Effects of Fungal Antagonists and Compost Age on Suppression of Rhizoctonia Damping-Off in Container Media Amended with Composted Hardwood Bark

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Journal Series Article 6-83.

Supported in part by grants from the Ohio Florists' Association and The Fred C. Gloeckner Foundation, Inc., 15 E. 26th Street, New York, NY 10010.

Salaries and research support provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

The authors are indebted to C. A. Musselman for technical assistance.

Accepted for publication 18 May 1983.

ABSTRACT

Nelson, E. B., Kuter, G. A., and Hoitink, H. A. J. 1983. Effects of fungal antagonists and compost age on suppression of Rhizoctonia damping-off in container media amended with composted hardwood bark. Phytopathology 73:1457-1462.

A total of 331 fungal isolates from container media amended with composted hardwood bark (CHB) were tested for their ability to suppress Rhizoctonia damping-off. Members of the genera *Trichoderma, Gliocladium, Penicillium, Mortierella, Paecilomyces, Geomyces*, and *Ophiostoma* were among the most effective fungi. Isolates of *Trichoderma hamatum* and *Trichoderma harzianum*, the most abundant taxa isolated from suppressive container media, were generally the most efficaceous antagonists. However, isolates of *Trichoderma* and other genera obtained from conducive media were also capable of restoring suppression to heated media (60 C) amended with mature CHB. Induction of suppression by *T. harzianum* was influenced by the degree of decomposition (age) of the organic component in the container medium. In container media amended with mature CHB, the population levels of *T. harzianum* increased from 10^2 colony-forming units (CFU)/g (dry weight) to 10^4–10^6 CFU/g (dry weight) after 14 days and high levels of disease suppression were observed. However, similar inoculum levels did not induce suppression in a Canadian peat medium or in media amended with fresh or green CHB even though *T. harzianum* reached high population levels in these media. It was concluded that the production of container media that were consistently suppressive to *R. solani* required not only the addition of antagonists, but also the introduction of the antagonist into an environment that favored antagonistic activity.

The suppressiveness of container media amended with composted hardwood bark (CHB) to Rhizoctonia damping-off varies with age of the compost and is microbial in nature (12,13). Previous work with fungi isolated from suppressive and conducive CHB media indicated that a relationship exists between certain fungi and disease suppression (10). Although differences in total numbers of fungi did not account for variation in suppressiveness, quantitative differences in the relative abundance of certain taxa separated suppressive from conducive media amended with CHB (10). High densities of *Trichoderma* spp. in relation to other fungi characterized media amended with suppressive composts. They were the most common fungi isolated from the radish rhizosphere and from *Rhizoctonia solani* Kühn inocula incubated in media amended with suppressive but not with conducive CHB. Finally, different species of *Trichoderma* were found in media amended with different sources of CHB (10).

The relative antagonistic effects of *Trichoderma* and other fungal isolates from green (conducive) and mature (suppressive) composts are not known. This study was initiated to evaluate isolates of fungi from suppressive and conducive composts for their ability to induce suppression to Rhizoctonia damping-off. In addition, the efficacy of antagonists in media amended with green or mature composts was examined to better understand the relationship between organic matter quality and disease suppression.

MATERIALS AND METHODS

Two types of composted hardwood bark (CHB), ie, field and bin CHB, were used throughout this work. The composting procedures used for production of these composts (8,10) are representative of two different methods used in Ohio nurseries. Each was prepared once in 1980 and again in 1981. Different ages (green versus mature) of bin compost were obtained by collecting 30-L samples at 3-wk intervals from a composting mass in an aerated bin (8,10). Samples were stored at −7 C until used in experiments. Green and mature field CHB samples were obtained from windrows in a nursery (8,10).

In some experiments, fresh (uncomposted) hardwood bark (FHB), obtained from Paygro, Inc., 11000 Huntington Rd., South Charleston, OH 45368, and a peat container medium (CP), with Canadian peat as the sole organic component, were used. The pH of the CP medium (50% peat, 50% perlite, v/v) was adjusted to 6.2 with a 1:1 (v/v) mixture of hydrated lime and dolomitic limestone. All CHB types, as well as FHB, were mixed with peat and perlite (5:3:2, v/v) as described previously (12) to adjust the air-filled pore space at container capacity (10-cm-tall column) to 15–20% (calculated from soil moisture desorption curves).

Suppressiveness to Rhizoctonia damping-off was determined with a radish (*Raphanus sativus* L.) seedling bioassay (7,12). Inoculum of *R. solani* was produced in a chopped potato-soil mixture (CPS) as described previously (9,12). To reduce variability in bioassays, inoculum was prepared by grinding air-dried CPS in a mortar and pestle followed by sequential sieving through 2.0 mm and 1.0 mm sieves. Pieces remaining on the 1.0-mm sieve were then used to infest container media. This second sieve, which eliminated particles <1.0 mm in diameter, significantly increased the sensitivity of this assay originally developed by Henis et al (7). Radish seed (cultivar Early Scarlet Globe; 97% germination; 32 seeds per 10-cm-diameter pot) were placed at a mean distance of 1.4 cm (0.9–3.2) from each other in pots containing approximately 400 ml of container media. Seeds were covered with 1.0 cm of container medium and the pots were incubated at 26 C under continuous illumination (2,500 lux). Pots were saturated every other day with tap water and allowed to drain. The percolation rates of the

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container media were >2.5 cm/min. After 7 days, the number of healthy seedlings in each pot (five pots per treatment) was recorded and disease incidence determined (disease incidence = mean percent damped-off seedlings).

Fungal isolates tested in bioassays were obtained by dilution plating on acidified potato-dextrose agar (APDA) and by baiting from container media prepared from various batches of CHB (10). Isolates of the predominant fungal taxa were stored on potato-dextrose agar (PDA) slants at 4 C until comparisons of their antagonistic activity could be made. The relative suppressiveness of the media from which fungal isolates were obtained have been published previously (10).

The ability of fungi to induce suppression was tested with spore suspensions of various fungal isolates prepared by placing the colonized agar from 21-day (25 C) PDA cultures in 200 ml of sterile distilled water. After vigorous stirring and filtration through two layers of cheesecloth, the filtrate was centrifuged (10,000 g, 10 min) and the pellet resuspended in sterile tap water (pH 6.8). Spore concentrations were determined with a hemacytometer. In preliminary experiments, the performance of potential antagonists in conducive media amended with heated (60 C for 5 days) green (3-wk-old) CHB was not consistent. However, when isolates were added to media amended with mature CHB (>6-mo-old) made conducive by heating, consistant results were obtained. Several preliminary trials showed that levels of suppression obtained in heat-treated compost were similar to those obtained with the antagonists in mature composts removed from the center of hot (55–65 C) compost piles after peak heating. Therefore, all fungal isolates were evaluated for their antagonistic effects in heat-treated mature CHB-amended container media. The final spore concentrations were 10^5–10^7 colony forming units (CFU) per gram (dry weight) of container medium. Inoculum of *R. solani* (1.2 g sieved CPS per 2 L of container medium) and a slow release fertilizer (10 g Osmocote, 14-14-14 per liter from Sierra Chemical Co., Milpitas, CA 95053) (12) were then added to the samples followed by vigorous shaking to distribute all amendments uniformly.

In experiments designed to examine population development of *Trichoderma harzianum* Rifai (isolate #738) in media amended with various ages (maturity levels) of CHB, 2-L samples were placed in polyethylene bags and heated (60 C for 5 days). Duplicate samples were left unheated. Spore suspensions (~10 CFU/g dry weight of container medium) of *T. harzianum* were then added to both heated and unheated media followed by vigorous shaking to distribute inocula of *Trichoderma* uniformly. Amended container media were then incubated at 24–26 C and two 10-g samples were removed from each treatment after 0, 2, 5, 8, and 14 days.

Populations of *T. harzianum* were monitored in the various container media on the semi-selective medium of McFadden and Sutton (11). After 14 days, inoculum of *R. solani* and slow release fertilizer were added to the samples followed by vigorous shaking to distribute these amendments uniformly. The pH of FHB, 3-, 6-, and 44-wk-old CHB-amended media were 5.0, 5.2, 5.7, and 6.2, respectively, and were determined by adding one volume of container medium to two volumes of distilled water. Ammonium nitrogen (NH_4^+ – N) levels in 3-, 6-, and 44-wk-old composts, determined by the method of Bremner (1), were 10, 2, and <2 ppm, respectively.

In all experiments, container media prepared from heated suppressive (rendered conducive due to this heat treatment) and unheated suppressive bin CHB were included as controls. Since disease incidence varied in both treatments and controls, results were usually standardized by expressing data as a percentage of the original suppressiveness (unheated control) eliminated by heat (heated control) which could be restored by adding a potential antagonist. Percent restoration of suppression was then calculated by using the formula: $(DH-DA)\ (100)/(DH - D)$ where DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated CHB container medium amended with a potential antagonist, and D = disease incidence in the container medium amended with unheated suppressive CHB.

Each type of compost was prepared twice over a 2-yr period and all experiments were repeated at least twice. Data were analyzed using simple *t*-tests, analysis of variance, and regression analysis where appropriate. Means were separated using the LSD test and Duncan's new multiple range test.

RESULTS

A total of 331 fungal isolates recovered by dilution plating from CHB-amended media were tested for their ability to suppress Rhizoctonia damping-off. Results for isolates of the predominant species isolated from media prepared with the suppressive or conducive field or bin CHB are presented in Fig. 1. The ability to induce suppression varied considerably among all 43 isolates (24 species) tested with only 14 isolates inducing significant levels of suppression.

Isolates inducing significant levels of suppression were recovered from suppressive as well as conducive CHB-amended media. For example, isolates of four species (*Mortierella vinacea* Dixon-Stewart, *Penicillium ochro-chloron* Biourge, *Mortierella isabellina* Oudem., and *Ophiostoma stenoceras* (Robak.) Nannf.) from conducive media amended with field composts (Fig. 1A) and isolates of two species (*Trichoderma hamatum* (Bonord.) Bain. and *Penicillium montanense* Christensen & Backus) from suppressive media amended with field composts (Fig. 1B) induced levels of suppression greater than those found in the heated controls.

Fungal isolates from conducive media amended with (3-wk-old) bin composts were generally not as antagonistic as isolates from suppressive media amended with 44-wk-old composts (Fig. 1C and 1D). None of the isolates from conducive media restored more than 50% of the levels of suppression found in suppressive media amended with unheated CHB. One isolate of *T. harzianum* and one of *Aspergillus fumigatus* Fresen. actually increased disease (ie, negative restoration values). On the other hand, isolates of *T. harzianum* and *Trichoderma koningii* Oudem., from suppressive media amended with bin CHB, induced levels of suppression equal to or better than those found in suppressive media amended with unheated CHB (Fig. 1D). Isolates of *Geomyces pannorum* var. *pannorum* (Link) Sigler & Carmichael and *Geomyces pannorum* var. *asperulatus* (Sigler & Carmichael) van Oorschot from suppressive media amended with bin CHB also induced significant levels of suppression.

Differences among all effective isolates became more readily apparent when isolates were ranked in order of their efficacy of restoring suppression (Table 1). Generally, individual isolates from suppressive media amended with CHB had higher levels of antagonistic activity than those from conducive sources. *Trichoderma* spp. (*T. harzianum, T. koningii*, and *T. hamatum*) induced the highest levels of suppression. However, when isolates of a single species from different sources were compared, no consistent relationship between source and antagonistic activity was apparent. For example, although *G. pannorum* var. *pannorum* from suppressive media amended with bin CHB induced higher levels of suppression than isolates of the same species from conducive media amended with CHB, an isolate of *P. ochro-chloron* from conducive media amended with field CHB induced higher levels of suppression than did isolates of this species from suppressive media.

Twenty-one fungal isolates recovered from inocula of *Rhizoctonia* incubated in suppressive or conducive media amended with CHB were tested for ability to suppress Rhizoctonia damping-off. Only isolates of *T. harzianum* and *O. stenoceras* from conducive media amended with CHB induced significant levels of suppression (Fig. 1E). On the other hand, isolates of *T. hamatum, T. koningii, Gliocladium virens* Miller, Gidden & Foster, *Mortierella zychae* Linnem., and *Mortierella alpina* Peyronel from suppressive media amended with CHB induced significant levels of suppression (Fig. 1F). Isolates of *G. virens* and *M. zychae* were rarely found on dilution plates and, therefore, had not been tested previously. *Trichoderma* spp. had the highest antagonistic activity of fungi recovered from inocula of *Rhizoctonia*. Although isolates from suppressive media induced the greatest levels of suppression (Table 2), it was not possible to identify a consistent relationship

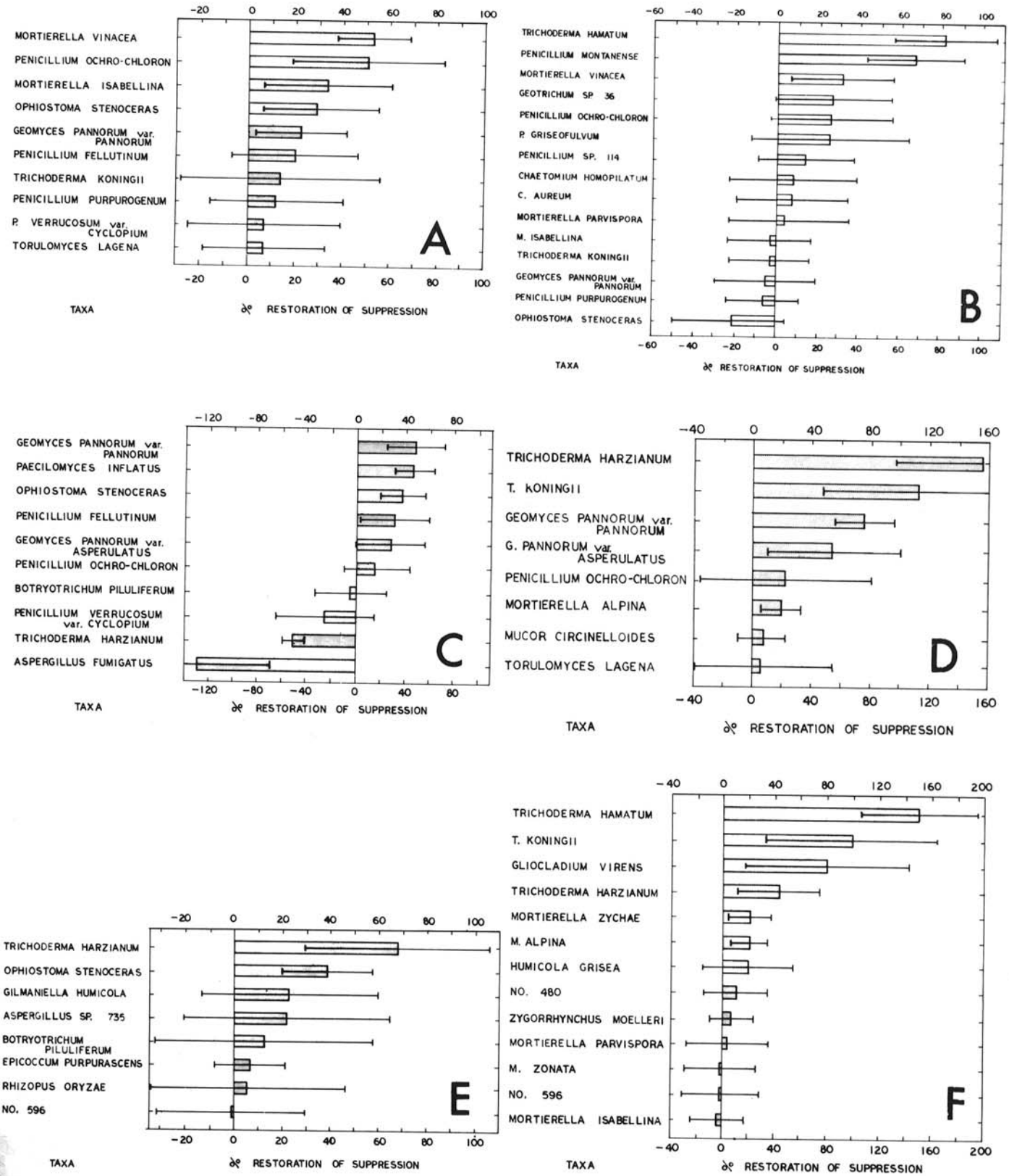

Fig. 1. Restoration of suppression of damping-off of radish by *Rhizoctonia solani* induced by fungal isolates from suppressive and conducive container media amended with composted hardwood bark (CHB). **A,** Isolates from conducive media amended with field CHB. **B,** Isolates from suppressive media amended with field CHB. **C,** Isolates from conducive media amended with bin CHB. **D,** Isolates from suppressive media amended with bin CHB. **E,** Isolates from inocula of *Rhizoctonia* incubated in conducive media amended with bin CHB. **F,** Isolates from inocula of *Rhizoctonia* incubated in suppressive media amended with field CHB. Percent restoration of suppression = $(DH-DA)\times 100/(DH-D)$ in which DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated medium to which the antagonist has been added, and D = disease incidence in unheated (suppressive) media. Bars represent confidence intervals, $P = 0.05$.

between the levels of antagonistic activity and source when only single isolates of individual species were compared.

The antagonistic activities of the groups of *Trichoderma* species (81 isolates) isolated from suppressive and conducive media are compared in Table 3. Although the mean level of suppression induced by all species was apparently greater for isolates from suppressive media than from those from conducive media, these differences were not significant ($P = 0.05$) because of the high variability among isolates from a single source.

By comparing the levels of suppression induced by different *Trichoderma* species groups from all sources (277 isolates), some differences were readily apparent (Table 4). Although differences were not significant ($P = 0.05$), isolates of *T. hamatum* appeared to induce higher levels of suppression than other species groups. In addition, a higher percentage of isolates of *T. hamatum* (51%) induced levels of suppression greater than or equal to those found in unheated suppressive media amended with CHB. Only 35.0, 16.7, and 10.0% of the *T. koningii, Trichoderma viride* Pers. ex Gray, and *T. harzianum* isolates induced similar levels of suppression, respectively.

Addition of increasing levels of *T. harzianum* (isolate #738) to heated suppressive media amended with bin CHB and to unheated Canadian peat (CP) media induced correspondingly higher levels of suppression (Fig. 2). Addition of only 10^2CFU/g dry weight to heated media amended with mature CHB induced a level of suppression that did not differ from that found in the most suppressive media. Much higher levels (10^8CFU/g dry weight) were required to induce equivalent levels of suppression in CP media.

The levels of suppression induced by *T. harzianum* in CHB-amended media varied with age of CHB (Table 5). Only media amended with mature (44-wk-old) CHB were highly suppressive. As observed in previous experiments, addition of *T. harzianum* to unheated mature CHB media did not significantly decrease damping-off. However, significantly higher levels of suppression (lower disease incidence) were induced by *T. harzianum* in heated mature media. On the other hand, addition of *T. harzianum* had no significant effect on damping-off in media amended with FHB or any of the green (3- and 6-wk-old) batches of CHB regardless of whether or not these media had been heated previously.

Population levels of *T. harzianum* increased in media amended with FHB and all ages of CHB (Fig. 3). After 14 days, populations had increased considerably in media amended with FHB and 6-wk-old CHB, whereas the population increase in media amended with 44-wk-old CHB was less pronounced. The lowest population levels developed in 3-wk-old CHB media. Regression analyses of these data and comparison of slope values showed that populations of *T. harzianum* developed at a significantly lower rate in media amended with 3- or 44-wk-old CHB than in media amended with FHB or 6-wk-old CHB. Regression coefficients for FHB, 3-, 6-,

TABLE 1. Restoration of Rhizoctonia damping-off suppression induced by individual isolates of predominant fungal taxa isolated from suppressive and conducive media amended with hardwood bark composts

Isolate	Source[a]	Mean percent restoration of suppression[b]
Trichoderma harzianum	SB	155.8
Trichoderma koningii	SB	111.8
Trichoderma hamatum	SF	81.5
Geomyces pannorum var. *pannorum*	SB	76.5
Penicillium montanense	SF	67.0
Geomyces pannorum var. *asperulatus*	SB	55.9
Mortierella vinacea	CF	52.8
Penicillium ochro-chloron	CF	50.9
Geomyces pannorum var. *pannorum*	CB	48.8
Paecilomyces inflatus	CB	48.2
Ophiostoma stenoceras	CB	38.3
Mortierella isabellina	CF	34.2
Ophiostoma stenoceras	CF	28.9
Geomyces pannorum var. *pannorum*	CF	23.0
Mortierella alpina	SB	20.9

LSD ($P = 0.05$) = 28.9

[a] SB = suppressive bin compost; SF = suppressive field compost; CB = conducive bin compost; and CF = conducive field compost.

[b] Percent restoration of suppression = $(DH-DA)\times(100)/(DH-D)$ in which DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated CHB container medium amended with a potential antagonist, and D = disease incidence in the container medium amended with unheated suppressive CHB.

TABLE 2. Restoration of suppression to Rhizoctonia damping-off induced by individual isolates of predominant fungal taxa recovered from *Rhizoctonia* inocula incubated in suppressive and conducive media amended with composted hardwood bark (CHB)

Isolate	Source[a]	Mean percent restoration of suppression[b]
Trichoderma hamatum	S	149.9
Trichoderma koningii	S	98.7
Gliocladium virens	S	80.5
Trichoderma harzianum	C	67.4
Trichoderma harzianum	S	43.6
Ophiostoma stenoceras	C	39.4
Mortierella zychae	S	22.0
Mortierella alpina	S	21.6

LSD ($P = 0.05$) = 26.7

[a] S = suppressive, C = conducive CHB

[b] Percent restoration of suppression = $(DH-DA)\,(100)/(DH-D)$ in which DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated CHB container medium amended with a potential antagonist, and D = disease incidence in the container medium amended with unheated suppressive CHB.

TABLE 3. Restoration of suppression to Rhizoctonia damping-off of radish induced by *Trichoderma* species isolated from conducive and suppressive container media amended with composted hardwood bark (CHB)

Species	Conducive media: Isolates tested (no.)	Conducive media: Restoration of suppression (%)[a]	Suppressive media: Isolates tested (no.)	Suppressive media: Restoration of suppression (%)
T. hamatum	6	50.9 ± 19.9	19	77.3 ± 26.1
T. harzianum	13	42.8 ± 21.3	12	63.7 ± 38.1
T. koningii	12	42.0 ± 15.7	13	56.5 ± 20.0
T. viride	2	28.1 ± 14.9	4	36.7 ± 53.8

[a] Percent restoration of suppression = $(DH-DA)\,(100)/(DH-D)$ in which DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated media amended with mature CHB inoculated with *Trichoderma* (10^5 colony forming units per gram dry weight), and D = disease incidence in the unheated suppressive container medium. Means are followed by confidence intervals, $P = 0.05$.

TABLE 4. Restoration of suppression to Rhizoctonia damping-off of radish induced by isolates of *Trichoderma* species

Species	Isolates tested (no.)	Mean restoration (%)[a]	Effective isolates (%)[b]
T. hamatum	105	61.4 ± 37.6	51.0
T. koningii	88	49.8 ± 29.6	35.0
T. harzianum	78	34.7 ± 18.7	10.0
T. viride	6	38.7 ± 39.6	16.7

[a] Isolates were added at 10^5 colony forming units per gram (dry weight) to media amended with heated (60 C) mature composted hardwood bark (CHB). Percent restoration of suppression = $(DH-DA)(100)/(DH-D)$ in which DH = disease incidence in the heated (60 C) control, DA = disease incidence in the heated CHB container medium amended with a potential antagonist, and D = disease incidence in the container medium amended with unheated suppressive CHB.

[b] Effective isolates = isolates inducing levels of suppression higher than levels found in unheated CHB-amended media.

and 44-wk-old CHB were 0.51 ($R^2 = 94.8$), 0.36 ($R^2 = 64.9$), 0.59 ($R^2 = 96.9$), and 0.24 ($R^2 = 90.0$), respectively.

DISCUSSION

Results of this study support previous work which indicated that suppression of Rhizoctonia damping-off in container media amended with CHB was due to particular antagonistic microorganisms (10). However, it is also apparent that the activity of the antagonists, when added to heated media, was strongly affected by age of compost used in preparation of container media. Therefore, disease suppression is dependent not only on the presence of populations of antagonistic microorganisms, but also on undefined factors associated with compost age.

A wide variety of fungi recovered from container media amended with hardwood bark composts induced significant levels of suppression when added to heated media. However, only a small percentage of the abundant fungi isolated from any given batch of CHB-amended medium were efficacious antagonists. In general, the fungal isolates from conducive media were not as antagonistic as those from suppressive media. Members of the genera *Trichoderma, Gliocladium, Penicillium, Mortierella, Paecilomyces, Geomyces*, and *Ophiostoma* had the highest levels of antagonistic activity. Some of these (*Trichoderma* and *Gliocladium* spp. in particular) have been reported to be antagonists of *R. solani* as well as other root-infecting fungi (2,4,15).

The high levels of antagonistic activity among isolates of *T. hamatum* recovered from suppressive media amended with field CHB, in addition to the high population level of this species in this medium (10) and its association with inocula of *Rhizoctonia* as well as the radish rhizosphere (10), suggests that it played a major role in disease suppression. Similarly, antagonistic activity of isolates of *T. harzianum* from suppressive media amended with bin CHB (10) and high population levels of this species in suppressive as compared to conducive media amended with bin CHB, suggests that it was responsible for disease suppression in media amended with the natural bin composts.

The low level of suppression reported previously (10) for media amended with batches of either green bin or field CHB could not be attributed to the absence of antagonistic fungi or to low levels of antagonistic activity among fungi present in these media. Isolates of *Trichoderma*, as well as other fungi which restored significant levels of suppression to heated media, were obtained from media amended with green as well as mature CHB (Fig. 1). Furthermore, isolates of *Trichoderma* from media amended with green CHB were as effective in inducing suppression as those isolated from mature CHB media (Table 3).

We reported previously that there were increases in the relative abundance of *Trichoderma* spp. in the rhizosphere of radish in suppressive media in contrast to conducive media (amended with green compost). Therefore, we suggested that the limited development of populations of *Trichoderma* in conducive media

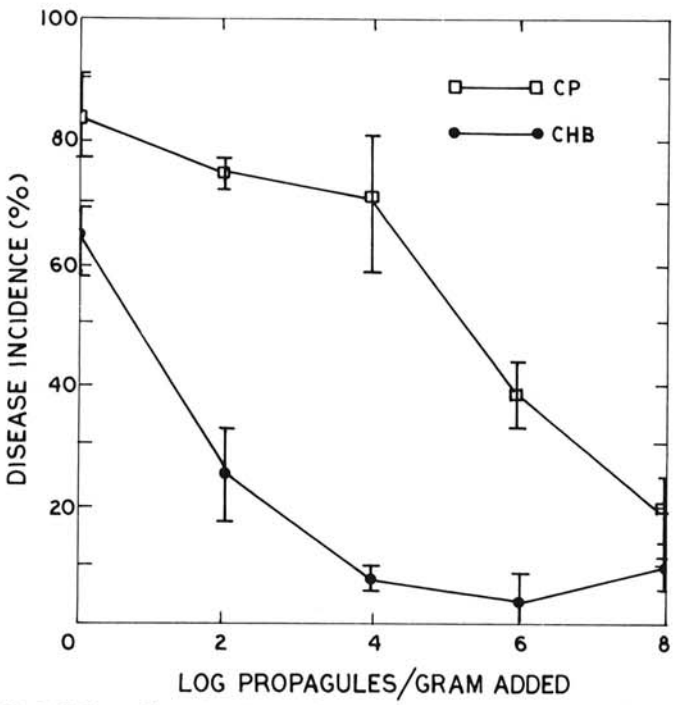

Fig. 2. Effects of inoculum levels of *Trichoderma harzianum* (isolate #738) on induction of suppression to Rhizoctonia damping-off of radish in container media amended with heated (60 C) mature (CHB) or Canadian peat (CP).

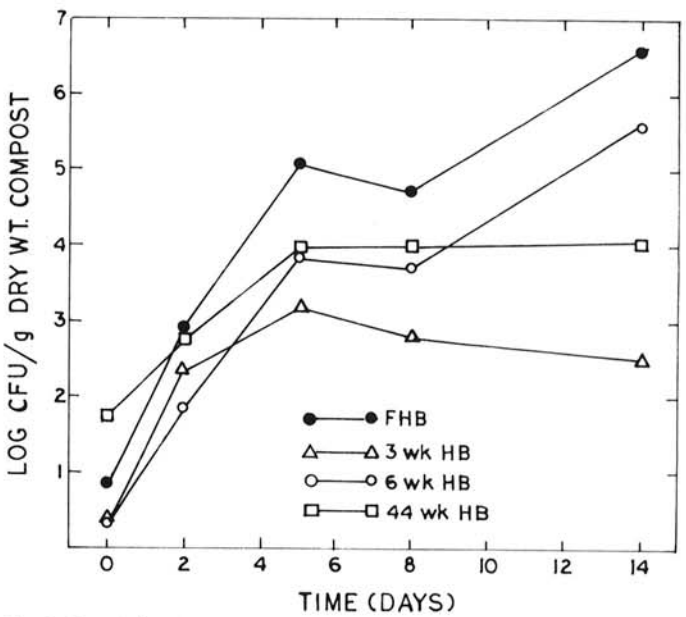

Fig. 3. Population development of *Trichoderma harzianum* (isolate #738) in container media amended with hardwood bark (HB) at various stages of decomposition. FHB = fresh (uncomposted) hardwood bark; 3-wk HB, 6-wk HB, and 44-wk HB represent hardwood bark composted for 3, 6, and 44 wk, respectively.

TABLE 5. Effect of compost age on the induction of suppression to *Rhizoctonia* damping-off by *Trichoderma harizanum* (isolate #738)

	Disease incidence							
	25 C				60 C[b]			
Trichoderma	Compost age (wk)				Compost age (wk)			
added[a]	0	3	6	44	0	3	6	44
−	79.2 a[c]	79.9 a	70.8 ab	45.0 b	73.4 ab	85.7 a	73.0 ab	68.5 ab
+	73.7 ab	74.0 ab	58.1 ab	52.3 ab	53.6 b	68.2 ab	58.1 b	30.3 c

[a] Initial levels added were 10^2 colony forming units per gram dry weight.
[b] See Fig. 3 for the population development of *T. harzianum* in each age of CHB-amended medium.
[c] Represents mean percent damping-off after preincubation of CHB-amended media with or without *T. harzianum*; numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

were responsible for low levels of disease suppression (10). However, results obtained with an isolate of *T. harzianum* added to heated media amended with composts of different ages indicated that differences in disease suppression were not correlated with the occurrence of high populations of *T. harzianum*. *T. harzianum* reached population levels in media amended with green (3 wk) CHB that were similar to those in mature (44 wk) CHB (Fig. 3), yet suppression was observed only in media amended with mature CHB (Table 5). The numbers of propagules of *Trichoderma* were even higher in the heated media amended with FHB (fresh hardwood bark) and 6-wk-old CHB, but these media were conducive or only mildly suppressive. Therefore, disease suppression appeared to depend not only on the presence of potential antagonistic microorganisms in CHB-amended media but also on factors which affected the antagonistic activity of these organisms.

The activity of antagonists was determined in heated (60 C, 5 days) media in which populations of competing microorganisms were reduced or eliminated. To the uninitiated this would appear to be a poor method for determining their activity. However, the results obtained with heated media are identical to results obtained recently with antagonists added directly to full size compost piles, probably because comparable temperatures are produced for several days in mature composts after turning of piles (H. A. J. Hoitink, *unpublished*).

Isolates of *Trichoderma* from all four species groups tested (*T. hamatum, T. harzianum, T. koningii*, and *T. viride*) restored significant levels of disease suppression to media amended with mature CHB. The results of in vivo assays of 277 isolates indicate that antagonistic activity is widespread in the genus *Trichoderma*. This is in agreement with other studies which were based on smaller numbers of isolates (3,4). Superior antagonistic strains were selected from each species group and the replacement of mixed populations of *Trichoderma* spp. of various levels of antagonistic activity by a single isolate consistently produced higher levels of disease suppression. Frequently the level of suppression in the antagonist-fortified mature compost-amended medium was significantly higher than that in unfortified media. Thus, controlled production of suppressive container media appears to be desirable and feasible.

Production of container media that were consistently suppressive to *R. solani* required not just addition of the antagonist to compost but also the introduction of this antagonist into an environment (mature CHB) which favored antagonistic activity. Low population levels (10^2CFU/g dry weight, Fig. 3) of *T. harzianum* added to mature CHB-amended media induced high levels of suppression. These same population levels did not induce suppression in peat media or in FHB or green CHB-amended media. The peat medium was rendered strongly suppressive, but only after the addition of very high inoculum levels (10^8CFU/g dry weight). Effects of age (maturity) and quality of amendments or the amount and degree of decomposition of crop residues have largely been overlooked in studies on efficacy of antagonists. Additional research may eventually explain some of the variability typically encountered in trials with biocontrol agents.

Age of compost was a useful parameter for predicting development of suppression in the hardwood bark composts used here. However, composting procedures vary and seasonal effects on the rate of decomposition for each system also vary significantly (5,6,14). A given age, therefore, may not always be a reliable indicator of suppressiveness.

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