, CPB, RPB, and CP are presented in Fig. 1. These data represent results from two experiments which were combined and analyzed ($P = 0.05$) using analysis of variance. The total amounts of damping-off in media containing CHB were significantly less than in all other media. The CPB medium (50-0) and the mixtures of pine barks (RPB and CPB) and peat (25-25) were not significantly different from the CP medium. RPB, however, was significantly less suppressive than CHB.

Effect of composted hardwood bark-peat ratio on damping-off. No significant ($P = 0.05$) amount of damping-off was observed in the 50-0 medium at any time following inoculation (Celosia assay). Substitution of CHB with 28, 50, 72, and 100% CP resulted in a directly proportional increase in damped-off row lengths (Fig. 2). The greatest amount of damping-off was observed in the 0-50 medium. The effect of decrease in the CHB-CP ratio on damping-off was more noticeable at 6, 7, and 8 days after inoculation (Fig.3). It was delayed in media containing 100% (50-0) and 86% (36-14) CHB in the organic component, whereas the increase of damping-off in media containing lower levels of CHB was similar to that in the CP (0-50) medium.

Effect of seedling density on damping-off. In three of four media tested (50-0, 25-25, and 0-50), seedling density did not affect the length of row in which damping-off occurred (Celosia assay, Table 1). Significantly ($P = 0.05$) more damping-off was observed at higher seedling densities in the 36-14 medium. For example, at seedling rates of 735 seedlings per square meter there was more damping-off than at the seedling rate of 245 seedlings per square meter.

To examine growth of *R. solani* in the CHB-CP and the CP media, flats were inoculated but not seeded with Celosia. After 8 days, 0.5-cm-diameter corkborer samples were taken at 1.0-cm intervals from the "unseeded row" and plated on a selective medium (13). In both the CHB-CP and CP media, *R. solani* was isolated from samples at distances up to 3.0 cm from the inoculum source (four flats with two "rows" per flat).

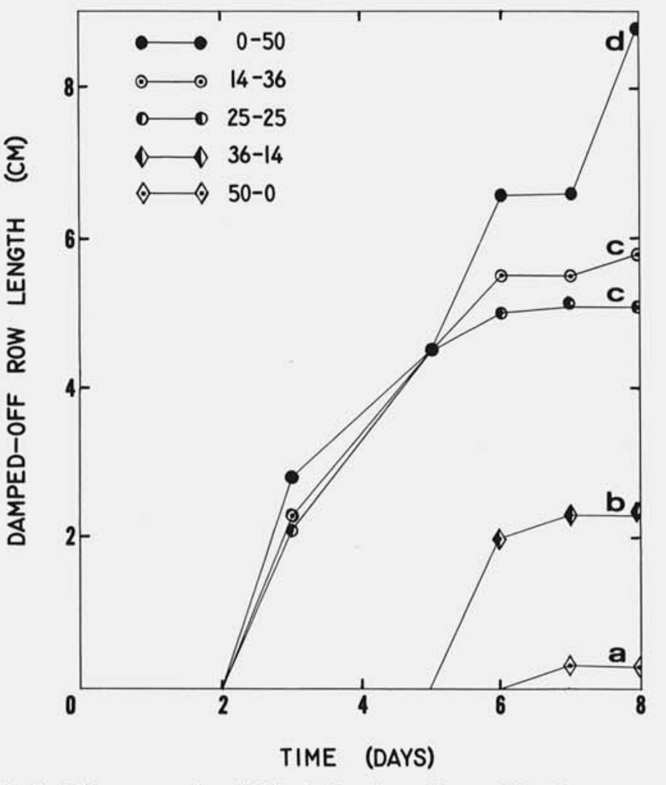

Fig. 3. Daily progression of Celosia damping-off caused by *Rhizoctonia solani* in various composted hardwood bark-Canadian peat container media. Eighth day data points followed by the same letter are not significantly different ($P = 0.05$) according to the Duncan's new multiple range test. Differences were similar on days 5, 6, and 7.

Substitution of part or all of the CHB with CP affected the length of the seedling row damped-off at all seedling densities tested. An increase in the percent CP in the organic component resulted in a corresponding increase in damping-off. For example, the mean lengths of rows in which damping-off occurred in the 50-0, 36-14, 25-25, and 0-50 CHB-CP media were 6.6, 7.9, 8.4, and 11.8 cm, respectively, at the highest density tested (Table 1). Similar readings were found at the other densities. Regression analyses of these data are presented in Fig. 4. Regression coefficients were not significantly different ($P = 0.001$) from zero. The intercept value for the 50-0 medium was significantly lower than those for 25-25 and 0-50 CHB-CP media. Data for the 36-14 medium are not shown in Fig. 4 but the slope value and intercept were not significantly different ($P = 0.05$) from the 50-0 medium.

Relationship between inoculum density and disease incidence. The addition of increasing numbers of small (<590 μm) propagules to the CHB medium (50-0) or the CP medium (0-50) resulted in increasing amounts of damping-off (radish assay, Fig. 5). Similar results were obtained in three other trials with either large or small propagules. Damping-off was most severe in CP medium. Significantly more propagules were needed in the CHB medium to produce disease equal to that in the CP medium. For example,

TABLE 1. Effect of seedling density on Celosia damping-off caused by *Rhizoctonia solani* in various composted hardwood bark-Canadian peat container media

Seedling density (plants/m^2)	Composted hardwood bark-peat ratio[a]			
	50-0[b]	36-14	25-25	0-50
245	5.8[c]	4.7	7.4	11.5
315	5.1	5.6	5.8	10.5
385	4.1	5.8	7.8	10.9
455	5.3	7.6	8.7	9.7
525	6.9	7.5	7.3	10.1
595	7.2	8.9	8.9	11.2
665	5.1	8.5	7.9	10.3
735	6.6	7.9	8.4	11.8

[a] Based on a volume-volume ratio; the remaining 50% of the container medium consisted of neutral aggregates (ie, perlite, sand).

[b] 50-0 = 50% composted hardwood bark (CHB)-0% Canadian peat (CP) in the organic component.

[c] Indicates mean damped-off row length (cm). LSD ($P = 0.05$) = 2.05.

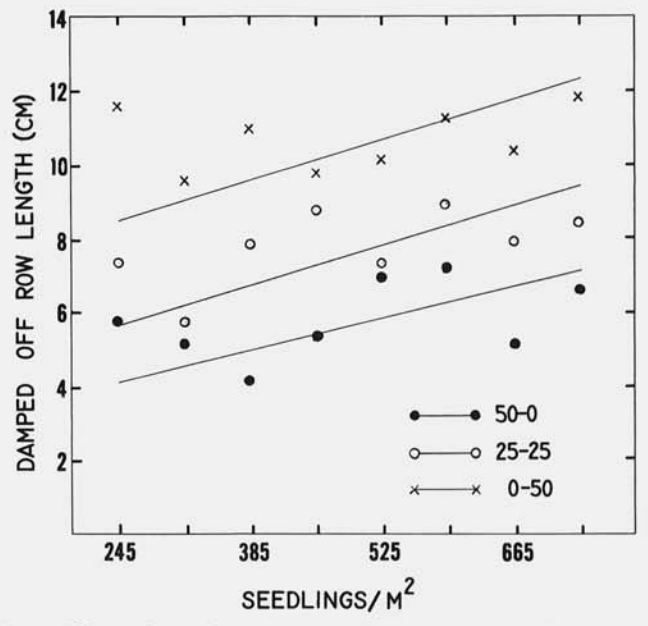

Fig. 4. Effect of seedling density on Celosia damping-off caused by *Rhizoctonia solani* in composted hardwood bark (CHB, 50-0), Canadian peat (CP, 0-50), and composted hardwood bark-Canadian peat mixtures (25-25). 50-0 (slope = 0.0042, r = 0.55); 25-25 (slope = 0.0054, r = 0.60), 0-50 (slope = 0.0056, r = 0.53).

approximately 10 propagules per gram (p/g) container medium produced 50% damping-off in the CP medium. Approximately 25 p/g produced 50% damping-off in the CHB medium.

DISCUSSION

Our results of Rhizoctonia damping-off suppression in CHB as compared to peat media supports previous findings (6,17). Damping-off in CHB was suppressed in both the radish and the Celosia assay. Since the Celosia assay cannot be used for inoculum density studies, and since quantitative aspects of the radish assay have been defined (8), the latter was used in several of the experiments reported in this paper. Batches of CHB varied in suppressiveness (mean length of rows in which damping-off occurred varied from 0.3–4.0 cm). The cause of this variability, at least in part, was microbial in origin and will be discussed in a future report. Raw hardwood bark was not tested for its effect on damping-off because of its high total cellulose content, which caused acute and chronic nitrogen deficiency in test plants.

The decrease in suppression in CHB media amended with increasing amounts of CP agrees with grower experiences in Ohio. Growers who substituted more than 50% of the CHB with CP to increase water retention of the media experienced losses induced by *R. solani* and other soilborne pathogens.

The effect of composting on the suppressiveness of pine bark is not understood. However, allelopathotoxins present in hardwood bark may inhibit growth of seedlings (16,18). Inhibitors of microorganisms also are present in tree barks (12,14). Some of these inhibitory effects are negated by composting (14,16,18). We found that RPB was slightly suppressive to Rhizoctonia damping-off whereas CPB was not. Perhaps inhibitors of *R. solani* in pine bark were therefore destroyed during the composting process.

Seedling density often has been ignored as a factor in suppression of damping-off but it may have profound effects on these diseases. For instance, Pythium (*P. irregulare*) damping-off of garden cress is transmitted readily between host plants at high seedling densities (3). Increased distances between plants at low densities reduces the probability of successful transmission (4). This effect of transmission is similar to that produced by increasing inoculum density. Results in inoculated flats with seedlings indicate that seedling densities within the range studied (245–735 seedlings per square meter; maximum distance between seeds = 2.0–0.0 cm) had no effect on Rhizoctonia damping-off of Celosia in either a CHB or CP medium (Fig. 4). Since *R. solani* colonized CHB and CP up to 3 cm from the inoculum source, interplant distances >3.0 cm (ie, densities much lower than 245 seedlings per square meter) would need to be tested to observe any effect on damping-off.

The importance of inoculum density as well as propagule size to diseases caused by *R. solani* is well known (7,9). At low inoculum densities, CHB was suppressive to Rhizoctonia damping-off of radish as compared to CP. However, CHB failed to suppress damping-off at inoculum densities >20–25 propagules per gram of container medium (Fig. 5). This effect is consistent with that reported for a Rhizoctonia-suppressive soil (19, Fig. 4). It also agrees with a previous report on Rhizoctonia crown and root rot of Poinsettia in which several inoculum levels were used (6).

The relationship between inoculum density and disease incidence in either CHB or CP was not affected by propagule size. At any given inoculum density, large or small propagules produced the same amount of disease. These results are different from those reported for soils (5,19). Chet and Baker (5) found that soil developed suppressiveness during monoculture to large propagules of *R. solani* only at pH 6.5 or lower, but not at pH 8.1. The same effect was reported for small propagules at all pH values tested (5). Since the pH of the CHB media tested ranged from 5.5–7.5, pH may not be a limiting factor in the suppression of large propagules of *R. solani* in CHB.

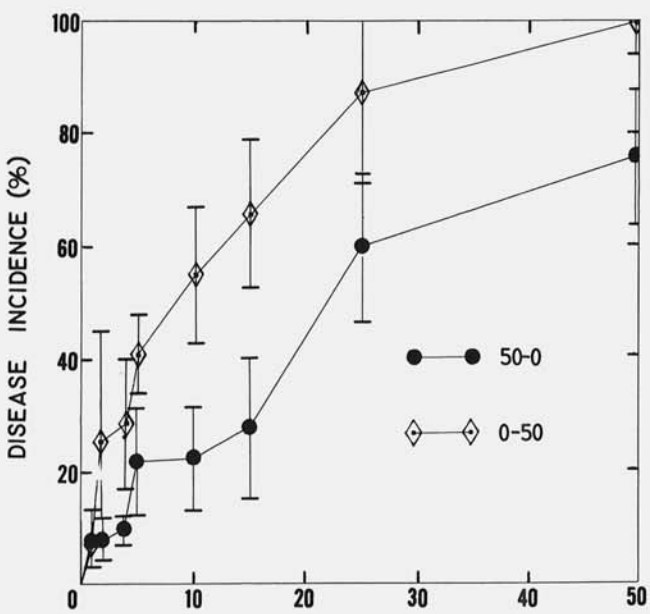

Fig. 5. Relationship between inoculum density and radish damping-off caused by *Rhizoctonia solani* in composted hardwood bark (CHB, 50-0) and Canadian peat (CP, 0-50) container media. Bars represent 95% confidence intervals.

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