

The Role of Microorganisms in the Suppression of *Rhizoctonia solani* in Container Media Amended with Composted Hardwood Bark

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ABSTRACT

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Suppression of *Rhizoctonia* damping-off in container media amended with mature (>11-wk-old) composted hardwood bark (CHB) was eliminated by heat (60 C) and gamma radiation (275 krad). Media amended with green (<11-wk-old) CHB were only slightly suppressive and heating had no effect on the resulting incidence of damping-off. Suppression could be reestablished in heated mature CHB media by adding 10% (v/v) unheated mature CHB or 10^7 colony-forming units (CFU) of *Trichoderma harzianum* per gram of container medium. Propagules of *Rhizoctonia*

solani were killed rapidly in unheated media containing mature CHB and in heated mature CHB media amended with *T. harzianum*, but a high percentage of propagules remained viable in media amended with either green or heat-treated mature CHB. Results suggest that suppression of *Rhizoctonia* damping-off of radish in container media amended with mature CHB is induced by microbial activity, whereas the low level of suppression observed in green CHB-amended media may involve chemical inhibitors.

Several diseases caused by soilborne plant pathogens may be suppressed in container media amended with composted hardwood bark (CHB) (6,8,14,22,23,27,28). Hoitink et al (14) showed that leachates from raw (<11-wk-old) CHB are suppressive to *Phytophthora* root rot of lupine contain substances that inhibit sporangium formation and lyse zoospores of *Phytophthora cinnamomi* Rands. They suggested that microbial inhibitors play a role in suppression. Similarly, Spencer and Benson (27) reported that leachates from CHB inhibit linear growth of *P. cinnamomi* and *P. citricola* Sawada. Inhibition of these fungi, however, was not detected in 2-yr-old CHB (14). On the other hand, there is evidence that antagonists present in CHB may play a major role in the suppression of *Pythium* damping-off of tomato (22). Although the suppression of *Rhizoctonia solani* Kühn in CHB-amended container media has been studied in some detail (8,23,28), the mechanism of suppression has not been investigated.

The purpose of this study was to determine whether microorganisms were responsible for the suppression of *R. solani* by CHB.

MATERIALS AND METHODS

Batches of CHB were obtained from Paygro Inc., South Charleston, OH 45368, and various Ohio nurserymen. The effect of compost age on suppression was determined with CHB prepared in an aerated reactor (15) for 3 wk followed by additional composting in an insulated bin (1.2 × 1.8 × 2.7 m). A 15-cm-diameter flexible perforated tube was placed through the center of the composting mass (3.4 m³) to maintain aerobic conditions (15). The CHB was removed from the bin at 4-wk intervals, blended, and returned to the bin to insure that all particles were exposed to elevated temperatures (40–65 C) that developed during decomposition by thermophilic microorganisms. Prior to placing the CHB back into the bin, samples were collected and moisture levels were adjusted (50–55%; w/w).

Container media consisted of mixtures of various ages of CHB, Canadian peat (CP), and perlite (5:2:3, v/v). The effect of heating on suppression in some tests was examined in other media in which CHB was replaced with 0, 28, 50, 72, and 100% CP. The organic components (CHB and CP in these media were mixed with perlite and sharp silica sand [5:3:2, v/v]). These media were designated as 50-0 (0% CP in the organic component), 36-14 (28% CP in the organic component), 25-25 (50% CP in the organic component), 14-36 (72% CP in the organic component), and 0-50 (100% CP in the organic component). Media pH readings ranged from 5.5 to 7.1. The air-filled pore space at container capacity (10-cm-tall column) of all media ranged from 15 to 20%.

In most experiments, inoculum of *R. solani* isolate R-19 (23) was produced on a chopped potato-soil mixture (17,23). The inoculum density was determined on 2% water agar by dilution plating (3). Although this method of determining inoculum density of *R. solani* underestimates the actual inoculum density (13), it was used because a pellet-sampling device could not be used to assay populations of *R. solani* due to the large particle sizes in CHB. In some experiments, inoculum consisted of 11-mm-diameter colonized agar disks taken from 72-hr cultures grown on potato-dextrose agar (PDA) at 25 C. Celosia and radish bioassays (11,23,28) were used to determine the suppressiveness of container media.

In the Celosia assay, inoculum was placed at the head of each seedling row (two rows per flat) at seedling emergence (23,28). The mean damped-off row length after 8 days (26 C, 25,000 lux) was used to determine the suppressiveness of the container medium. In the radish assay, seed (*Raphanus sativus* 'Early Scarlet Globe,' 97% germination, 32 seeds per 10-cm-diameter pot) was placed randomly in each pot and covered with 1.0 cm container medium (11,23). 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 was determined. The causal agent of damping-off was verified routinely by plating diseased seedlings on PDA.

Trichoderma harzianum Rifai (tb-1), isolated from mature CHB, was grown on PDA at 25 C for 7 days. Colonized agar was removed from plates and stirred in 200 ml of sterile distilled water.

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The spore suspension was poured through two layers of cheesecloth and centrifuged (10,000 *g* for 10 min). Pellets were resuspended in sterile tap water (pH 6.8) and spore concentrations were determined with a hemacytometer. Spore suspensions were added to container media to yield a final concentration of 10^7 CFU/g dry wt of container medium. Samples were shaken vigorously in polyethylene bags to distribute the spores uniformly throughout the container medium.

Heat and radiation treatment of container media. Container media (2 L per polyethylene bag; 50–55% moisture) were incubated at 25, 40, or 60 C for 5 days or autoclaved at 121 C for 1 hr on 3 consecutive days. This long heating period was chosen to simulate conditions in compost piles. Media were then amended with slow-release fertilizer (23) and used immediately.

CHB samples were irradiated in polyethylene bags (1 L of CHB per bag) in a Tech/Ops Laboratory Irradiator (#600-892, Picker X-ray Corp., White Plains, NY 10600) equipped with a ^{137}Cs radiation source. Irradiation dosage rate was 6,400 Rads/hr for 43 hr, equivalent to a gamma radiation dose of 275 krad. The irradiated CHB samples were then used in container media.

Survival. Container media (100 ml) were placed on 9-cm-diameter moisture tension plates (9) and incubated in the dark at 26 C. Water column lengths were adjusted to provide a moisture tension of –22 mb. This tension was chosen because it represents the moisture tension commonly found in container media just after irrigation and drainage (container moisture capacity). Samples were equilibrated 24 hr before *R. solani* was introduced into the media. For this survival study, *R. solani* was grown on chopped potato pieces (50 g) in 1-L flasks. After 14 days (25 C), colonized potato pieces were air-dried for 48 hr in a laminar-flow hood, ground with a mortar and pestle, and sieved sequentially through 850- μm and 600- μm sieves. Inoculum remaining on the 600- μm sieve was removed and sandwiched between two layers of nylon screen (Nitex HC 3-500; Tetko Inc., Elmsford, NY 10523). Each sandwich (25 \times 25 mm) contained ~50 individual pieces of

inoculum. The nylon screen layers were stapled together to prevent loss of inoculum pieces. Sandwiches (five per tension plate) were arranged on the surface of the medium and covered with 1 cm of additional container medium. The added medium was rewetted from the bottom to reestablish the moisture tension and the plates were covered with loosely fitting polyethylene bags to reduce evaporation.

To determine the competitive saprophytic ability of *R. solani* in the various container media, fresh potato pieces were sterilized, dried, ground, sieved, and placed in sandwiches 3 cm from sandwiches containing potato pieces colonized by *R. solani*. Sandwiches were recovered after 7, 14, 21, and 28 days and rinsed for 2 min in sterile distilled water. Pieces were removed and plated on a Rhizoctonia-selective medium (17) and on acidified PDA. Percentage of recovered pieces from which *R. solani* grew was noted after 48 hr of incubation.

All experiments were replicated at least four times and were performed twice. Results were analyzed by analysis of variance and linear regression analysis where appropriate. Means were separated by using the LSD test or Duncan's new multiple range test.

RESULTS

Effect of heat and gamma radiation on suppression. Damped-off row lengths (Celosia assay) were significantly greater in unheated (25 C) media containing high levels of peat (ie, 25-25, 14-36, and 0-50) than in media containing high levels of CHB (ie, 50-0, 36-14) (Fig. 1). Damped-off row lengths after 7 days were 5.4, 7.1, 9.1, 9.6, and 11.7 cm for the 50-0, 36-14, 25-25, 14-36, and 0-50 container media, respectively. A similar relationship among media was observed after heating to 40 C. However, after heating to 60 or 121 C, suppressiveness was eliminated and damped-off row lengths among media did not differ ($P=0.05$) (Fig. 1). Similar results were

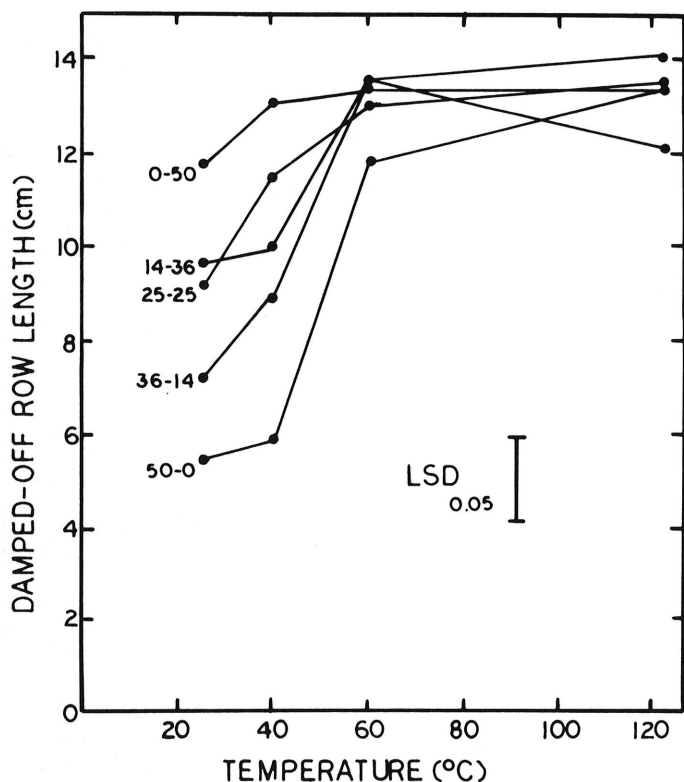


Fig. 1. Effect of heating several composted hardwood bark (CHB)-Canadian peat (CP) media for 5 days at various temperatures on Rhizoctonia damping-off of Celosia (121 C = media autoclaved for 1 hr on 3 consecutive days). 50-0 = only CHB in the organic component; 0-50 = only CP in the organic component. $\text{LSD}_{P=0.05} = 1.8$.

TABLE 1. Effect of heat and gamma radiation treatments on suppression of Rhizoctonia damping-off of radish in container media amended with composted hardwood bark (CHB)

Container medium ^w	Treatment	Damping-off (%)
Mature compost	25 C	18.6 a ^z
	60 C ^x	63.1 b
	121 C ^y	78.7 b
	275 krad	77.9 b
CP (control)	121 C ^y	97.1 c

^wMature compost contained 36% CHB (aged > 1 yr) and 14% Canadian peat (CP); remaining 50% of medium consists of neutral aggregates (ie, 30% perlite and 20% sand). CP = 50% CP, 30% perlite, 20% sharp silica sand.

^xDry heat for 5 days.

^yAutoclaved for 1 hr on 3 consecutive days.

^zNumbers followed by the same letter are not significantly different ($P = 0.05$) according to the Duncan's new multiple range test.

TABLE 2. Reestablishment of a Rhizoctonia-suppressive factor in media prepared with heated mature composted hardwood bark (CHB) by the addition of 10% (v/v) unheated mature CHB

Container medium ^x	Treatment	Damping-off (%)
Mature CHB	Unheated	32.4 a ^z
	60 C ^y + 10%	
	Unheated	31.5 a
	60 C ^y	51.0 b
CP (control)	Unheated	70.3 c

^xMature CHB medium contained 36% CHB aged > 1 yr and 14% Canadian peat (CP); remaining 50% of medium consisted of neutral aggregates (ie, 30% perlite and 20% sand). Medium CP contained 50% CP, 30% perlite, and 20% sand.

^yDry heat for 5 days.

^zNumbers followed by the same letter are not significantly different ($P = 0.05$) according to the Duncan's new multiple range test.

obtained when these experiments were repeated.

Effects of heating on suppression detected with the Celosia assay were similar to those in the radish assay (Table 1). A low level of damping-off (18.6%) was observed in the unheated CHB medium after 7 days. However, heating to 60 or 121 C followed by inoculation 2 days after these treatments resulted in significantly greater amounts of damping-off (63.1 and 78.7%, respectively).

Treatment of the CHB with 275 krad of gamma radiation resulted in a significant increase in damping-off to levels equivalent to those observed after heat treatments (Table 1). Disease incidence in the autoclaved CP medium was significantly greater than that in autoclaved or gamma-irradiated CHB medium. Similar data were obtained when these experiments were repeated.

Reestablishment of suppression. Addition of 10% (v/v) unheated CHB to a heated (60 C) CHB-CP medium (36-14) restored suppression levels equivalent to those observed in unheated CHB (Table 2). Incubation of the heated container medium amended with 10% unheated mature CHB for 7 days resulted in a damping-off level of 31.5% compared with 32.4% in the unheated control. The highest levels of damping-off were again observed in the CP (0-50) medium. The addition of inoculum of *T. harzianum* tb-1 effectively restored suppression to heat-treated CHB (Fig. 2). Heating (60 C) increased the incidence of damping-off from 25.2 to 65.2%. Addition of isolate tb-1 restored suppressiveness to levels similar to those in the unheated CHB. Addition of this isolate to unheated mature CHB did not significantly alter the resulting level of damping-off (Fig. 2).

Effect of composting time on suppression. CHB, regardless of age, was more suppressive to *R. solani* than was CP (Fig. 3). After 3 wk of composting, percentages of damping-off were 66.1 and 96.1% in the CHB and CP media, respectively. Similar levels of damping-off were observed after composting for 7 wk. After 11 wk, however, a significant increase in suppression was observed over that observed at 7 wk. Percentages of damping-off after 7 and 11 wk were 65.7 and 23.2%, respectively. Further composting (up to 23 wk) did not significantly increase the level of suppressiveness over that observed at 11 wk.

Heat treatment (60 C) did not significantly reduce the level of suppression observed after 3 and 7 wk of composting (Fig. 3). However, significant increases in damping-off were observed (after heating) in samples collected after 11, 15, 19, and 23 wk of

composting. For example, percentages of damping-off in CHB samples composted 23 wk were 18.6 and 88.0% in unheated (25 C) and heated (60 C) samples, respectively. Similar results were obtained with a second batch of compost.

Survival. A significantly greater percentage of propagules of *R. solani* remained viable during the 28-day period in container media prepared from green CHB (3-wk-old) than in any other medium (Fig. 4A). Percentages of surviving propagules did not differ significantly in media prepared from mature CHB (> 1 yr old) and in *Trichoderma*-amended mature CHB at any time during the experimental period. Rates of decline in propagule viability were significantly greater in media amended with mature or *Trichoderma*-amended CHB than in green or heat-treated CHB media (Fig. 4B). The time required for a 50% loss in propagule viability (TS₅₀, estimated from regression lines) was 2, 3, 6, and 12 days for the *Trichoderma*-amended, mature, heat-treated, and green CHB media, respectively. TS₅₀ did not differ (*P* = 0.05) between the *Trichoderma*-amended and mature CHB-amended media. However, the TS₅₀ for the heat-treated CHB was significantly greater (*P* = 0.05) than those for the *Trichoderma*-amended and mature CHB media, but significantly less than that for media amended with green CHB. Individual propagule pieces could no longer be detected after 21 days in mature or *Trichoderma*-amended CHB media. However, individual pieces could be detected after 28 days in media amended with green or heat-treated CHB.

Uninfected potato pieces incubated in media amended with green and heat-treated CHB and placed 3 cm from potato pieces infected with viable *R. solani* became colonized by *R. solani* (Table 3). After 7 days, 93 and 89% of the pieces were colonized by *R. solani* in media amended with green or heat-treated CHB, respectively. On the other hand, *R. solani* was not isolated from potato pieces incubated 7 days in media amended with mature or *Trichoderma*-amended CHB. Similar results were obtained in repeated experiments.

DISCUSSION

Biocidal treatments such as heat (60 C) and gamma radiation (275 krad) destroyed suppressiveness to Rhizoctonia damping-off

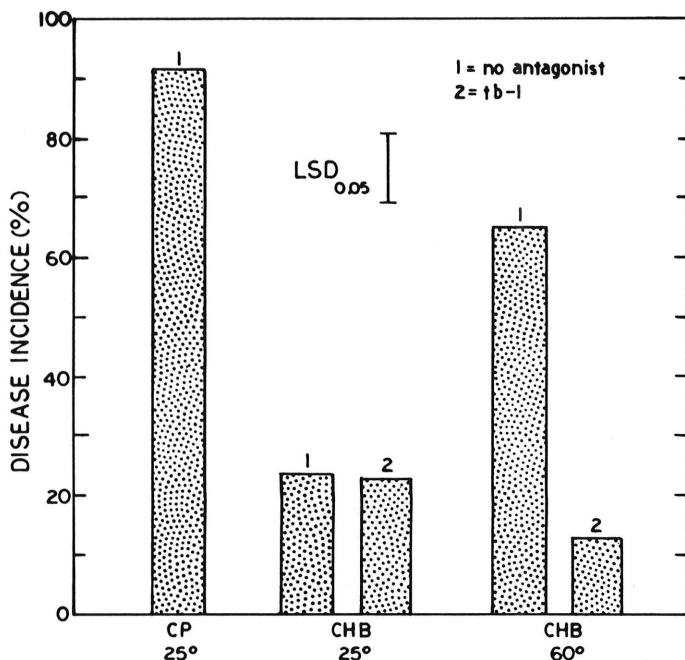


Fig. 2. Effect of *Trichoderma harzianum* (isolate tb-1) on the suppression of Rhizoctonia damping-off of radish in heated and unheated composted hardwood bark. Canadian peat included as a standard. LSD_{*P* = 0.05} = 11.7.

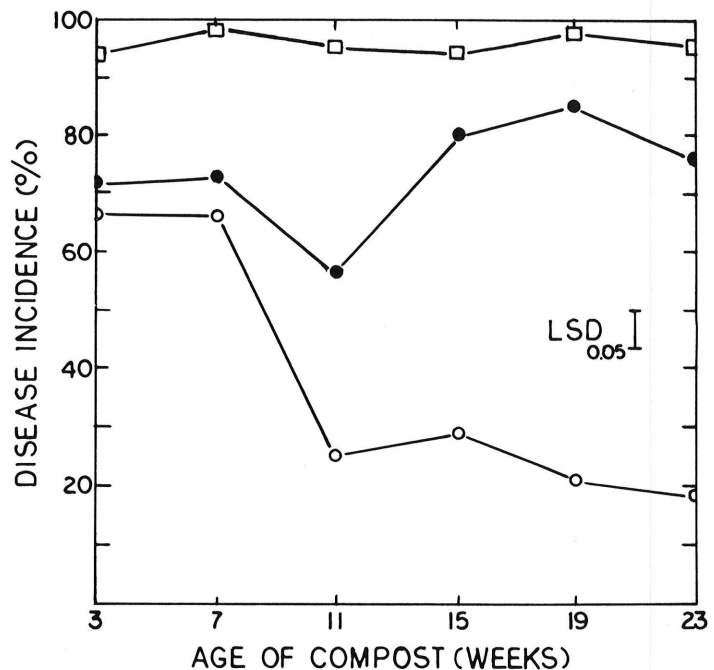


Fig. 3. Effect of compost age on the suppression of Rhizoctonia damping-off of radish. ○—○ = unheated composted hardwood bark (CHB); ●—● = heated (60 C) CHB; □—□ = Canadian peat standard. LSD_{*P* = 0.05} = 8.1.

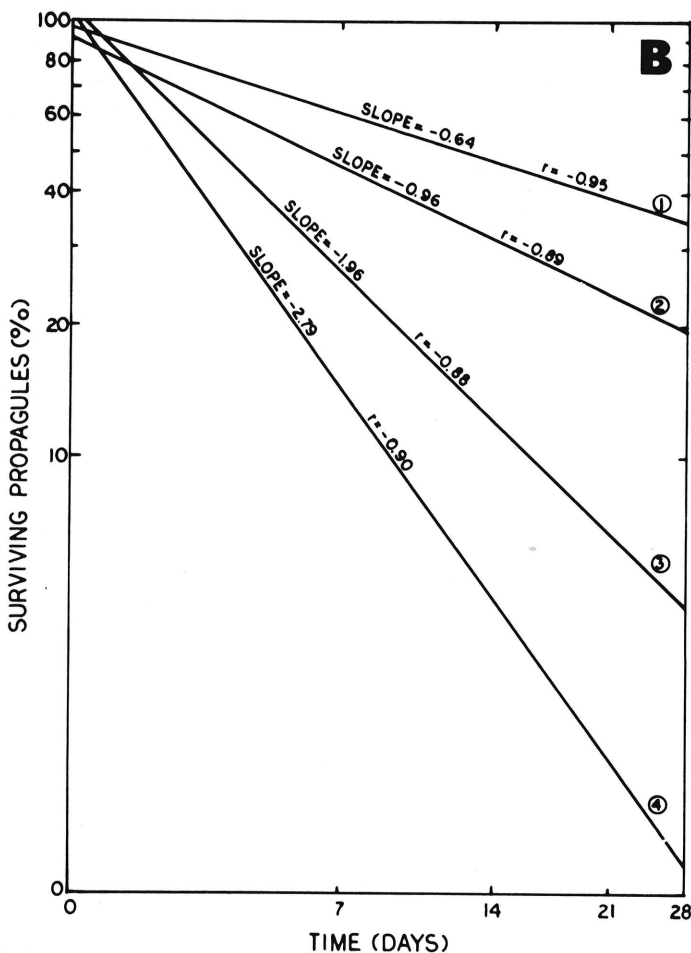
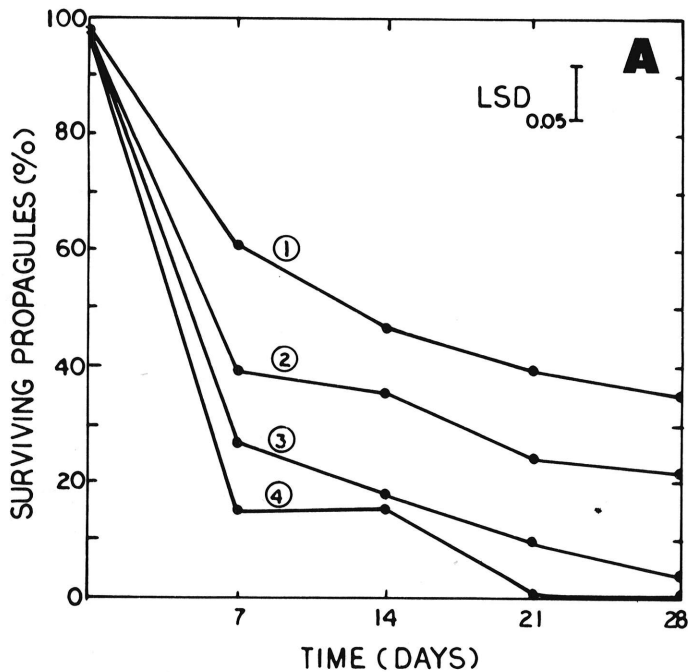


Fig. 4. Survival of *Rhizoctonia solani* at 26 C and -22 mb moisture tension in media prepared from various types of composted hardwood bark (CHB). 1 = green CHB; 2 = heated (60 C) mature CHB; 3 = unheated mature CHB; 4 = heated (60 C) mature CHB recolonized with *Trichoderma harzianum* (isolate tb-1): A, arithmetic plot of raw data; LSD_{p=0.05} = 10.6. B, log_e-log_e transformed plot of predicted survival based on regressing log_e (survival) on log_e (time).

TABLE 3. Colonization of potato pieces by *Rhizoctonia solani* in container media amended with different types of composted hardwood bark (CHB)

HB type ^w	Colonization ^x (%)
Green CHB	92.8 a ^z
Heat-treated mature CHB	89.3 a
Mature CHB	0.0 b
Mature CHB amended with <i>Trichoderma</i> ^y	0.0 b

^wMedia consisted of 70% organic components and 30% perlite, green CHB (composted 3 wk), mature CHB (composted >1 yr).

^xPercentage of potato pieces containing viable *Rhizoctonia solani* after 7 days of incubation.

^y10⁷ CFU of *Trichoderma harzianum* (isolate tb-1) per gram dry wt of container medium.

^zNumbers followed by the same letter are not significantly different ($P = 0.05$) according to the Duncan's new multiple range test.

in media amended with mature CHB or mixtures of mature CHB and CP. These treatments also reduced the numbers of microorganisms isolated by dilution plating procedures (E. B. Nelson, *unpublished*). The reduced microbial population following heat and gamma radiation treatments suggests that suppressiveness in mature CHB-amended media may be due to microorganisms. Furthermore, the addition of small amounts of suppressive CHB to media amended with heated conducive CHB conferred suppressiveness. The amount of suppressive CHB added was too small to explain the observed effect on suppression alone (23), suggesting that the microorganisms responsible for suppression multiplied in the conducive CHB-amended media. Similar phenomena have been observed with biologically suppressive soils (12,20,21,25).

Compared to CP media, green CHB-amended media were only slightly suppressive to *Rhizoctonia* damping-off. Heating to 60 C did not destroy this effect. Similar results on heating of green and mature CHB have been obtained with *Fusarium* wilt of flax (6) and *Phytophthora* root rot of lupine (H. A. J. Hoitink, *unpublished*). Green CHB, therefore, may not be a suitable substrate for microorganisms involved in suppression or these microorganisms simply are not present in green CHB due to initially high temperatures during composting.

As the CHB in this study aged, it became more suppressive to *Rhizoctonia* damping-off. The most noticeable increase in suppression occurred after 11 wk of composting. It is well known that physical and chemical properties of composts change with age (5,18,19,24,26). These changes are accompanied by successions of microorganisms (1,2,4,24,29). After peak heating (15,24), compost is recolonized by a diverse microbial community (24,29). Many of these are antagonists (E. B. Nelson, G. Kuter, and H. A. J. Hoitink, *unpublished*). It is not surprising, therefore, that suppression of *Rhizoctonia* damping-off increased (from 66.1 to 18.6% disease incidence) as the CHB aged. In mature CHB, *Trichoderma* spp. are quite abundant (15) and their hyperparasitic nature is well known (7,10). The similarity in survival curves between the mature and *Trichoderma*-amended CHB may indicate that hyperparasitic microorganisms such as *Trichoderma* spp. may play a major role in the suppressiveness of media amended with mature CHB.

Attempts have been made in the past to artificially recolonize compost with specific antagonists to produce a predictably suppressive compost (16,22). The results were inconsistent, however. In this study, recolonization of mature CHB with *T. harzianum* yielded consistently suppressive composts with levels of suppression equal to or better than suppressive "natural" CHB. Much of the variability experienced in previous studies (16,22) also was eliminated. The behavior of *Trichoderma* spp. and other microbial populations during recolonization of CHB and their role in the suppression of *R. solani* is currently being investigated.

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