Disease Control and Pest Management

Interactions Between Thermophilic Fungi and
Trichoderma hamatum in Suppression of Rhizoctonia Damping-Off
in a Bark Compost-Amended Container Medium

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ABSTRACT

Chung, Y. R., and Hoitink, H. A. J. 1990. Interactions between thermophilic fungi and *Trichoderma hamatum* in suppression of Rhizoctonia damping-off in a bark compost-amended container medium. Phytopathology 80:73-77.

The ability of *Trichoderma hamatum* to induce suppression of Rhizoctonia damping-off in a container medium prepared with mature, composted, hardwood tree bark removed from various temperature zones in compost piles varied significantly. Efficacy of the biocontrol agent in medium amended with compost from a 40–50 C zone was reduced significantly as compared with that of the medium containing higher-temperature composts. Population densities of *T. hamatum* or *Rhizoctonia solani* did not differ in the medium prepared with compost from the various temperature zones. Population densities of thermophilic bacteria and fungi were significantly higher in the 40–50 C compost. *Humicola* spp. were the predominant fungal taxa isolated from compost in this temperature zone, but they were unable to grow on potato-dextrose agar (PDA) at 25 C. Some isolates of *Humicola* significantly reduced efficacy but not population densities of *T. hamatum* in the medium. Bacteria isolated from the 40–50 C zone did not reduce efficacy of the biocontrol agent. Damping-off was suppressed by *T. hamatum* if the medium containing 40–50 C compost or compost fortified with *Humicola* spp. was incubated for three or more weeks at 25 C before bioassays were performed. We propose that some thermophilic fungi naturally present in 40–50 C compost temporarily interfered with the biocontrol activity of *T. hamatum* after compost-amended medium was first prepared.

Suppression of diseases caused by *Rhizoctonia solani* Kühn with *Trichoderma harzianum* or *T. hamatum* (Bonord.) Bain. as biocontrol agents has been reported for field and ornamental crops (1,6–10,18). However, *Trichoderma* spp. are not effective in all soil environments (11,12). Hubbard et al (12) showed that *T. hamatum* failed to protect pea seeds from rots caused by *Pythium* spp. in New York soils low in available iron (<4 μg of Fe/g of soil). They suggested that fluorescent pseudomonads present on the seed coat inhibited growth of *T. hamatum*. Other reports showed that *R. solani* was not suppressed in field soils, even in the presence of a high population density of *Trichoderma* (11,17). In bark-compost medium infested with *Trichoderma* spp., variability in efficacy also occurs. Propagules of *R. solani* were not eradicated from, nor was Rhizoctonia damping-off suppressed in, a container medium amended with fresh hardwood bark, despite a high population density of isolate 382 of *Trichoderma hamatum*, a specific biocontrol agent of *R. solani* (20). Conversely,

in medium amended with mature composted hardwood bark, lower population densities of the antagonist developed, but the disease was suppressed and the pathogen was eradicated (20).

More recently, Chung et al (3) showed that the cellulose concentration in a bark-compost medium affects the inoculum potential of *R. solani* and therefore affects disease severity even in the presence of a microflora capable of inducing suppression. Finally, Chung et al (4) showed that even fresh hardwood bark was rendered suppressive to Rhizoctonia damping-off provided that adequate time was allowed for the antagonist, i.e., *Trichoderma*, to fully colonize the substrate before *R. solani* was introduced. A high positive correlation existed between the population of *Trichoderma* and disease severity only if precolonization occurred.

In this study, we examine interactions between *T. hamatum* and thermophilic fungi present in mature bark composts and the effects of the interactions on suppression of Rhizoctonia damping-off in a compost-amended container medium.

MATERIALS AND METHODS

Preparation of container medium. A compost-amended container medium was prepared by mixing composted hardwood bark, Canadian sphagnum peat, and perlite (5:2:3) as described previously (16). The air-filled pore space of this container medium ranged from 15 to 20% at container capacity (10-cm tall column), and its pH ranged from 5.5 to 6.4. Slow-release fertilizer Osmocote (14-14-14, 3–4 mo release) (Sierra Chemical Co., Milpitas, CA) was added at 17.5 g/L of the container medium immediately before bioassays were initiated. Compost (12–16 wk old) was removed from varying temperature zones within compost piles. Care was taken to reduce contamination from zone to zone. The arbitrarily chosen temperature zones were: the outer zone of the pile, with a temperature <40 C; two zones deeper into the pile, with temperatures of 40–50 C and 50–60 C; and the center zone, with a temperature >60 C (Fig. 1). Container medium prepared with low-temperature edge composts (<40 C) or high-temperature center composts (>60 C) were suppressive and conducive to Rhizoctonia damping-off, respectively (19).

Bioassay. The ability of isolate 382 of *T. hamatum* to induce suppression of Rhizoctonia damping-off was determined with a radish (*Raphanus sativus* L. 'Early Scarlet Globe'; 97% germination) bioassay. Inoculum of *R. solani*, produced in a chopped-potato and soil mixture (13), was air dried and sieved to yield 1- to 2-mm soil inoculum pieces (19). Container medium was infested with 0.4 g of *Rhizoctonia* soil inoculum per 1 L of medium. *T. hamatum* was added at an initial population density of 2×10^5 colony-forming units (cfu) per gram dry weight of container medium as described previously (16). The container medium then was distributed in pots and seeded with radish as described previously (16). Control treatments were medium without *R. solani* and/or isolate 382 of *T. hamatum*. Pots were incubated in a growth chamber, as described previously (16), and watered daily. After 7 days, seedlings were rated on a disease severity scale where 1 = symptomless, 2 = diseased but not damped-off, 3 =
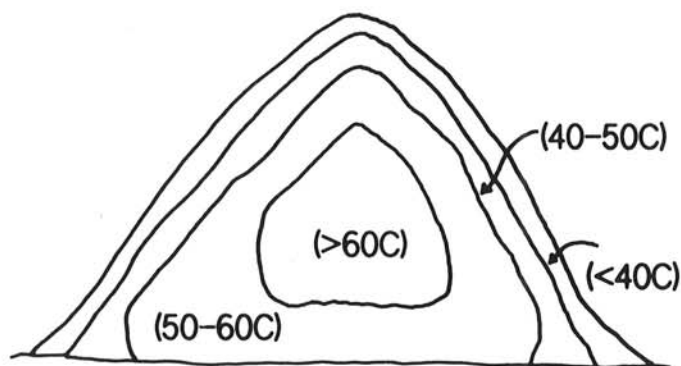
postemergence damping-off, and 4 = preemergence damping-off. After the first disease harvest, the container medium was replanted with radish, and disease severity was rated again after another 7 days. Mean disease severity was based on five replicates of 32 plants each. Completely randomized designs were used in all bioassays. One-way analysis of variance was performed with the MINITAB computer program. Separations of means were based on least significant difference at $P = 0.05$. The entire experiment was performed six times with four different batches of compost over a 3-yr period.

Microbial activity. Microbial activity in the bark-compost medium prepared with composts from the four temperature zones was determined by measuring the rate of hydrolysis of fluorescein diacetate (FDA) (21). Triplicate 5-g (wet weight) container medium samples were placed in 250-ml Erlenmeyer flasks containing 20 ml of potassium phosphate buffer (60 mm, pH 7.6). One hundred microliters of FDA stock solution (2 mg/ml of acetone) was added to the compost-buffer suspension, and the mixture was shaken on a rotary shaker for 20 min at 25 C. The reaction was stopped by adding 20 ml of acetone to the mixture, and then the mixture was filtered through a prepleated filter paper to remove container medium residues. Absorbance of the filtrate was measured at 490 nm to determine the concentration of fluorescein; the reaction mixture without FDA was run as a control for each sample. Standard curves were prepared for each container-compost medium with 0, 5, 10, and 15 μg of hydrolyzed FDA per mililter of buffer solution (2). Microbial activity was expressed as micrograms of hydrolyzed FDA per gram dry weight of container medium per minute. One-way analysis of variance was performed as described above for bioassays. This experiment was repeated once.

Microbial populations. Population densities of thermophilic and mesophilic bacteria, fungi, and actinomycetes in compost samples from the four temperature zones were measured on diluted (1/10) trypticase soy broth agar (TSA), on PDA amended with 50 mg of streptomycin sulfate and 50 mg of chloramphenicol, and on colloidal chitin agar, respectively. All plates were incubated at temperatures corresponding to the mean compost temperatures (25, 45, 55, and 65 C) of the zones from which the samples had been removed. All colonies were counted after 5 days of incubation. Means were based on three dilution plates, for four dilution series for each group of microorganisms.

Fungal colonies isolated from compost removed from the 40–50 C temperature zone were purified on PDA (incubated at 45 C) and identified to genus. The mean relative density (percent) of the predominant fungal taxa isolated from this zone was determined with procedures described for bark compost by Kuter et al (15). Bacterial colonies recovered on diluted (1/10) TSA were purified and stored at 4 C as described previously (16). This entire experiment was repeated once.

Interactions between thermophilic microorganisms and *T. hamatum*. The effect of various thermophilic fungi and bacteria isolated from the 40–50 C zone on the antagonistic activity of *T. hamatum* was determined in the edge-compost container medium rendered conducive by autoclaving (30 min at 120 C). All thermophilic fungi were grown for 7 days at 45 C on PDA. Spores and hyphae scraped from the surface of the medium in plates (two plates per isolate) were suspended in 20 ml of sterile distilled water. Thermophilic bacterial cultures were grown for 2 days at 45 C on diluted (1/10) TSA. Bacterial colonies then were scraped from the surface of the medium in plates (one plate per isolate) and suspended in 20 ml of sterile distilled water. Each suspension was poured into a 2-L batch of the autoclaved bark compost medium in a polyethylene bag and mixed vigorously. Infested media were incubated for 7 days at 45 C. Thereafter, each bag was incubated for 2 hr at 25 C and then infested with isolate 382 of *T. hamatum* and with *Rhizoctonia* soil inoculum, as described above. Damping-off bioassays were performed and analyzed as described above. Control treatments were media not infested with a thermophilic fungus but infested or not infested with isolate 382 of *T. hamatum* and/or *R. solani*. Each bioassay was performed twice with each thermophilic fungal and bacterial

Fig. 1. Diagram illustrating temperature zones in a bark compost pile.

isolate, according to the procedure described above.

Population development of T. hamatum and R. solani. Population densities of *Trichoderma* were determined by the dilution plate method with a modified selective medium (5). The medium contained 3.0 g of glucose, 1.0 g of NH_4NO_3, 0.9 g of K_2HPO_4, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.15 g of KCl, 20 mg of $FeSO_4 \cdot 7H_2O$, 20 mg of $MnSO_4 \cdot H_2O$, 20 mg of $ZnSO_4 \cdot 7H_2O$, 30 mg of Rose bengal, and 20 g of agar per liter of distilled water. After autoclaving, 50 mg of chloramphenicol, 50 mg of streptomycin sulfate, 10 mg of metalaxyl (25.1% ai), and 0.1 g of PCNB (75% a.i.) were added. Plates were incubated 3 days at 25 C. Three subsamples were assayed from each container medium. For each treatment and incubation time, means and standard errors were calculated. The assays were performed twice.

The mean population density of *R. solani* was determined as described previously (3). Container medium samples were air dried for 24 hr at 25 C, and a 1-g subsample was added to 10 ml of a warm (50 C) selective agar medium and dispensed into a petri dish (three subsamples per treatment). After the agar had solidified, 15 disks were removed from plates with a 6-mm-diameter cork borer and placed on 2% water agar in a petri dish. Plates were incubated for 24 hr at 25 C, and growth of *R. solani* from disks was rated where 0 = no growth, 0.3 = 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. Ratings for all disks on a plate were then added and multiplied by the 14.3 correction factor as described previously (3) to account for the remainder of the 1.0 g of infested sample suspended in agar that was not plated out as disks. The mean number of colony-forming units per gram of air-dried container medium was based on three subsamples per treatment. For each treatment and incubation time, means and standard errors were calculated. The assay was performed twice.

RESULTS

Efficacy of T. hamatum in container medium amended with composts from various temperature zones. Container medium prepared with composted bark removed from the four temperature zones differed in suppressiveness to Rhizoctonia damping-off (Fig. 2). Disease severity in the medium infested with *R. solani* only and prepared with composts from the 40–50 C, 50–60 C, and center (>60 C) zones of a compost pile was significantly ($P = 0.05$) higher than that of the medium prepared with compost from the edge (<40 C) of the pile. Disease severity in the conducive
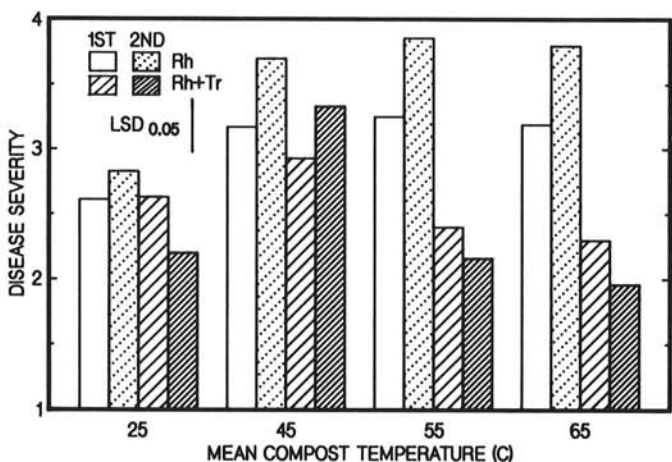
medium not infested with *T. hamatum* increased significantly ($P = 0.05$) with replanting; the edge (<40 C) medium remained suppressive (Fig. 2).

The addition of *T. hamatum* to container medium amended with composted bark from the 50–60 C and center (>60 C) zones induced a moderate reduction in disease in both the first and second plantings. However, isolate 382 of *T. hamatum* was consistently less or not effective in the medium prepared with bark compost from the 40–50 C zone. Disease severity in the edge compost (<40 C) medium was consistently low in all treatments (Fig. 2). Results were consistent in all six trials with the four different bark compost piles over a 3-yr period.

Trichoderma populations. Population densities of *T. hamatum* consistently increased significantly ($P = 0.01$) with incubation time in all bark compost treatments. Results of one experiment are presented in Figure 3. After 1 wk, population densities of *T. hamatum* reached 1.8×10^6 cfu/g dry weight in a medium containing compost from all the high-temperature zones (>40 C); no significant differences in population densities among these treatments were found. However, in the edge-compost medium (<40 C) that was colonized by a mesophilic microflora, the total population density of *Trichoderma* was significantly ($P = 0.05$) lower (4.4×10^5 cfu/g dry weight) than that of all other treatments after 6 days of incubation.

Rhizoctonia populations. Population density of *R. solani* did not differ significantly in samples of medium amended with composts from the three high-temperature zones (>40 C) (Fig. 4). Population density in the edge-compost medium could not be determined because of interference by the population of *Trichoderma* spp. naturally present (14). Population trends observed in a second experiment were the same.

Microbial activity. Microbial activity in the edge-compost (<40 C) bark medium was significantly higher ($P = 0.05$) than that in the 50–60 C compost medium but was not significantly different from that in the 40–50 C compost medium (Table 1). No microbial activity was detected in the high-temperature center (>60 C) compost medium. In a second experiment, similar values were obtained.

Microbial populations. Population densities of both mesophilic and thermophilic bacteria were significantly higher in the edge (<40 C) and 40–50 C compost samples than in the higher-temperature zones (>50 C) (Table 2). Population densities of thermophilic fungi in the 40–50 C zone were significantly ($P = 0.05$) higher than those in the higher temperature (50–60 C) zone. Mesophilic fungi were most abundant in the edge compost (<40 C) zone. Population densities of thermophilic actinomycetes were found in all high-temperature zones (>40 C) and were lowest

Fig. 2. Ability of isolate 382 of *Trichoderma hamatum* to induce suppression of Rhizoctonia damping-off in a bark compost medium containing compost removed from various temperature zones. Rh, infested with soil inoculum of *R. solani* (0.4 g/L) only; Rh+Tr, infested with inoculum of *R. solani* and isolate 382 of *Trichoderma hamatum* (2×10^5 cfu/g dry weight). Disease severity, on a scale of 1 to 4, where 1 = symptomless, 2 = diseased but not damped-off, 3 = postemergence damping-off, and 4 = preemergence damping-off, was rated after first bioassay (1ST) and after replanting (2ND), respectively.
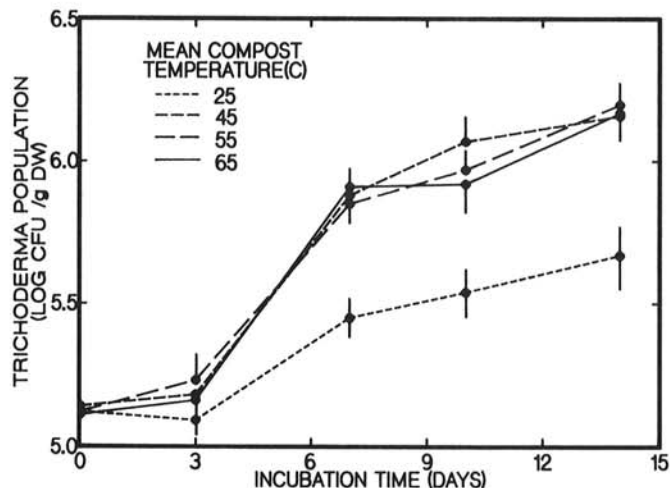

Fig. 3. Population density of isolate 382 of *Trichoderma hamatum* in bark-compost media prepared with compost removed from four temperature zones in a compost pile. Population density in the edge compost medium (25 C) represents the sum of the population density of naturally occurring *Trichoderma* spp. plus that of isolate 382 of *T. hamatum*. Bars indicate standard errors.

in the 40–50 C zone. Mesophilic actinomycetes were highest in the 40–50 C zone.

Humicola spp. were isolated most frequently from the 40–50 C zone, with a mean relative frequency of 75.3% among 129 fungal isolates obtained in one experiment (Table 3); similar values were found in a second experiment. *Penicillium, Mucor, Aspergillus,* and other unidentified fungi also were isolated, but at lower frequencies. Thermophilic isolates of *Humicola* spp. obtained from the 40–50 C zone, identified on PDA at 45 C, were not able to grow at 25 C on PDA during a 14-day incubation period.

Effect of selected thermophilic microorganisms on the activity of *T. hamatum*. None of the bacterial isolates obtained from the 40–50 C zone reduced the efficacy of isolate 382 of *T. hamatum* in paired radish bioassays. Although actinomycetes were obtained from this temperature zone, they were not evaluated for their effects on *T. hamatum*. Among 40 thermophilic fungal isolates tested in radish bioassays, several isolates of *Humicola* spp. and one isolate of *Penicillium* significantly ($P = 0.05$) reduced the efficacy of isolate 382 of *T. hamatum* in suppression of Rhi-

zoctonia damping-off in the autoclaved bark-compost medium (Table 4). The population density of *T. hamatum* increased from 2×10^5 to 7×10^6 cfu/g dry weight by the end of the 7-day period when paired in bioassays with the isolates of *Humicola* spp. or *Penicillium* sp. This final population density was not significantly different ($P = 0.05$) from that of controls not infested with the isolates of *Humicola* spp. or *Penicillium* sp.

To examine the long-term effect of isolates of *Humicola* spp. on biocontrol efficacy of *T. hamatum*, samples of medium containing a compost infested with a *Humicola* isolate and the biocontrol agent were incubated at 25 C for 3 wk before radish
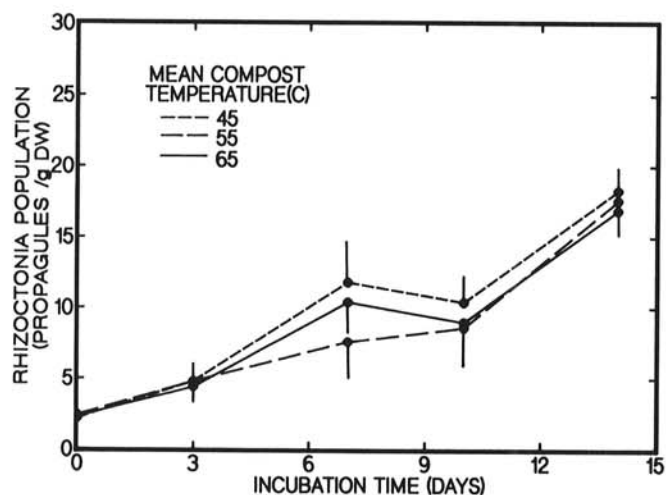

Fig. 4. Population density of *Rhizoctonia solani* in bark-compost media prepared with compost removed from four temperature zones of a compost pile. Bars indicate standard errors.

TABLE 3. Mean relative frequency of fungal taxa in bark compost removed from the center edge zone (40–50 C) of a compost pile

Fungi	Relative frequency (%)[a]
Penicillium spp.	15.5
Humicola spp.	75.3
Aspergillus spp.	4.8
Mucor spp.	1.0
Other	3.4

[a] Based on a total of 129 fungal isolates.

TABLE 4. Effect of selected thermophilic fungi isolated from the center edge layer (40–50 C) of compost piles on the ability of isolate 382 of *Trichoderma hamatum* to suppress Rhizoctonia damping-off of radish in an autoclaved bark-compost container medium

Fungal isolate	Inoculum of *Rhizoctonia*[a]	Inoculum of *Trichoderma*[b]	Disease severity[c]	Population density of *Trichoderma*[d]
...	−	−	1.1	−
...	+	−	3.3	−
...	+	+	2.7	7.0
Humicola #4	+	−	3.2	−
Humicola #4	+	+	3.1	6.9
Humicola #5	+	−	3.2	−
Humicola #5	+	+	3.4	6.9
Humicola #7	+	−	3.2	−
Humicola #7	+	+	3.4	6.9
Penicillium #10	+	−	3.4	−
Penicillium #10	+	+	3.3	6.9
LSD$_{0.05}$			0.4	0.2

[a] Infested with 0.4 g of *Rhizoctonia* soil inoculum per 1 L of container medium.
[b] Infested with 2×10^5 cfu of *T. hamatum* 382 per gram dry weight of container medium.
[c] Mean disease severity of five replicates (32 plants per replicate) according to the rating scale: 1 = symptomless; 2 = diseased but not damped-off; 3 = postemergence damping-off; and 4 = preemergence damping-off.
[d] Log cfu/g dry weight of container medium; determined 7 days after incubation at 25 C. Treatments not assayed for population density of *Trichoderma* are identified with a minus sign.

TABLE 1. Microbial activity in container medium samples amended with composted bark removed from four temperature zones of a compost pile

Compost temperature (C)	Microbial activity[a]
<40	2.17 ± 0.35
40–50	1.94 ± 0.41
50–60	1.53 ± 0.26
>60	0.00
LSD$_{0.05}$	0.51

[a] Represents μg of fluorescein diacetate hydrolyzed at 25 C min^{-1} g^{-1} dry weight of container medium, plus or minus the standard error.

TABLE 2. Microbial populations in bark-compost media prepared with composts removed from four temperature zones in a compost pile

Compost temperature (C)	Population (log cfu/g dry weight)					
	Bacteria		Fungi		Actinomycetes	
	CT[a]	RT[b]	CT	RT	CT	RT
<40	...	7.3 ± 0.1[c]	...	5.5 ± 0.2	...	4.5 ± 0.1
40–50	7.3 ± 0.1	7.0 ± 0.2	4.9 ± 0.1	4.4 ± 0.1	5.5 ± 0.1	5.8 ± 0.2
50–60	6.7 ± 0.2	6.8 ± 0.1	4.1 ± 0.1	ND[d]	6.1 ± 0.1	4.4 ± 0.1
>60	6.3 ± 0.1	6.6 ± 0.1	ND	ND	6.1 ± 0.2	3.9 ± 0.3
LSD$_{0.05}$	0.2		0.4		0.3	

[a] CT = Population densities determined after 5 days of incubation at mean compost temperature.
[b] RT = Population densities determined after 5 days of incubation at room temperature (25 C).
[c] Mean population densities followed by standard deviation, based on three replicates per treatment.
[d] Population density <10^2 cfu/g dry weight.

bioassays were performed. Disease severity in the medium infested with isolate 382 of *T. hamatum* was 2.5 and not significantly different from that in the medium infested with *Humicola* spp. and isolate 382 of *T. hamatum*. The medium, therefore, was suppressive. Disease severity in the conducive controls (not infested with *Humicola* or isolate 382 of *T. hamatum*) was significantly ($P = 0.05$) higher, ranging from 3.1 to 3.3. In a second experiment, similar differences were observed.

DISCUSSION

Container media prepared with compost removed from the high-temperature center of the pile, whether hardwood bark compost or sewage sludge compost, are conducive to Rhizoctonia damping-off of radish (14,19). Infestation of such conducive media with an effective biocontrol agent such as isolate 382 of *T. hamatum* induces suppression to Rhizoctonia damping-off (15,19). However, efficacy of strains of *Trichoderma* in a conducive bark-compost medium is not consistent. Kwok et al (16) showed that efficacy is improved if a mixture of antagonists is introduced. The reduced ability of *T. hamatum* to induce suppression in the 40–50 C zone (Fig. 2) as compared with its ability in compost of higher temperatures may explain part of the variability obtained when *T. hamatum* is used as the sole antagonist.

Lack of significant differences in population densities of *T. hamatum* among media prepared with 40–50 C, 50–60 C, and center (>60 C) compost suggests that reduced efficacy was not a result of growth inhibition of *T. hamatum* in the 40–50 C medium. Population density of *R. solani* in the three high-temperature compost-amended media also did not differ significantly (Fig. 4). This indicates that the greater disease severity in the 40–50 C compost medium was not a result of improved pathogen growth but was caused by some other factor(s).

Microbial activity in the 40–50 C compost medium, which did not differ significantly from that in the 50–60 C medium, indicated that the reduced efficacy of *T. hamatum* was not attributable to altered general microbial activity (Table 1). Rather, it suggests that the reduced efficacy of *T. hamatum* could be caused by a specific subcomponent of the microflora in that temperature zone.

Although significantly higher population densities of both thermophilic bacteria and fungi were found in the 40–50 C zone than in the other high-temperature zones (Table 2), bacterial isolates from this zone did not negate the efficacy of *T. hamatum* as a biocontrol agent. Conversely, some isolates of thermophilic fungi, including isolates of *Humicola* spp., which predominated in the 40–50 C zone and were most effective in reducing efficacy of *T. hamatum*, played perhaps the greatest role in variability encountered with this biocontrol agent.

The decrease in disease severity with incubation in the 40–50 C bark-compost medium infested with *T. hamatum* and fortified with *Humicola* spp. suggests that the phenomenon is of short duration. It may partially explain the 3- to 4-wk period required to induce consistently high levels of suppression to Rhizoctonia damping-off in a container medium containing a mixture of composts from all temperature zones of a pile (14).

Most thermophilic fungi isolated from the 40–50 C zone were identified to genus (Table 3). Species diversity in this zone was much less than that of the low-temperature edge compost, as described by Kuter et al (15). *Humicola* spp., isolated most frequently from the 40–50 C zone, were unable to grow at 25 C. This suggests that the *Humicola* spp. did not grow in the 40–50 C compost medium during the disease severity bioassay because the prevailing temperature in this bioassay was 25 C. Possibly, these thermophilic fungal isolates, which were no longer in an optimal environment for growth, served as a better food base for *T. hamatum* than the active propagules of *R. solani*. The

result would be decreased hyperparasitism of *R. solani* and the observed increase in disease severity.

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