

Effects of Organic Matter Decomposition Level and Cellulose Amendment on the Inoculum Potential of *Rhizoctonia solani* in Hardwood Bark Media

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ABSTRACT

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Container media amended with fresh hardwood bark (~ 45% cellulose, w/w) were conducive to *Rhizoctonia* damping-off of radish. Those amended with hardwood bark compost were suppressive. Significantly higher cellulase activity was present in the fresh bark medium colonized by *R. solani* than in the bark compost medium. The population of *R. solani* increased in the fresh bark but not in the bark compost medium. Addition of low levels of cellulose (5%, w/w) to the bark compost medium decreased

damping-off. High levels of cellulose (20%, w/w) established high cellulase levels in the *Rhizoctonia*-infested bark compost media, increased the population of *R. solani*, and negated suppression. Even in the presence of a suppressive microflora, the high cellulose amendment increased conduciveness. We conclude that high cellulose levels increase the inoculum potential of *R. solani*, resulting in increased damping-off severity.

Rhizoctonia solani Kühn is a destructive, versatile, and widespread pathogen of various plants. It has high competitive saprophytic ability in soil (10,26,28). However, *R. solani* cannot colonize soil devoid of a food base (5,8,25). Bateman (3,4) showed that *R. solani* can utilize cotton fibers, filter paper, and carboxymethyl cellulose as sole sources of carbon, by producing cellulase in culture. It also can utilize cellulose in soil (7,12,13). Nutrient availability and soil organic matter decomposition level, therefore, are primary factors affecting growth and saprophytic colonization of *R. solani* and, consequently, its inoculum potential in soil (13,25,31).

During the past decade, a variety of organic wastes have been used in container media for the production of ornamental plants (14). Nelson et al (23,24) reported that *Rhizoctonia* damping-off of radish was suppressed in container media amended with mature bark compost but not with fresh bark. They also found that survival of propagules of *Rhizoctonia* was significantly better in fresh bark media than in mature bark compost media. Cellulose is broken down through activity of cellulolytic microorganisms during composting (1,11). Populations of *R. solani*, therefore, could be affected directly by the maturity level (cellulose content) of composts. The microflora in composts changes as the level of decomposition increases (9,14,27). This also may affect the ability of *R. solani* to colonize compost-amended substrates or to cause disease. The relative contribution of these factors to disease severity is unknown.

Suppression of *Rhizoctonia* damping-off in bark compost media has been attributed to the activity of *Trichoderma* spp., other fungi (17,24), and their interaction with bacterial antagonists (16). Unidentified components of this soil microflora may be involved as well. In this paper, we report population development of *R. solani* in substrates amended with cellulose and with bark composts of various maturity levels. These populations were related to cellulase activity produced in these substrates.

MATERIALS AND METHODS

Preparation of container media. Freshly hammermilled hardwood tree bark (mostly *Quercus* spp.), obtained from Paygro, Inc.,

South Charleston, OH, was mixed with nitrogen and water and then composted in windrows, as described previously (22). During this process approximately 40% of the carbon, mostly in the form of cellulose, is volatilized as carbon dioxide (1,6). Bark-amended container media were prepared by mixing fresh bark or bark composts of various maturity levels with Canadian sphagnum peat and perlite (5:2:3, v/v). Fresh bark medium was prepared with bark that had been removed from trees less than 1 wk before. Unless specified otherwise, the bark compost medium was prepared with 44-wk-old compost. The pH of all media ranged from 6.0 to 6.4. Slow-release fertilizer was added to all treatments immediately before planting, as described previously (23,24). In some experiments, container media were heated in an oven (5 days, 60 C) to simulate effects of self-heating on the compost microbiota that occur naturally in compost piles. This procedure destroys most of the suppressive effect of the bark compost medium to *Rhizoctonia* damping-off (17). In such experiments, additional container media also were incubated at 25 C. Media incubated at 25 C remained suppressive, therefore, and served as controls.

Bioassay. A radish bioassay was used to determine the suppressiveness of container media to *Rhizoctonia* damping-off (24). Radish seeds (*Raphanus sativus* L. 'Early Scarlet Globe,' 97% germination) were placed at equal spacing in each of five, 10-cm-diameter, pots (10-cm depth, 500-ml capacity, 32 seeds/pot) and covered with a 1-cm layer of container medium. Soil inoculum of *R. solani*, produced in a chopped-potato soil mixture (15), was air dried and screened to yield 1–2-mm pieces of inoculum (22). Unless specified otherwise, the container medium was infested with 0.4 g of this soil inoculum per liter. The experimental design was a randomized factorial. Pots were incubated and watered in a growth chamber at 25 C under continuous illumination. After 7 days, each plant was rated according to a disease severity scale in which: 1 = symptomless, 2 = diseased but not damped-off, 3 = postemergence damping-off and 4 = preemergence damping-off. Mean disease severity was based on five replicates. Analysis of variance of data was performed by using MINITAB computer program. Means were compared with and *F* protected LSD. Each experiment was repeated at least once.

Population development of *R. solani*. Populations of *R. solani* were examined in container media prepared with fresh bark (<1-wk-old) and with 4-, 11-, 22-, 30-, 43-, and 54-wk-old

composts. In addition, populations were determined in the 44-wk-old bark compost medium amended with various levels of cellulose. Whatman CF11 cellulose powder was added at concentrations of 0, 5, 10, and 20%, on a dry weight basis. All media were prepared in 2-L volumes and mixed thoroughly. Separate 2-L volumes of media were heated 5 days at 60 C or incubated at 25 C as controls. Thereafter, media were infested with soil inoculum of *R. solani* as described above or not infested as a control. Treated 2-L volumes of media were distributed in pots (five pots/treatment) and seeded with radish as described above. A duplicate set of treatments was not seeded.

To determine the mean population density of *R. solani*, container media samples were air dried 24 hr at 25 C. A 1-g subsample then was added to 10 ml of a warm (50 C) selective agar medium and dispensed into an 8.8-cm-diameter petri dish (three subsamples/treatment). The selective medium contained 50 mg of chloramphenicol, 50 mg of streptomycin sulfate, 1.25 mg of benomyl (2.5 mg of Benlate 50W, DuPont), 10 mg of metalaxyl (40 µl of Ridomil 2E, 25.1% a.i., Ciba-Geigy Corp.), and 20 g of agar per liter of distilled water. After the agar had solidified, 15 disks were removed from plates with a 6-mm-diameter cork borer and placed on the surface of 2% Difco water agar in an 8.8-cm-diameter petri dish. The plates were then incubated 24 hr at 25 C. Thereafter, growth of *R. solani* from disks was rated as follows: 0 = no growth; 0.3 = mycelial growth from one or two points of a disk; 0.6 = disks with three to five radial growth zones, and 1.0 = growth surrounding the entire disk. A correction factor was determined by dividing the area of the plate (60.79 cm²) in which the sample had been dispensed by the total area of the 15 disks (4.24 cm²). Ratings for all 15 disks on a plate then were added and multiplied by the correction factor (14.34) to account for the remainder of the 1 g of infested sample suspended in agar that was not transferred with the disks. This product was considered to be a relative estimate of the mean number of propagules per gram air dried of container medium. The mean number of propagules, thus, was based on three samples per treatment.

Cellulase activity. Cellulase activity in the container media was determined by following release of glucose from carboxymethyl cellulose (sodium salt, medium viscosity, Sigma Chemical Co., St. Louis, MO). Container media samples (three replicates of 5 g wet weight each) were placed in a 50-ml beaker. Toluene (0.5 ml) was mixed thoroughly with this sample to minimize microbial activity during the enzyme assay (18). Without added toluene, the enzyme reaction did not have zero-order kinetics. After a 15-min incubation at 37 C, 10 ml of warm (37 C) 0.1 M acetate buffer (pH 5.4), containing 1% carboxymethyl cellulose, was added. This suspension was then mixed thoroughly again and incubated 4 hr at 37 C. The reaction was stopped by placing the beaker in a boiling water bath for 10 min. Thereafter it was cooled in an ice bath and 10 ml of sterilized distilled water was added to dilute the reaction mixture. This suspension was filtered through prepleated filter paper (Schleicher & Schuell Inc., Keene, NH). The amount of glucose released from the substrate into the filtrate was determined with a glucose oxidase reagent (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's procedure. Depending on cellulase activity, samples were diluted further with distilled water and then examined for glucose concentrations. Samples to which carboxymethyl cellulose had not been added were run concurrently as controls. Cellulase activity was expressed as micrograms of glucose released per gram dry weight of container medium per 4 hr. The amount of glucose in the control treatment was subtracted from that of each sample. In a preliminary test, validity of the enzyme assay was tested for linearity with respect to time using regression analysis. All assays were conducted within the linear portion of the cellulase-time relationship. The MINITAB computer program was used to analyze data.

RESULTS

Population development of *R. solani*. The mean number of propagules of *R. solani* in the fresh bark medium (< 1 wk old) that was planted with radish increased significantly ($P=0.01$) from the

initial level of 5/g to a level of 48/g within 1 wk of infestation (Fig. 1). In the unplanted fresh bark medium, population development also was extensive, but not significantly different from that in the planted fresh bark medium. In the planted bark compost media that were prepared with various compost maturity levels, population development of *R. solani* was much less extensive. Without planting, population development did not occur in these media.

High populations of *R. solani* developed in the heat-treated fresh bark medium (Fig. 2). During the first week, planting had no effect on population development. Thereafter, significantly ($P=0.01$) higher populations developed in the planted medium. In contrast, in the heated bark compost medium not planted with radish, populations of *R. solani* declined to a low level within 7 days. Thereafter, populations remained just above detectable levels. In the planted bark compost medium, which had been heated to render it conducive to disease, populations of *R. solani* did not change with time. These results were verified in a second experiment.

Cellulose amendment had a significant effect on population development of *R. solani* in the heated bark compost medium not planted with radish (Fig. 3). After 3 days, populations in the 10 and 20% cellulose treatments were significantly ($P=0.05$) higher than those in the unamended heated control. After 14 days, the population of *R. solani* in the 20% cellulose treatment was 20-fold

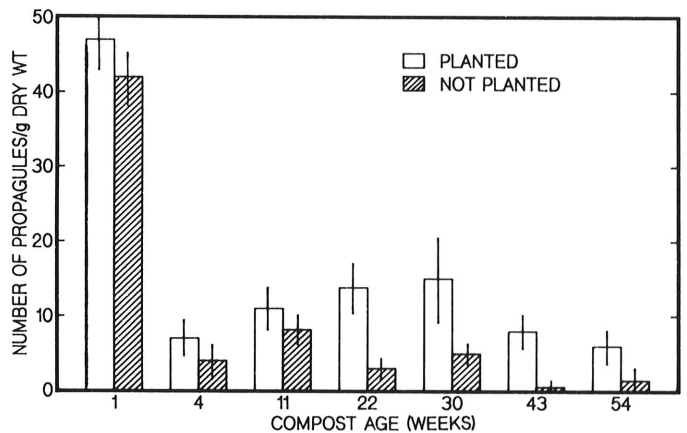


Fig. 1. Population development of *Rhizoctonia solani* in container media amended with fresh bark or bark compost of various maturity levels. Means of three replicates were determined 7 days after infestation (0.6 g of soil inoculum per liter) of media. Vertical bars represent standard deviation.

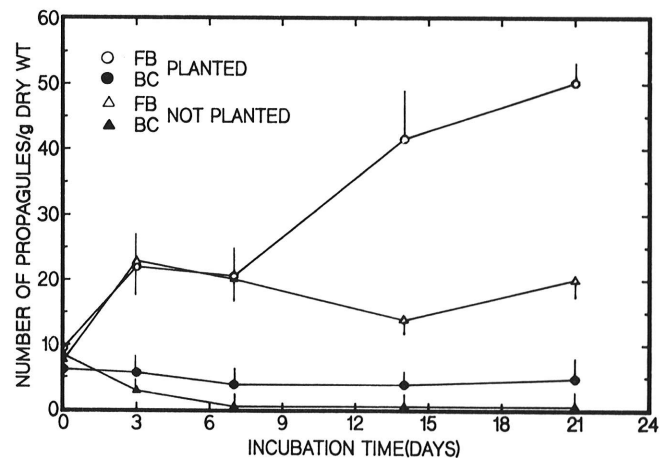


Fig. 2. Population development of *Rhizoctonia solani* in heated (5 days, 60 C) fresh bark and bark compost media planted or not planted with radish. FB = Fresh bark (< 1 wk old) medium, BC = Bark compost (44 wk old) medium. Media were infested with 0.4 g of soil inoculum per liter. Vertical bars represent standard deviation.

higher than the initial level. Populations in the bark compost medium not planted with radish and not amended with cellulose were similar to those presented above for this treatment with another batch of compost (Fig. 2). Similar trends were found in a second experiment on population development of *R. solani*. Populations of *R. solani* in the unheated (25 C) cellulose-amended bark compost medium were not determined because *Trichoderma* also colonized the agar disk and thus interfered with counts of propagules of *R. solani*. Unfortunately, selective media for isolation of *R. solani* that inhibit growth of *Trichoderma* are not available.

Cellulase activity. Immediately after preparation of the heated fresh bark medium, cellulase activity was below or just at detectable levels (Fig. 4). After 3 and 7 days and regardless of planting, cellulase activity in the fresh bark medium infested with *R. solani* was significantly ($P = 0.01$) higher than in the noninfested control. Highest cellulase activity was present in the medium not planted with radish (Fig. 4A). In contrast, in the heated bark compost medium, cellulase activity, although low initially, also remained low thereafter, even if infested with *R. solani*. Planting with radish had no effect on cellulase activity in this heated bark compost medium (Fig. 4B).

In the fresh bark medium that was not heated, cellulase activity was at detectable levels after the medium was first prepared (Fig. 5). High levels of cellulase activity were detected 7 days after the medium was prepared. Planting with radish or infestation of the medium with *R. solani* did not have a significant effect on cellulase activity until after 14 days. At 21 days, cellulase activity in the medium infested with *R. solani* was significantly higher ($P = 0.05$) than that in the noninfested control (Fig. 5B). Just after container media were prepared, cellulase activity in the suppressive bark compost medium (not heated) was significantly ($P = 0.01$) higher than that in the fresh bark medium (Fig. 5). Levels of activity did not change significantly with time. Infestation of this suppressive medium with *R. solani*, or planting with radish, did not have a significant effect on cellulase activity.

Amendment of the heated bark compost medium with cellulose (20%, w/w), significantly ($P = 0.01$) increased cellulase activity over that in nonamended control treatments (Fig. 6). This increase was significantly ($P = 0.01$) early (3 days) after infestation with *R. solani*. In the noninfested control, cellulase activity did not increase until after 7 days. In the control treatments not infested and not amended with cellulose, activity was low and similar to that in equivalent treatments shown in Figure 4A.

Disease severity in bark compost media amended with cellulose. Cellulose amendment affected severity of *Rhizoctonia* damping-off in both the suppressive bark compost medium incubated at 25 C and in the heated bark compost medium (conductive) (Table 1). In the unamended suppressive medium, with a disease severity of

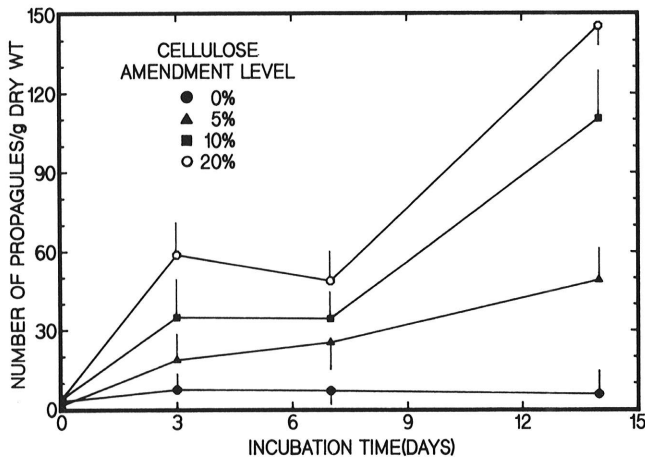


Fig. 3. Population development of *Rhizoctonia solani* in a heated bark compost (44 wk old) medium amended with various levels of cellulose. Media were infested with 0.4 g of soil inoculum per liter on day 0 and seeded with radish on day 3. Vertical bars represent standard deviation.

2.3, a 5% cellulose amendment significantly ($P = 0.05$) reduced that disease severity value to 1.8. In contrast, the highest amendment level (20%) consistently increased the disease severity. In the heated medium (conductive), even the lowest cellulose amendment level (5%) induced a significantly ($P = 0.05$) higher level of conductivity (the disease severity level was raised from 2.9 to 3.6). The highest cellulose amendment level (20%) raised the disease severity in the heated medium to 3.9. Essentially none of the seedlings survived with this treatment.

DISCUSSION

Trends in population development of *R. solani* in the heated bark compost medium, regardless of planting, show that compost maturity or age indeed had an effect. *R. solani* colonized fresh but not composted bark-amended media. Therefore, the inoculum potential of *R. solani* in fresh and bark compost media differed. The conducive nature of the fresh bark medium, as opposed to suppression in the bark compost medium, at least in part was due to the ability of *R. solani* to increase its inoculum density in that medium but not in the bark compost medium. This relationship between inoculum density and disease severity is similar to that reported for field soils (2,13,19,31).

The increased cellulase activity in the fresh bark medium after it was infested with *R. solani* (Figs. 4 and 5), as compared with the bark compost medium, shows that *R. solani* was using cellulose as a substrate in the fresh bark medium but not in the bark compost medium. Also, the increase in populations of *R. solani* in the conducive heated bark compost medium as a result of cellulose amendment and the concomitant increase in cellulase activity in that medium thereafter suggest that carbon (cellulose) availability in the bark compost medium limited population development of *R. solani*. This appears to be the basis for the lower inoculum

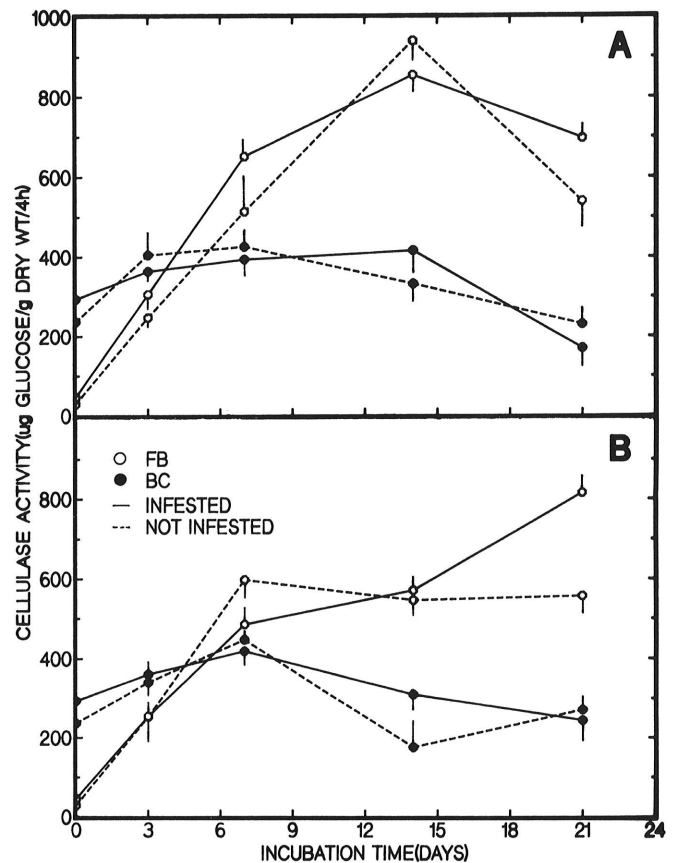


Fig. 4. Cellulase activity in heated (5 days, 60 C) container media infested with *Rhizoctonia solani*. FB = Fresh bark (< 1 wk old) medium, BC = Bark compost (44 wk old) medium; A, not planted and B, planted with radish. Infested with 0.4 g of soil inoculum per liter. Vertical bars represent standard deviation.

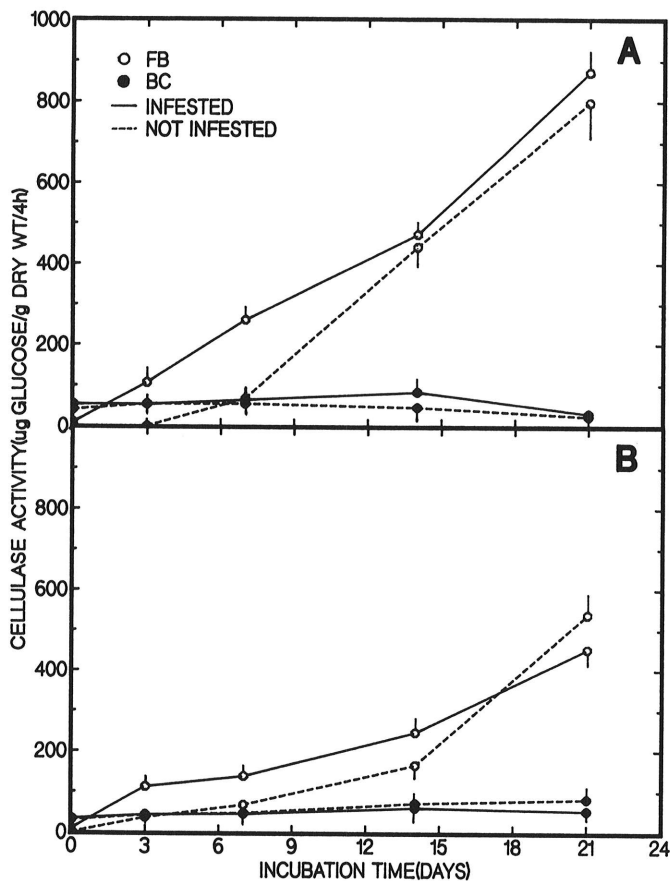


Fig. 5. Cellulase activity in unheated container media infested with *Rhizoctonia solani*. FB = Fresh bark (< 1 wk old) medium, BC = Bark compost (44 wk old) medium; A, not planted and B, planted with radish. Infested with 0.4 g of soil inoculum per liter. Vertical bars represent standard deviation.

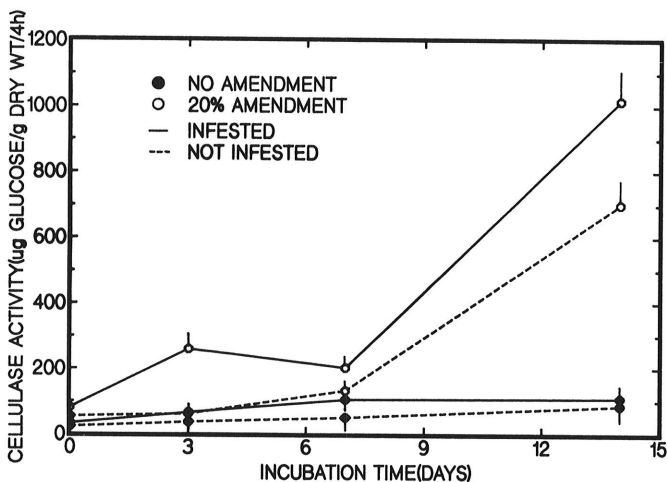


Fig. 6. Cellulase activity in a heated bark compost media infested with *Rhizoctonia solani* and amended with cellulose. Media were infested with 0.4 g of soil inoculum per liter on day 0 and seeded with radish on day 3. Vertical bars represent standard deviation.

potential of *R. solani* in the bark compost medium. This observation agrees with previous reports on disease severity obtained with this pathogen in field soils treated with amendments varying in stage of decomposition or in C/N ratios (9,10).

The lower cellulase activity observed in the heated fresh bark medium planted with radish (Fig. 4B) as compared with the activity in the absence of plants (Fig. 4A) appears to contradict data on *R. solani* population development in the presence or

TABLE 1. Effects of heat treatment at 60 C (BC60) versus incubation at 25 C (BC25) and cellulose amendment on *Rhizoctonia* damping-off severity of radish in bark compost media

| Treatment ^a | <i>Rhizoctonia</i> inoculum ^b | Cellulose amendment level (%) ^c | | | |
|------------------------|--|--|-----|-----|-----|
| | | 0 | 5 | 10 | 20 |
| BC25 (unheated) | + | 2.3 ^d | 1.8 | 2.2 | 2.8 |
| BC60 (heated) | + | 2.9 | 3.6 | 3.6 | 3.9 |

^aBC25 is an unheated bark compost medium incubated at 25 C then amended with cellulose just before infestation with *R. solani*. BC60 is a bark compost medium heated at 60 C for 5 days. Mean disease severity values in uninfested controls ranged from 1.0 to 1.1.

^b0.4 g of soil inoculum of *R. solani* was mixed per liter of container medium.

^cBased on dry weight (w/w).

^dDisease severity scale in which 1 = symptomless, 2 = diseased but not damped-off, 3 = postemergence damping-off, and 4 = preemergence damping-off. The interaction LSD_(0.05) = 0.4.

absence of plants in that medium (Fig. 2). Apparently the host plant served as a preferred food base for the pathogen in that conducive medium. Glucose released as radish root exudates (20) into the container medium may have repressed cellulase synthesis by *R. solani* (11,29). The high cellulase activity detected in the fresh bark medium (Fig. 5) may have been produced by cellulolytic microorganisms other than *R. solani* that were present in fresh bark. Relatively stable intermediate levels of cellulase activity detected in the bark compost medium throughout this work may have been due to the accumulation and stabilization of cellulase produced by microorganisms active during composting (18).

The decreased disease severity, observed after amendment of the suppressive bark compost medium (BC25, Table 1) with the lowest level of cellulose (5%, w/w), is similar to that reported by Rouse and Baker (30) for field soil. They proposed that nutrient competition was the mechanism responsible for the decreased disease severity. Suppression of *Rhizoctonia* damping-off in this bark compost medium has been attributed to the activity of fungal antagonists, including *Gliocladium virens* and *Trichoderma* spp. (17,24). Activity of several bacterial antagonists alone or in combination with *T. hamatum* (16), and possibly other microorganisms, may be involved as well. Nelson et al (23) demonstrated that propagules of *R. solani* were eradicated from this suppressive medium. *Trichoderma* spp. and *G. virens* were isolated from killed propagules of *R. solani*, suggesting that hyperparasitism was involved. Interestingly, the high amount of cellulose (20%, w/w) destroyed the suppressive effect, this in spite of the presence of these antagonists in this bark medium. It appears, therefore, that these very high cellulose levels negated efficacy of the biocontrol agents.

Unfortunately, we could not determine the effect of cellulose on populations of *R. solani* in the naturally suppressive container medium because *Trichoderma* overgrew *R. solani* in the assay plate. We hypothesize, however, that the increased disease severity in the fresh bark medium, at least in part, is due to an increased saprophytic activity of *R. solani* in the presence of high amount of cellulose. It is also possible that high cellulose levels, which result in the production of high amounts of glucose, repressed chitinase production (21), thus reducing hyperparasitism of *R. solani* by *Trichoderma*, which could also lead to an increased disease severity.

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