Fungal Populations in Container Media Amended with Composted Hardwood Bark Suppressive and Conducive to Rhizoctonia Damping-Off

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ABSTRACT

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Sample fungal populations (200–300 isolates each) were isolated from container media suppressive and conducive to *Rhizoctonia* amended with hardwood bark composts (CHB) produced in windrows (field compost) or in a reactor (bin compost). Sample populations from suppressive and conducive batches of container media were compared to determine the relationship between fungi and disease suppression. Although the total numbers of fungi and pH of the media containing CHB from the two compost sources differed, these differences could not account for variations in suppressiveness. Fungal populations isolated from both suppressive and conducive media were dominated by hyphomycetes, ascomycetes, and zygomycetes with 34 taxa accounting for >80% of over 2,000 isolations. Quantitative differences in the relative abundance of taxa differentiated suppressive and conducive batches. Principal components analysis demonstrated relationships among species and disease suppressiveness. High relative densities of *Trichoderma hamatum* characterized populations isolated from suppressive container media amended with field compost, whereas *T. harzianum* was the predominant species in suppressive media amended with bin composts. Conducive media, however, had high populations of *Penicillium verrucosum* var. *cyclopium* or varieties of *Geomyces pannorum*. In addition, *Trichoderma* spp. were the most abundant taxa in populations isolated from radish rhizospheres and inocula of *Rhizoctonia* planted and incubated in suppressive media. Associations between populations of *Trichoderma* spp. and suppression suggested that antagonism toward *Rhizoctonia solani* by these fungi may have accounted for the reduction in disease. Disease suppression was not associated with a single fungal taxon. Furthermore, quantitative differences in sample populations isolated from suppressive and conducive container media indicated that the lack of suppression in some media was due to factors that limited development of high populations of *Trichoderma* or interfered with the antagonistic activity of these fungi.

Container media amended with composted hardwood bark (CHB) suppress a variety of soilborne plant pathogens (4,9,21,22,39,40). Recent research on suppression of Rhizoctonia damping-off has shown that microbial populations within media containing CHB are responsible for disease control (32,33). Heat (60 C) and gamma radiation (275 krad) treatments which reduced microbial populations eliminated disease suppression. Suppression was restored by adding 10% (v/v) unheated compost or propagules of selected fungi isolated from suppressive media (32).

Nelson and Hoitink (33) have observed variations in suppressiveness of container media amended with CHB. Media amended with green composts (<11 wk old) are significantly less suppressive than those containing mature composts (>11 wk old). They have suggested that such differences in suppressiveness are the result of colonization of mature composts by antagonistic microorganisms. This hypothesis is consistent with other reports on soils suppressive to *Rhizoctonia* which have attributed suppression to populations of mycoparasitic fungi (5,24,25,28,35).

The purpose of this study was to compare both qualitatively and quantitatively fungal populations in suppressive and conducive container media amended with CHB. By enumeration of sample populations isolated from suppressive and conducive media prepared from batches of composts obtained from the same source, it was not only possible to ascertain whether differences in the occurrence and densities of fungal taxa were associated with suppressiveness, but also to identify the fungi which might be responsible for such suppression. In addition, comparison of sample populations isolated from media amended with CHB from different sources provided a means for determining whether certain taxa were uniquely associated with suppressiveness.

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MATERIALS AND METHODS

Batches of two composted hardwood bark (CHB) formulations were obtained: bin compost, pH 5.2–5.7 (Paygro, Inc., 11000 Huntington Rd., South Charleston, OH 45368) and field compost, pH 7.1–7.2 (Warner Nursery, Route 6, Willoughy, OH 44094). Both composts were prepared from a mixture of hardwood tree species (largely *Quercus* spp.) (32). The bin CHB was amended with nitrogen and phosphorus as described previously and composted in an aerated tank or reactor vessel (21) for 3 wk, followed by additional composting in an insulated bin with a concrete floor (33). A 15-cm-diameter flexible porous tube was placed through the center of the composting mass (3.4 m^3) to maintain aerobic conditions (21). The CHB was removed from the bin at 4-wk intervals, rewetted to 55% moisture (w/w), and mixed to ensure that all particles were exposed to thermophilic decomposition (40–65 C). Prior to placing the CHB back into the bin, 30-L samples were removed and stored at −7 C until use. The field CHB was amended with nitrogen and phosphorus as outlined previously (21) and prepared in windrows. In contrast to the bin compost, field compost was located on a soil base and thus was susceptible to inoculation from soil and an adjacent flood plain forest. Both types of CHB were prepared twice (once in 1980 and again in 1981) and used in repeated trials. All composts were mixed with Canadian peat and perlite (5:3:2, v/v) before use in experiments to obtain a container medium with 15–20% air-filled pore space at container capacity (10-cm-tall column).

Suppressiveness of container media to Rhizoctonia damping-off was determined with a radish (*Raphanus sativus* L.) seedling bioassay. The isolate of *Rhizoctonia solani* Kühn and the assay were described previously (19,32). Fungal populations were isolated by dilution plating from container media amended with CHB prior to assaying for disease suppression and again at the end of the 7-day assay. In addition, when disease ratings were made,

populations of fungi were also isolated from the radish rhizosphere and roots. Serial dilutions were prepared from a 10-g sample of roots and adhering container media processed for 3 sec in a Waring blender. Replicate samples were set aside for moisture determinations. Aliquots (0.1 ml) were plated on four to five plates containing 15 ml of acidified Difco potato-dextrose agar (APDA). After 48 hr at 22 C, plates with 20–100 colonies were chosen for isolations. From each plate, hyphal tips were removed from 20–55 colonies with the aid of a dissecting microscope and placed onto potato-dextrose agar (PDA) slants. Attempts were made to obtain between 100–300 random isolations from each sample of CHB-amended media. A total of over 2,000 isolations were made from various batches of container media.

The number of species obtained was plotted against the number of isolations to determine whether the sample populations were of adequate size. Although the total number of species isolated from the suppressive container media amended with bin and field CHB (Fig. 1) differed, relatively few taxa were added as the number of isolations increased above 200. Most importantly, the number of species isolated as the sample population was increased above 150 accounted for less than 10% of all colonies appearing on dilution plates. Thus, sample populations isolated in this study appear to be of sufficient size to represent the most abundant taxa in each container medium.

Additional isolations were made from inocula of *Rhizoctonia* incubated in suppressive and conducive media amended with CHB that had been adjusted to −22 mb moisture tension by the use of tension plates. Survival of these inocula in container media has been described previously (33). Inocula were prepared by growing *R. solani* on chopped potatoes (50 g in a 1-L flask). After 14 days (25 C) colonized potato pieces were air-dried 48 hr in a laminar flow hood, ground in a mortar and pestle, and sieved sequentially through 850- 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 × 25 mm) contained ~50 individual pieces of inoculum. The nylon screen layers were then stapled together to prevent loss of inoculum pieces and arranged on the surface of the container media. Sandwiches were covered with an additional 1.0 cm container medium, reequilibrated to a moisture tension of −22 mb and incubated in a growth chamber at 26 C. Sandwiches were removed after 7, 14, 21, and 28 days and rinsed for 2 min in sterile distilled water to remove as many surface contaminants as possible. Inocula were then removed from screens and placed on APDA. After 48 hr, hyphal tips emerging from inocula were removed and transferred to PDA slants. A total of 391 and 220 isolations were made from inocula incubated in suppressive media amended with field CHB and conducive bin CHB, respectively.

After 7–10 days of incubation (22 C), slant cultures were sorted into presumptive species groups based on gross cultural morphology. Representative cultures were given identification numbers and one or two cultures were set aside for detailed examination. Isolates of some genera (eg, *Trichoderma, Penicillium*, and *Mortierella*) were not always readily classifiable to species by gross morphological characteristics. Therefore, each slant culture was identified using standard mycological procedures. All isolates of *Trichoderma* were identified to species groups according to Rifai (37). The penicillia were treated according to Raper and Thom (36) with the exception of members of the section fasiculata which were identified according to Samson et al (38).

For each sample population isolated from different container media, the number of isolates of each species was recorded separately. The relative density of each species within the sample population was then calculated by using the formula: Relative Density = (number of isolates of a given species ×100)/(number of all isolations in the sample population). To compare the abundance of species, mean relative densities (based on all isolations from container media and radish rhizosphere and roots) were calculated.

The similarity between sample populations was determined by using mean relative densities and similarity coefficients (8,17). The coefficient was calculated by using the formula $2W/(A + B)$, in which W is the sum of the lower densities of those species in common, A is the total relative density of one sample population, and B is the total relative density of the other. Sample populations with identical composition would have a value of 1.0, whereas sample populations that shared no species in common would have a value of 0.

The relative densities of the 33 most commonly isolated species were determined for each of 12 sample populations isolated from CHB-amended media. Five of the sample populations were isolated from media amended with field CHB and seven from the bin CHB. Suppressive and conducive batches were represented equally. A Q-type principal components analysis (PCA) of the data was performed by using methods previously described (16,30,34). The fundamental objective of PCA is to reduce the dimensions of a data matrix in such a way that the relationships among either the container media or the species is more readily interpretable. This is achieved by replacing species values with the scores of the uncorrelated principal components defining the relationships among the container media (26). The analysis was based on the matrix of container media correlation coefficients. The component coefficients, as well as the variance of each component, were determined with the BMD-P4M procedure of the Biomedical Computer Programs (10). To interpret the derived principal components, factor loadings were calculated as functions of component coefficients and are measures of the correlation between individual container media and components. No rotation of the coefficients was performed.

Analysis of variance was used to evaluate differences in relative densities of the predominant taxa among sample populations isolated from container media at various times. Means were separated according to Duncan's new multiple range test ($P = 0.05$). Although percentage values were transformed (arc-sin $\sqrt{[X/100]}$), in which X is relative density, there was no effect on the separation of the means and thus, the nontransformed data are presented.

RESULTS

Levels of damping-off were nearly equal in suppressive media amended with CHB from the different sources (Table 1). In addition, disease levels in the conducive media were comparable to those of suppressive media that were made conducive by heat treatments.

The total numbers of fungi, the age, and the acidity of each batch of container medium are given in Table 2. Although the exact age of the field CHB could not be determined (~42-wk), it was conducive at the time of collection and still undergoing thermophilic decomposition. Samples (600 L) of this conducive medium

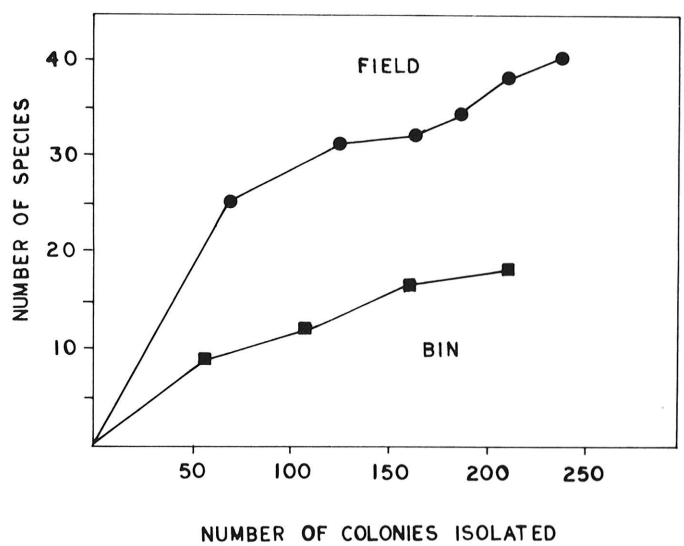

Fig. 1. Relationship between number of species and isolations for sample fungal populations isolated from suppressive container media amended with field (windrow)- and bin-composted hardwood bark.

incubated at 25 ± 1 C became suppressive after 6 wk. In contrast, suppressive and conducive media amended with bin CHB were prepared from compost which differed greatly in age. The conducive batches had been composted for only 3 wk (~55 C), whereas the suppressive batches were 44 wk old (<43 C). Media amended with CHB from the different sources differed in pH and total numbers of fungi. However, there were only slight differences between suppressive and conducive media amended with CHB from the same source.

A total of more than 2,000 colonies, representing 37 genera of fungi, were isolated from all CHB-amended media. The distribution of genera within sample populations isolated from suppressive and conducive batches was similar (Table 3). For example, hyphomycetes accounted for the majority of genera isolated and made up nearly equal percentages of sample populations in media amended with both suppressive and conducive CHB. Most of the remaining genera were either zygomycetes or ascomycetes. A small percentage of isolates were sterile and/or could not be readily identified.

Thirty-four taxa, comprising 16 genera, appeared with sufficient regularity that they accounted for over 80% of the sample populations isolated from each medium. The distribution of these taxa between suppressive and conducive media amended with CHB from the two sources is given in Table 4. Differences in species distributions between suppressive and conducive media amended with CHB were quantitative. Many taxa were isolated from both suppressive and conducive batches with six of the 10 most abundant taxa (*Penicillium ochro-chloron, Trichoderma harzianum, Geomyces pannorum* var. *pannorum, Mortierella isabellina, Ophiostoma stenoceras*, and *Trichoderma koningii*) found in all four batches. Only a few fungi (*Mortierella alpina, Mucor circinelloides*, and *Gliocladium virens*) were found in both suppressive media, but were absent from conducive CHB-amended media. Only one taxon, *Penicillium verrucosum* var. *cyclopium*, had a unique association with conducive media.

Although media amended with CHB from different sources shared many species, there appeared to be an association of certain taxa with media amended with composts from the same source.

TABLE 1. Disease levels in heated (60 C) and unheated (25 C) container media amended with hardwood bark composts (CHB) suppressive (S) and conducive (C) to Rhizoctonia damping-off

CHB Source	Disease incidence (%)	
	25	60
Field (S)	22.0	84.7
Field (C)	86.1	88.2
Bin (S)	21.3	82.0
Bin (C)	85.3	87.3
LSD ($P = 0.05$) = 11.8		

TABLE 2. Numbers of fungi in and pH of suppressive (S) and conducive (C) container media amended with various ages of composted hardwood bark (CHB)

CHB source	Age (weeks)	pH	Total fungal colonies/g dry weight
Field (C)	42	7.2	3.8×10^5
Field (S)	48	7.0	3.0×10^5
Bin (C)	3	5.5	1.4×10^6
Bin (S)	44	5.7	1.6×10^6

TABLE 3. Distribution of fungal genera in suppressive (S) and conducive (C) container media amended with hardwood bark composts

Class	Number of genera		Percent colonies[a]	
	S	C	S	C
Hyphomycetes	19	17	70.7	71.8
Zygomycetes	7	4	20.4	14.5
Ascomycetes	5	6	7.4	9.7
Sterile/Unidentified	···	···	1.5	4.0
Totals	31	27	100	100

[a] Based on 1,304 and 968 colonies isolated from suppressive and conducive media, respectively.

TABLE 4. Mean relative density (%) of predominant fungal taxa in *Rhizoctonia* suppressive (S) and conducive (C) container media amended with hardwood bark composts from two sources

Taxa	Source of compost			
	Field		Bin	
	C	S	C	S
1. *Penicillium ochro-chloron* Biourge	8.9	9.1	19.6	26.8
2. *Trichoderma harzianum* Rifai aggr.	0.3	1.7	2.9	38.2
3. *Geomyces pannorum* var. *asperulatus* (Sigler & Carmichael) van Oorschot.	0.0	0.0	25.0	10.9
4. *Penicillium verrucosum* var. *cyclopium* (Westling) Samson, Stolk & Hadlok	24.9	0.0	5.4	0.0
5. *Trichoderma hamatum* (Bonord.) Bain. aggr.	1.8	20.8	0.0	0.0
6. *Geomyces pannorum* var. *pannorum* (Link) Sigler & Carmichael	18.5	1.8	3.0	0.5
7. *Mortierella isabellina* Oudem.	7.4	9.2	2.3	0.2
8. *Ophiostoma stenoceras* (Robak.) Nannf.	4.4	3.8	9.6	0.6
9. *Mortierella alpina* Peyronel	0.0	1.5	0.0	9.7
10. *Trichoderma koningii* Oudem	2.3	2.9	2.4	0.9
11. *Mortierella parvispora* Linnem.	6.2	5.6	0.2	0.0
12. *Chaetomium aureum* Chivers	0.7	9.1	0.2	0.0
13. *Zygorrhynchus moelleri* Vuill.	0.6	9.1	0.2	0.0
14. *Penicillium griseofulvum* Dierckx	0.0	7.0	0.0	0.0
15. *Trichurus spiralis* Hasselbr.	0.0	0.0	6.2	0.0
16. *Mortierella vinacea* Dixon-Stewart	1.9	2.3	0.0	0.0
17. *Penicillium fellutanum* Biourge	1.3	0.6	1.4	0.0
18. *Mucor hiemalis* Wehmer	0.3	2.6	0.0	0.0
19. *Torulomyces lagena* Delitsch	1.7	0.1	0.0	1.6
20. *Penicillium purpurogenum* Stoll	1.1	1.7	0.0	0.0
21. *Rhizopus oryzae* Went & Prinsen Geerlings	0.0	0.6	1.5	0.0
22. *Mucor circinelloides* van Teigh.	0.0	0.1	0.0	1.9
23. *Trichoderma viride* Pers ex Gray aggr.	0.3	0.3	1.1	0.0
24. *Botryotrichum piluliferum* Sacc. & March.	0.0	1.3	0.2	0.0
25. *Mariannaea elegans* (Corda) Samson	2.0	0.1	0.0	0.0
26. *Gliocladium virens* Miller, Giddens & Foster	0.0	0.1	0.0	1.2
27. *Penicillium odoratum* Christensen & Backus	0.5	0.8	0.0	0.0
28. *Mortierella ramanniana* (Moller) Linnem.	0.0	1.1	0.0	0.0
29. *Chaetomium homopilatum* Omvik (?)	0.0	1.0	0.0	0.0
30. *Penicillium citrinum* Thom	0.0	1.0	0.0	0.0
31. *P. montanense* Christensen & Backus	0.0	1.0	0.0	0.0
32. *Geotrichum* sp.	0.1	0.6	0.0	0.0
33. *Aspergillus fumigatus* Fresen.	0.0	0.0	1.1	0.0
34. *Paecilomyces inflatus* (Burnside) Carmichael	0.0	0.0	5.6	0.0
35. Others	14.8	11.8	6.6	7.5

TABLE 5. Similarity coefficients[a] for sample fungal populations isolated from suppressive (S) and conducive (C) container media amended with hardwood bark composts

Compost source	Field (S)	Field (C)	Bin (S)	Bin (C)
Field (S)	1.00			
Field (C)	0.38	1.00		
Bin (S)	0.20	0.12	1.00	
Bin (C)	0.23	0.29	0.36	1.00

[a] Similarity coefficient = $2W/(A + B)$; in which W = the sum of the lower relative densities of species common to both sample populations, A = total relative density of all species in the first sample population and B = the total relative density of all species in the second sample population.

Table 5 gives the results of a pairwise comparison of the four batches of media made by using similarity coefficients calculated from the mean relative densities. Populations isolated from suppressive and conducive media amended with CHB from the same source were more similar than suppressive media amended with CHB from different sources. Conducive media amended with CHB from different sources had only a slightly higher degree of similarity than the suppressive media. However, there were quantitative differences between sample populations isolated from suppressive and conducive media. For example, *Trichoderma hamatum* was frequently isolated from suppressive media amended with field CHB, but occurred only infrequently in conducive media. *T. harzianum* was particularly abundant in suppressive media amended with bin CHB, whereas high populations of *Geomyces pannorum* var. *pannorum* (field compost) and *G. pannorum* var. *asperulatus* (bin compost) characterized the conducive media.

Relationships between species abundance and disease suppression (Table 4) were based on mean relative densities of species from all isolations (before and after potting) from each batch of container medium. Principal-components analysis of the relative densities of the 34 taxa in each of the 12 sample populations provided a method to more objectively determine relationships, not only among species, but also among certain species and suppressiveness. Species were separated according to their abundance in sample populations isolated from different batches of container media. As shown in Table 6, the first three principal components accounted for 67% of the total variance in the species-abundance data. The suppressive and conducive populations were best separated by the second and third principal components. For example, sample populations isolated from suppressive media amended with field CHB had, in general, the highest factor loadings in the second component, whereas the sample populations in conducive media amended with bin CHB had the most negative values. Similarly, sample populations from conducive media amended with field CHB had the most positive values in the third principal component, while those from suppressive media amended with bin CHB were the most negative. The first component, on the other hand, consisted of positive loadings for all sample populations, and thus was not useful for separation of the taxa.

Fig. 2 shows the position of the taxa listed in Table 4 in relation to the second and third principal components. In general, species with the highest relative densities (numbered 1–12 in Table 4) are farthest from the center whereas those with low densities are clustered near the center. Taxa predominating in suppressive media amended with either bin or field CHB (field or bin) were distributed in the lower and right portions of the plot in contrast to those taxa that were most abundant in the conducive container media and distributed in the upper and left portions. Species located in the upper and right corners were those that reached maximum densities in media amended with field CHB, whereas those in the bottom and left were most abundant in the container media amended with bin CHB.

Trichoderma spp. were characteristic of the populations isolated from suppressive media. *T. hamatum* was particularly abundant in media amended with field CHB, whereas *T. harzianum* was most common in the media amended with bin CHB. Although *T. koningii* and *T. viride* were frequently isolated, neither species was uniquely associated with any particular container medium.

In contrast to suppressive media, sample populations isolated from the conducive media were characterized by high numbers of *Penicillium verrucosum* var. *cyclopium* and *Geomyces pannorum*. Different varieties of *Geomyces pannorum* were typically isolated from the media amended with CHB from two different sources; var. *asperulatus* often appeared in media amended with bin CHB, whereas var. *pannorum* was commonly isolated from field CHB media.

The associations between species abundance and disease suppression described above were particularly evident when sample populations isolated at the end of the seven day assay from radish rhizosphere and roots and container media were compared with populations isolated prior to infesting media with *R. solani* and planting with radish. Although sample populations isolated before and after potting were generally similar, some taxa increased in relation to others. For example, Table 7 shows changes in the relative density of the 14 most abundant taxa isolated from suppressive media amended with field CHB. Only two taxa, *Mortierella alpina* and *Mucor hiemalis*, that were abundant at the end of 7 days, were not isolated prior to potting. The most notable change was the significant increase in the relative density of *T. hamatum*. Such a marked increase was not observed in conducive media amended with field CHB. Similar comparisons made between populations isolated from media amended with bin CHB showed increases in *T. harzianum* in suppressive but not in conducive batches.

TABLE 6. Factor loadings[a] for principal components ordination of sample fungal populations isolated from suppressive (S) and conducive (C) container media amended with hardwood bark composts

	Principal components		
Compost source	1	2	3
Field S	0.49	0.60	0.20
S	0.56	0.65	−0.02
S[b]	0.17	0.59	0.06
C	0.12	−0.04	0.73
C	0.31	0.36	0.67
Bin S	0.81	−0.15	−0.24
S	0.72	−0.21	−0.44
S[b]	0.87	−0.16	−0.33
C	0.44	−0.51	0.17
C	0.27	−0.66	0.57
C	0.73	0.29	0.08
C[b]	0.61	−0.41	0.26
Cumulative % of total variance explained	33	52	67

[a]Correlations between component and compost are functions of the component coefficients (30).
[b]Sample population isolated from rhizosphere and roots.

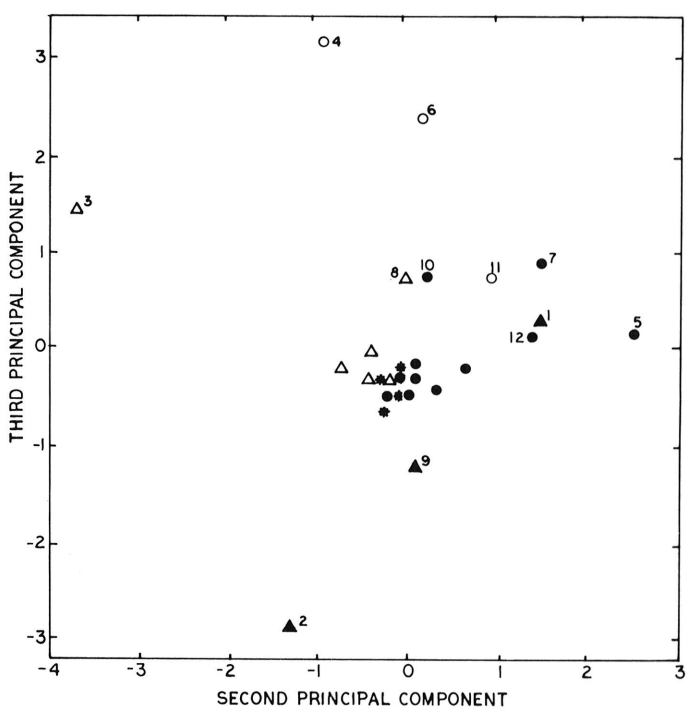

Fig. 2. Principal component scores for 34 taxa isolated from suppressive (S) and conducive (C) container media amended with composted hardwood bark. The most abundant taxa are numbered (1–12) in agreement with Table 4. Symbols indicate compost amended medium in which each taxon was most abundant: ● = Field (S); o = Field (C); ▲ = Bin (S); Δ = Bin (C); * = more than one species.

Sample fungal populations recovered from *Rhizoctonia* inocula (391 isolations) further indicated an association between high levels of *Trichoderma* and disease suppression. For example, many taxa which had colonized inocula recovered from suppressive media amended with field CHB (*T. hamatum, T. koningii, Zygorrhynchus moelleri, T. harzianum, Mortierella parvispora, M. zonata* Linnem. ex Gams, *Gliocladium virens* Miller, Gliddens & Foster, *Mortierella isabellina, Humicola grisea* Traaen, and *Mortierella zychae* Linnem.) were also isolated by dilution plating. However, *Trichoderma* species, in particular *T. hamatum* (40.4% relative density), dominated. In contrast, other fungi which were also abundant in sample populations obtained on dilution plates (eg, *Penicillium* and *Chaetomium* spp.), were rarely isolated, if at all, from inocula.

Inocula placed in a conducive medium amended with bin CHB were not colonized by *Trichoderma* to the same degree. For example, only 13% of the fungal isolates recovered from inocula incubated in a conducive medium prepared from bin CHB were *Trichoderma* species (*T. harzianum*), whereas 75% were *Botryotrichum piluliferum*. Most importantly, *Rhizoctonia* could be isolated after 28 days from only 3% of the inocula buried in the suppressive medium, whereas 35% of the inocula placed in the conducive medium contained viable *Rhizoctonia*.

DISCUSSION

The characteristic sample populations isolated from CHB-amended container media were to some degree influenced by the choice of isolation methods. Colonies that appeared on dilution plates most likely originated from spores or other dormant propagules, and therefore, absolute numbers of fungal colonies are not necessarily indicative of activity. Thus, it was not surprising that differences in total numbers of fungi (Table 2) among the various batches had little association with suppression. In addition, the predominance of hyphomycetes and zygomycetes (Table 3) is typical for sample populations isolated by dilution plating. Nevertheless, sample populations obtained from dilution plates are not merely a random assemblage of spores, but rather represent a community of organisms adapted to the environment from which they were isolated (7,17). Differences in the relative abundance of various taxa are indicative of differences in spore population levels. Although the species lists are not inclusive of the entire mycoflora, they include the taxa which grow rapidly and sporulate profusely in culture and thus are most easily used in laboratory studies on biological control.

Many of the fungi isolated from CHB-amended media are readily isolated from soils. However, despite quantitative variation among the various batches, there appears to be a distinctive fungal community associated with container media amended with CHB. For instance, although species of *Penicillium, Trichoderma, Mortierella*, and *Chaetomium* were often isolated from CHB media, other common soil fungi such as *Fusarium, Aspergillus*, and *Oidiodendron* were infrequently obtained. Furthermore, many fungi which are commonly associated with the initial stages of decomposition of plant litter were rarely isolated from CHB-amended media. For example, *Aureobasidium, Cladosporium*, and *Alternaria* are among the earliest and most abundant invaders of a wide variety of plant remains (14,23), yet apparently could not colonize CHB as readily as other fungi.

Populations isolated from CHB-amended media share some taxa with fungal populations described from other composts. For example, *Aspergillus fumigatus* and *Trichurus* spp., which were primarily isolated from green bin CHB-amended media, are reported to be common colonists of spent mushroom compost (27) and composted municipal wastes (42). The isolation of other fungi may be related to the ability to withstand high temperatures. For example, species of *Mortierella* in the section micromucor (*M. isabellina, M. ramanniana*, and *M. vinacea*) were among the most common taxa isolated (Table 4) and are capable of surviving temperatures (70 C for 30 min) which are lethal to many other fungi (2). Although less frequently isolated from CHB-amended media, a number of ascomycetes (eg, *Thielavia, Eupenicillium*, and *Talaromyces*), which have heat-resistant spores (43), were among the few fungi isolated from media amended with CHB made conducive by heat treatments (60 C for 5 days).

The number and distribution of genera in sample populations isolated from suppressive and conducive batches (Table 3) were remarkably similar. However, the sample populations isolated from suppressive media amended with different batches of CHB appeared to differ markedly in species diversity. Media containing field CHB yielded 38 species per 210 isolations in contrast to only 18 species for the bin CHB media (Fig. 1). This difference may be due to differences in the composting procedure and exposure to potential colonists. The field CHB was prepared in open windrows on soil, whereas the bin CHB was produced within enclosed concrete containers.

By comparing populations isolated from various batches of CHB-amended media (Table 4) it was possible to make some generalizations concerning species distributions and disease suppressiveness. First, differences between suppressive and conducive media were quantitative. There were few taxa which were abundant in suppressive and absent in conducive media.

TABLE 7. Relative densities of fungi isolated from suppressive container medium amended with field hardwood bark composts at potting and after 7 days incubation with *Rhizoctonia solani* and radish seedlings

Taxa	Mean relative density (%)		
	At potting	After 7 days of incubation	
		Nonrhizosphere	Rhizosphere
Penicillium ochro-chloron Biourge	16.8	12.5	7.8
Chaetomium aureum Chivers	16.0	8.7*[a]	2.7*[a]
Penicillium griseofulvum Dierckx.	10.3	0.0*	10.8
Mortierella isabellina Oudem.	9.8	7.1	10.6
M. parvispora Linnem.	7.8	4.6	4.4
Ophiostoma stenoceras (Robak) Nannf.	4.5	6.9	0.0
Geomyces pannorum var. *pannorum* (Link) Sigler & Carmichael	4.1	1.4	0.0
Trichoderma hamatum (Bonord.) Bain.	3.4	13.8*	45.8*
Penicillium purpurogenum Stoll	3.3	1.8	0.0
Trichoderma koningii Oudem.	3.2	1.8	3.6
Mortierella vinacea Dixon-Stewart	1.4	2.8	2.7
Trichoderma harzianum Rifai	0.7	3.6	0.8
Mortierella alpina Peyronel	0.0	4.2	0.0
Mucor hiemalis Wehmer	0.0	7.1	0.8
Others	18.7	24.2	10.0
Number of colonies isolated	237	113	314

[a] Mean relative densities followed by an asterisk in a single row are significantly ($P = 0.05$) different from the mean at potting according to Duncan's new multiple range test.

Rather, some fungi were more abundant in suppressive than in conducive media. Second, suppressive media amended with CHB from different sources were quantitatively less similar than were suppressive and conducive media prepared with CHB from the same source. Thus, disease suppression in media amended with CHB from different sources cannot be associated with the abundance of a single taxon. Although *T. hamatum* was particularly abundant in suppressive media amended with field CHB, high relative numbers of *T. harzianum* characterized suppressive media amended with bin CHB.

Principal components analysis (PCA) revealed relationships among the various taxa based on their relative densities in media prepared from various batches of CHB. Results of PCA (Table 6) support observations apparent in the data summarized in Table 4. However, through PCA the association between just a few taxa and suppressiveness was more apparent. Factor loadings clearly delineated differences between suppressive and conducive media. These factor loadings produced principal components (Fig. 2) in which taxa most commonly associated with suppressiveness (or conduciveness) were well separated from the other taxa. Other relationships also were apparent, including the association between CHB source and taxa relative density. Although PCA has not been widely used in analysis of data by plant pathologists, our results support those of others who have found it valuable in examining distributions of fungi (7,8,44).

The association between high numbers of *Trichoderma* spp. and suppressiveness provides circumstantial evidence that these taxa are involved in disease suppression. Evidence that these fungi are active within the medium amended with CHB is provided by comparing sample populations isolated before and after potting (Table 7) and from *Rhizoctonia* inocula. Although *Trichoderma* species were present in low numbers in conducive media amended with CHB, they did not increase significantly during the 7-day assay for disease suppression, suggesting that their activity was limited in these media. Thus, conduciveness was not due to the absence of particular organisms but rather factors which restricted their growth or activity.

Trichoderma species have been implicated as the agents responsible for suppression of *Rhizoctonia* elsewhere. For example, Chet and Baker (5) reported that *Trichoderma* spp. (apparently a mixture of *T. hamatum* and *T. harzianum*; R. Baker, *personal communication*) were responsible for the natural suppression of *Rhizoctonia* in a Colombian soil. Parasitism of *Rhizoctonia* by *Trichoderma* spp. has been widely reported and the addition of *Trichoderma* at high densities to soils has frequently resulted in disease reduction (6,11–13,18–20,29).

Trichoderma spp. were not the only fungi isolated from media amended with CHB that have been reported as antagonists or parasites of *Rhizoctonia*. *Gliocladium virens, Gliocladium roseum*, and *Talaromyces flavus* were all isolated and have been reported as parasites of *Rhizoctonia* (1,3,41) or particularly abundant in *Rhizoctonia*-suppressive soils (24,25,35). With the exception of *Gliocladium virens*, however, these were found infrequently and sporadically in suppressive media. *G. virens*, along with *Trichoderma* spp., was isolated from *Rhizoctonia* inocula and thus, may be an active mycoparasite in media amended with field CHB and partially responsible for the rapid decline of viable inocula.

Differences in composition of *Trichoderma* populations isolated from CHB-amended media from different sources likely reflect differential abilities of species to colonize CHB. The abundance of *T. hamatum* in the higher pH field CHB and the association of *T. harzianum* with more acidic bin CHB agrees with observations on colonization of sterilized soils of different acidities by different species groups of *Trichoderma* (15,31). However, differences in acidity cannot account for the variation in suppressiveness and numbers of *Trichoderma* spp. in media prepared from CHB obtained from the same source.

A variety of factors may be responsible for the low relative densities of *Trichoderma* in the conducive batches. For example, appropriate nutrients may not have been available or the presence of competing or antagonistic organisms may have limited the availability of nutrients. High relative densities of *P. verrucosum* var. *cyclopium* and varieties of *G. pannorum* in both conducive batches may have a significant negative effect on the development of high *Trichoderma* populations. On the other hand, high populations of mycoparasitic *Trichoderma* spp. in CHB-amended media may require the presence of other fungi. For example, the appearance of *Trichoderma* in fungal successions associated with decaying plant litter appears to be regulated by prior colonization by other fungi which serve as hosts (14).

The fungi isolated from CHB-amended media may obviously interact in a variety of ways, not only with each other, but also with other organisms (eg, bacteria and actinomycetes) not described in this paper. The observation that there were associations between the abundance of some taxa with disease suppression provides a first step in understanding the nature of disease suppressive environments. Although high populations of *Trichoderma* spp. were associated with suppression, no single species could account for suppressiveness in media amended with CHB from different sources. Lack of disease suppression in some CHB-amended media may result from lower populations of *Trichoderma*, or from differences in antagonistic activity of those isolates found within conducive media. Determinations of the antagonistic activities of fungi from various CHB-amended media should provide evidence needed to evaluate their roles in disease suppression.

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