Suppression of *Rhizoctonia solani* in Potting Mixtures Amended with Compost Made from Organic Household Waste

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ABSTRACT

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Compost made from organic household and garden waste was used to substitute part of the peat in potting mixtures used for growing woody ornamental nursery stock. The effects of amendment with compost on the colonization of potting mixture by *Rhizoctonia solani* (AG1) were studied in greenhouse experiments. A bioassay was developed using cucumber as a sensitive herbaceous test plant as a substitute for woody ornamental cuttings. Pathogen growth in the potting mixture was estimated by measuring the distance over which damping-off of seedlings occurred. Compost from two commercial composting facilities suppressed growth of *R. solani* in potting mixtures with 20% of the product when the compost was fresh (directly after delivery) or long matured (after 5 to 7 months of

additional curing). In contrast, short-matured compost (1 month of additional curing) from the same batches stimulated pathogen growth. In vitro mycelial growth of *R. solani* on mixtures with mature compost was inhibited by microbial antagonism. Compost-amended potting mixtures responded differentially to the addition of cellulose powder; the effect on suppressiveness depended on curing time and origin of the compost. In long-matured compost, suppressiveness to *R. solani* was associated with high population densities of cellulolytic and oligotrophic actinomycetes. The ratio of the population density of actinomycetes to that of other bacteria was around 200-fold higher in mature suppressive compost than in conducive compost.

Additional keywords: *Bacillus*, fluorescent *Pseudomonas*, *Trichoderma harzianum*.

In the Netherlands, an increasing amount of compost is produced since the separate collection of organic household waste (vegetable, fruit, and garden waste [VFG]) became compulsory. The compost is used primarily in agriculture. Extension of the use of compost to the horticultural industry as a substitute for part of the peat in potting mixtures would reduce the amount of peat imported and the use of fertilizers in container-grown crops. Large amounts of potting mixture are used in the production of woody ornamental nursery stock.

An important feature of compost in any culture is its effect on pathogens already present in the soil or potting mixture. Composts prepared from many types of waste products (e.g., tree bark, licorice root waste, grape marc, cattle manure, and municipal sewage sludge) microbially suppress soilborne pathogens (19,27,30-32). At the onset of the current study, the only record of disease suppression by compost made from organic household waste, in this case amended with 25% wood chips, was that of Schüler et al. (42). In that study, the compost was added to pasteurized sand; consequently, the results had little relevance for application of compost to soil under commercial conditions. Different mechanisms of suppression have been described for different pathogens (20,49). Whereas suppression of *Pythium* spp. and other pathogens was a rather consistent phenomenon, the response of *Rhizoctonia solani* to the amendment of media with hardwood bark compost was not (7,8,28,29,33,34).

Use of VFG compost in potting mixtures for woody ornamental nursery stock necessitated a study of its effects on pathogens oc-

curing on these crops. *R. solani* is an important pathogen causing rot of cuttings of woody ornamentals (11). The long-term goal of this study was to determine the influence of VFG compost on the development of disease caused by *R. solani* in woody ornamentals. Because of the costs and season-dependent availability of the woody host plants, we looked for an alternative herbaceous test plant for a greenhouse bioassay. Furthermore, we studied whether disease suppression was influenced by additional maturation or curing of the compost. Microbial properties of the compost that might play a role in disease suppression were investigated. Preliminary results of part of this study were reported earlier (45).

MATERIALS AND METHODS

Inoculum and host plant. *R. solani* AG1 (isolate 55), originally isolated from diseased cuttings of *Cotoneaster* sp. at the Research Station for Nursery Stock in Boskoop and also pathogenic to *Pyracantha coccinea* 'Orange Glow', *Erica × darleyensis* 'Jack H. Brummage', and *Juniperus squamata* 'Meyeri' (11), was grown on malt extract agar (E. Merck AG, Darmstadt, Germany) at 24°C. Mycelial disks 5 mm in diameter were taken from the colony margin of a 3-day-old culture and used as inoculum. The isolate was identified as anastomosis group 1 by D. E. Carling according to Carling (3). Seedlings of a number of herbaceous plants were tested to serve as substitutes for woody ornamentals in the assay. A plug of mycelium was placed against the stem base of each plant. *R. solani* was reisolated from infected plant parts on water agar (E. Merck AG) with 50 mg of oxytetracycline hydrochloride (Sigma Chemical Co., St. Louis) per liter. Bean (*Phaseolus vulgaris* 'N.H. Bruine') and wheat (*Triticum vulgare* 'Florida') showed dark-brown lesions on the stem base. Lupine (*Lupinus angustifolius* 'Kubesa') stems turned brown and then damped-off. Stems of cucumber (*Cu-*

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cumis sativus 'Lange Groene') seedlings showed discoloration, soft rot, and constriction of the stem base, followed by damping-off. This occurred rapidly and on both young and older seedlings. Therefore, cucumber was chosen as the test plant for the greenhouse experiments.

Compost and container media. VFG compost was obtained from two commercial composting facilities (coded P and C) in the province of North-Holland in the Netherlands where the source-separated household waste (vegetable, fruit, and garden waste) was composted under standardized conditions in an enclosed environment. During the composting process, the material was turned and moistened (when below 45% wt/wt) each week. At facility P, processing time was 6 weeks, and the fresh compost was screened through a 17-mm sieve before delivery. At facility C, processing time was 11 weeks, and the fresh compost was screened through a 10-mm sieve.

We collected the fresh compost and did an additional curing (maturation) in the greenhouse at 20°C with weekly moistening and biweekly turnings of heaps of about 40 cm high in a 100-cm-diameter plastic tub. Moisture content was kept at 40 to 50% (wt/wt), corresponding with moisture tensions around pF 1.7 (–5 to –7 kPa). In the bioassays, compost was used (i) directly after delivery from the facility (fresh or freshly delivered compost), (ii) after a short period of additional maturation (short matured or 1-month matured), and (iii) after a long period of additional maturation (long matured or 5- to 7-months matured). Samples to be used in experiments were composed of at least 20 cores per heap of compost. Before use, compost P was sieved additionally by hand to remove the 10- to 17-mm fraction. Numbers after the code for the compost facility indicate different compost batches used (Table 1). Samples of the batches were sent to a laboratory for soil chemical analyses. On the basis of dry weight, the contents in the compost batches ranged from 26 to 36 mg of CaO per g, 20 to 30 mg of CaCO₃ per g, 12 to 19 mg of K₂O per g, 3 to 5 mg of Cl per g, and 6 to 9 mg of P₂O₅ per g. The pH values of the composts were between 7 and 8. Additional data are given in Table 1.

Potting mixtures were prepared with light peat (Kekkila Finnpeat AO; Kekkila, Tuusula, Finland) and horticultural grade perlite (Agra perlite nr 3; Pull, Rhenen, the Netherlands). The two types of mixtures consisted of (i) 85% peat (vol/vol), 15% perlite (vol/vol), 1.5 g of N-P-K mixture (14-16-18) per liter, and 1 g of lime (Dolokal; Ankersmit Maalbedryven, Maastricht, the Netherlands) per liter; and (ii) 65% peat, 15% perlite, and 20% compost. Mixing and moistening was done in a concrete mixer. In the first two bioassays performed with long-matured (5 and 7 months) compost, the mixtures were stored for 1 week at 20°C before use. For the next full series of assays with fresh, short-matured, and long-matured compost, the mixtures were stored 1 to 2 days before use. Moisture content of the substrates during storage was 70 to 80% (wt/wt). Acidity of the substrates ranged from pH_{H₂O} 5.0 to 6.0.

Bioassay for assessing suppressiveness to *R. solani*. In commercial nurseries, *R. solani* commonly grows over the surface of the soil in trays with cuttings covered with polyethylene sheets. Therefore, we developed a bioassay in which mycelial growth from a point source was measured under similar conditions. Shallow trays with a volume of 3.2 liters were evenly filled with the potting mixtures. The volumetric weight of the potting mixtures was determined, and seven replicate trays were filled to the same weight for each of the potting mixtures. Cucumber seeds were disinfested by shaking in a 1% hypochlorite solution (1 min) followed by a thorough rinse with tap water. Using tweezers, the seeds were placed at a depth of 0.5 cm in five rows of 16 seeds each. Sowing distance was 2.2 cm within the row and 4.5 cm between rows. Each tray was placed in a dish in a greenhouse at 20°C, with a photoperiod of 16 h and a relative humidity of 90 to 100%. The trays had holes in the bottom and were watered from below. Water potentials were kept at values between –2 and –4 kPa. After emergence of the seedlings (4 days after sowing), the first seedling in every row was inoculated by placing a plug of mycelium at the stem base. Every 2 days, the number of infected seedlings per row was counted, and damped-off plants were cut off at 1 to 2 cm above the soil. The average distance of pathogen colonization along rows per tray was calculated 2 to 3 weeks after inoculation.

The first bioassay was performed with long-matured (5 months) compost C1 and the second with long-matured (7 months) compost P1 and C1. Thereafter, a series of experiments was performed with fresh, short-matured (1 month), and long-matured (5 months) compost P2 and C2. Finally, a series was performed with fresh, short-matured (1 month), and long-matured (3 months) compost C4 (Table 1).

The effect of free cellulose was examined with the short-matured and long-matured P2 and C2 compost by including a treatment in which 2% (wt/wt on a dry weight basis of substrate mixture) cellulose (grade CF11; Whatman International, Ltd., Maidstone, England) was added.

Randomized complete block designs were used in all experiments. There were seven replicate trays per potting mix. Analysis of variance (ANOVA) was performed to assess the effect of adding compost. The difference between the two origins of compost was tested using orthogonal contrasts. There was a factorial design in the experiments in which the cellulose treatment was included. Statistical analysis was performed using Genstat V (release 3; Genstat 5 Committee, Harpenden, England).

In vitro assay of suppression of mycelial growth on potting mixtures. Growth of *R. solani* on cellophane-covered compost or potting mixtures was determined in an in vitro assay. The assay was a modification of the method of Van der Hoeven and Bollen (46) for measuring antagonistic activity of the soil microflora and the agar-ring method (47). The ratio between growth on nonsterile

TABLE 1. Characteristics of compost used in the bioassays

Compost batch ^a	Time of delivery	Moisture content at delivery (%) ^b	Fresh compost ^c			Short-matured compost ^d			Long-matured compost ^e		
			Organic matter (%)	Total nitrogen (mg g ⁻¹ d.w.) ^f	C/N ratio ^g	Organic matter (%)	Total nitrogen (mg g ⁻¹ d.w.) ^f	C/N ratio ^g	Organic matter (%)	Total nitrogen (mg g ⁻¹ d.w.) ^f	C/N ratio ^g
P1	September 1993	25	42	17.4	12.5	... ^h	...	...	32	17.2	9.7
C1	September 1993	45	34	13.4	13.1	...	...	...	26	14.6	9.1
P2	August 1994	34	33	14.5	11.8	30	15.6	9.9	27	13.0	10.9
C2	August 1994	33	29	12.8	11.8	26	13.2	10.3	31	15.3	10.4
C4	November 1995	22	41	14.6	14.6	35	16.4	11.2	34	15.3	11.4

^a P and C represent two different commercial composting facilities. The number after P or C indicates different batches of compost.

^b Percentage of the wet product.

^c Directly after delivery.

^d Duration of additional curing was 1 month.

^e Duration of additional curing in the greenhouse was 5 and 7 months for P1 and C1, 5 months for P2 and C2, and 3 months for C4.

^f d.w. = dry weight.

^g C/N ratio estimated using C as 52% of the organic matter content.

^h Not included.

and sterile substrate (twice autoclaved for 1 h) gives a measure of the influence of biological factors on mycelial growth. Because of the spatial separation by the cellophane membrane, only antibiosis and competition for nutrients, but not mycoparasitism, are involved in the microbial antagonism measured.

Sterilized metal rings with a 7-cm diameter and a height of 7 mm were covered on one side with wet cellophane (Cuprophane, thickness 17.5 μm ; Akzo Nobel, Wuppertal, Germany) held in place by a rubber strap. The rings with the cellophane facing downwards were autoclaved in glass petri dishes with 5 ml of distilled water. The rings were then filled completely with the test substrate. Twenty milliliters of lukewarm water agar (2%) was added to solidify the substrate. After allowing compounds to diffuse from the solid substrate to the membrane for 24 h at 20°C, the rings were turned and an inverted disk (5-mm diameter) from the edge of a growing colony of *R. solani* on malt extract agar was placed on the cellophane membrane in the center of the ring. The dishes were incubated at 24°C in the dark. Six dishes per treatment were used. Because of the fast growth of the fungus, the colony diameter and the hyphal density (number of hyphae crossing a unit of length at 1 cm distance from the disk) were measured after 24 h. The product of the area of the colony (calculated from the measured radius) and the hyphal density was used as a parameter for mycelial growth. Relative values were calculated after randomly pairing a nonsterile with a sterile substrate. These data were analyzed using ANOVA.

Microbial analyses. *Bacterial and fungal populations.* The density of culturable groups of microorganisms, among which some that are known to contain antagonists (24,25,38), was determined. For the analyses of long-matured suppressive compost, two composite samples of each of the composts (P2 and C2, 5 months old) and of the peat-perlite mix were taken. At the end of the bioassay, four composite samples of the bioassay mixtures, with or without long-matured P2 and C2 compost, were taken (replicate trays). For the series of analyses of compost C4—fresh, 1-month matured, and 3-months matured—three composite samples per substrate were taken from the compost before use and also from the mixtures in additional trays of the bioassay at 10 days after inoculation of the cucumber seedlings.

To determine the population densities of different groups of bacteria and fungi, a sample of moist substrate corresponding to 2.5 g dry weight was suspended in 250 ml of 0.1% sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) in a 1-liter Erlenmeyer flask. The suspension was shaken for 30 min at 150 rpm (shaker type R05; Gerhardt, Bonn, Germany). Ten-fold dilution series were prepared with 0.1% water agar (Agar technical nr 3; Oxoid, Basingstoke, England), and 0.1 ml of the suspensions was pipetted onto five replicate plates per culture medium per dilution. Three or four dilutions were plated per series.

Copiotrophic (zymogenous) bacteria were isolated on nutrient agar (Oxoid) amended with 100 μg of cycloheximide (E. Merck AG) per ml. *Bacillus* spp. also were isolated on full-strength nutrient agar after suspensions were heated for 10 min at 80°C. Fluorescent pseudomonads were isolated under UV light after plating on King's B agar (E. Merck AG) amended with 100 μg of cycloheximide per ml, 50 μg of ampicillin (E. Merck AG) per ml, and 12.5 μg of chloramphenicol (Boehringer GmbH, Mannheim, Germany) per ml (16). Oligotrophic actinomycetes and bacteria were isolated on 0.01-strength nutrient agar (5,35) with 100 μg of cycloheximide per ml. Actinomycetes were distinguished from bacteria by examination under a dissecting microscope; a second evaluation was made after longer incubation. Cellulolytic actinomycetes and bacteria were isolated on cellulose agar consisting of 20 mg of fibrous cellulose powder (grade CF11; Whatman International, Ltd.) per ml; 1 mg of $(\text{NH}_4)_2\text{SO}_4$ and CaCO_3 per ml; 0.5 mg of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, NaCl, and K_2HPO_4 per ml; 100 μg of cycloheximide per ml; and 15 mg of agar per ml. Fungal counts were made on potato dextrose agar amended with 50 μg of oxytetracycline hydrochloride (Sigma Chemical Co.) per ml and 100 μg of valida-

mycin (Solacol liquid, 30 g a.i. liter⁻¹; Hoechst Schering AgrEvo, Haren, the Netherlands) per ml to restrict colony size and increase recovery (15). All plates were incubated at 24°C in the dark. The incubation periods were 1 week for the copiotrophic bacteria, 2 days for *Bacillus*, 2 weeks for the oligotrophic and cellulolytic microorganisms, 2 days for fluorescent pseudomonads, and 1 week for the fungi. The colony counts were converted to colony forming units (CFU) per gram dry weight of substrate.

ANOVA was performed on the log-transformed population densities. Analyses were made of (i) data of peat and long-matured (5 months) P2 and C2 compost; (ii) data of bioassay mixtures with or without long-matured P2 and C2 compost; and (iii) data of fresh, short-matured, and long-matured (3 months) C4 compost, used pure or as a bioassay mixture. Ratios of population densities were transformed and analyzed by ANOVA.

Trichoderma: Presence and establishment. *Trichoderma* spp. are known antagonists of *R. solani* and play an important role in the suppressive effects of composted hardwood bark (20). If *Trichoderma* were active in VFG compost, they would be present as mycelium rather than as spores. Therefore, we used the soil-washing method that is specific for quantifying fungi that occur as mycelium. A sample of compost equivalent to 5 g dry weight was washed through nested sieves with 1- and 0.5-mm mesh using a strong jet of water three times each for 30 s on the upper 1-mm sieve and an additional 30 s on the 0.5-mm sieve. The fraction contained on the smallest sieve was then washed three times for 1 min using a Vibro Mischer (Chemap AG, Männedorf, Switzerland). The sieve was placed on a wire frame in a 500-ml beaker with water that was renewed after every washing. The Vibro Mischer is fitted with a high-speed vibrating bar with a punched flat end, which whirls the soil particles in the water above the sieve. The third washing was done with sterile water. Soil particles were collected on sterile filter paper. For each sample, 100 organic particles were placed on a medium selective for *Trichoderma*, modified from Papavizas and Lumsden (37). The following antimicrobial agents were added to the basal medium: oxytetracycline hydrochloride (50 μg ml⁻¹, as a substitute for the four bacteriostatic agents used by Papavizas and Lumsden [37]), nystatin (20 μg ml⁻¹; Duchefa, Haarlem, the Netherlands), and sodium propionate (500 μg ml⁻¹; BDH Laboratory Supplies, Poole, England). As the fungicide chloroneb was no longer available from the producer, we used captan (20 μg ml⁻¹, 83% wettable powder; Ligtermoet Chemie, Roosendaal, the Netherlands). Captan was used in a *Trichoderma*-selective medium described by Smith et al. (44). Growth and spore germination of an isolate of *T. harzianum* were not affected on the captan-amended medium. Pentachloronitrobenzene (PCNB) was omitted, and for inhibition of rapidly growing fungi, Tergitol NP-10 (1 μl ml⁻¹; Sigma Chemical Co.) was added instead of alkyl alkaryl polyether alcohol. The petri dishes were incubated at 24°C in the light. The number of *Trichoderma* colonies was counted after 1 and 2 weeks. For identification, isolations were transferred to malt extract agar with 50 μg of oxytetracycline per ml. Two samples per batch were taken from long-matured (5 months) compost P2, C2, P3, and C3, peat-perlite mixture, and substrates at the end of a bioassay; nonamended peat-perlite mixture; and mixtures with 20% long-matured P2 and C2.

An experimental wettable powder formulation of *T. harzianum* (code 3003; Bioworks, Geneva, NY) was obtained to determine if *Trichoderma* could establish itself in the compost. The preparation contained 1×10^9 CFU g⁻¹. Fifteen milligrams was added to 500 ml of fresh compost C5 (39% moisture, wt/wt) in a 1-liter Erlenmeyer flask. As a control, a sterilized sample of the *Trichoderma* preparation was added. Three replicates were used per treatment. Samples were taken after 1 and 17 weeks of incubation at 24°C and analyzed with the soil-washing method.

Assays of compost stability. *Self-heating potential.* Compost stability depends on the degree to which the biodegradable organic matter in the composting material has been diminished (22).

Stability can be determined by measuring the parameters that result from microbial activity. When compost is mixed, the increased aeration will cause a resurgence of the temperature due to microbial activity and mainly depending on the amount of readily biodegradable compounds and moisture conditions. The maximum temperature reached can be used as a measure of the stability or maturity of the compost, according to the German classification that divides compost into five 'Rottegrades' from I for immature compost to V for mature compost (1). Thermal insulated vessels (Dewar vessels; KGW Isotherm, Schwerin, Germany), three per sample, were filled with 1 liter of compost (water potential -5 to -7 kPa; moisture content 40 to 50% wt/wt). Thermocouples were placed in the lower third of the vessel, and temperature was recorded every 10 min until a maximum was reached. The range of maximum temperatures ($^{\circ}\text{C}$) per stability class was 60 to 70 (I), 50 to 60 (II), 40 to 50 (III), 30 to 40 (IV), and 20 to 30 (V).

Fluorescein diacetate (FDA) hydrolysis. Estimation of the rate of hydrolysis of FDA is a rapid method for the assessment of general microbial activity in soils (41). We used a procedure slightly modified from Inbar et al. (23). A compost sample (1 g dry weight) at a moisture content of 40 to 50% was placed in a 100-ml Erlenmeyer flask. Ten milliliters of potassium phosphate buffer and 400 μg of FDA (Sigma Chemical Co.) in 0.2 ml of acetone were added. For each treatment, three replicates and one control without FDA were used. Reaction flasks were shaken (100 rpm) for 20 min in a water bath at 25°C . The reaction was ended by adding 10 ml of acetone to the suspension. The suspension was then filtered through a S&S folded paper filter (type 595 $^{1/2}$; Schleicher & Schuell, Inc., Dassel, Germany). The optical density of the filtrate was read at 490 nm using a Beckman DU-64 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The concentration of fluorescein in the filtrate was calculated by using a calibration line fitted for each compost sample analyzed. Data for the calibration lines were based on a series in which fixed amounts of FDA from the stock solution were added to 2.5 ml of phosphate buffer in screw-capped tubes in duplicate for each concentration. The tubes were tightly closed and kept for 60 min in boiling water to hydrolyze FDA. The content of the tubes was

then added to Erlenmeyer flasks containing 1-g (dry weight) samples of compost. The fluorescein was completely washed from the tubes using another 2.5 and 5.0 ml of buffer. The content of the Erlenmeyer flasks was then treated as described before; shaking, addition of acetone, filtering, and measuring of the absorbance. Whereas none of the composts posed problems in the analysis, the mixtures of compost and peat could not be analyzed because even a high dose of 400 μg of fluorescein to a 1-g dry weight sample was completely adsorbed to the organic matter of the peat. Inbar et al. (23) encountered the same problem with some of the peats that they used.

RESULTS

Effect of compost on disease incidence. Amendment of peat-perlite potting soil with 20% VFG compost suppressed growth of *R. solani*, depending on the degree of maturation of the compost (Fig. 1). Freshly collected compost of both installations conferred suppressiveness, whereas compost used after a short maturation period stimulated pathogen growth and infection. Long-matured compost consistently suppressed the pathogen. The growth rate of *R. solani* in the nonamended control substrate was not the same in the different experiments. To enable comparison of the different assays, distances of growth on compost-amended substrates were related to growth in the corresponding nonamended substrate. The relative values are presented in Figure 1. Data of the different experiments were analyzed separately; the *F* probabilities of the variance ratios of treatments are given in Table 2.

Effect of the addition of cellulose to compost on disease incidence. There was a significant interaction between added cellulose and the origin of the short-matured compost present ($P < 0.001$). Cellulose amendment conferred suppressiveness in the presence of short-matured compost C, but resulted in conduciveness in short-matured compost P (Table 2, Fig. 2). There was no significant interaction between cellulose and amendment of long-matured compost.

In vitro antagonism. Mycelial growth of *R. solani* was significantly decreased in the substrates with 20% of the long-matured

Fig. 1. The influence of amendment of 20% compost into potting soil on the growth of *Rhizoctonia solani* AG1 in the cucumber bioassay. The distance of pathogen growth was determined on the basis of the length of a row of cucumber seedlings that became infected when the first seedling in a row was inoculated. Batches of compost differed in origin (P and C) and in duration of additional curing (0, 1, 3, 5, and 7 months [m]). In the inset, the age of the long-matured compost is given between parentheses. Pathogen growth is expressed as a percentage of the distance of growth in the corresponding nonamended potting soil. Asterisks indicate a significant difference between growth in compost-amended versus nonamended substrate (*, **, and *** significant at $P = 0.05$, $P = 0.01$, and $P = 0.001$, respectively) (Table 2). N = not determined.

(4 months) P1 and C1 compost ($P = 0.07$) and the long-matured (2 months) P2 and C2 compost ($P < 0.001$) as compared with growth on nonamended peat (Fig. 3). This implies that in these compost-amended substrates the antagonistic activity or soil fungistasis is higher than in nonamended peat. Hyphal density of *R. solani* was affected more than radial extension of the colony.

Microbial populations in compost and compost mixtures. In the long-matured composts P2 and C2 (5 months) that were suppressive to *R. solani*, densities of actinomycetes, especially those growing on cellulose medium, were higher than in the three younger composts (C4) and peat (Fig. 4A). Densities of other bacteria were lowest in mature compost and peat (Fig. 4B). The relatively low

bacterial densities in fresh compost compared with short-matured compost could be caused by the dry conditions under which this product was sieved and delivered by the composting installation. Population densities were determined before the compost was moistened for additional curing. A significant difference between the long-matured composts P2 and C2 was only recorded for population densities of *Bacillus* spp. (Table 3). Overall, microbial populations were lower in the bioassay mixtures than in the pure compost (Fig. 4C and D), except for the fungi (Fig. 4C). Fluorescent *Pseudomonas* spp. were not detected in the pure substrates ($\leq 10^4$ CFU g⁻¹), but increased in the mixture during the bioassay. Mean densities in compost-amended mixtures and peat were 10^7 CFU g⁻¹ and 0.4×10^5 CFU g⁻¹, respectively. For both 5-month-matured composts, a 10-fold higher density of oligotrophic bacteria was found in the mixture than in the pure composts (Fig. 4A and C).

When ratios of population densities of specific groups in the microflora are considered, there were large differences between the suppressive 5-month-matured composts and the shorter-matured ones and peat (Fig. 5). The mean ratio of cellulolytic actinomycetes to cellulolytic bacteria in the 5-month-matured compost was 160 to 550 times higher than those in the other substrates. For the oligotrophic populations, the pattern was similar. The ratio between cellulolytic and copiotrophic bacteria was smallest in the two mature composts. The same was found for the ratio of oligotrophic and copiotrophic bacteria in pure substrates, but not in the bioassay mixtures.

Presence and establishment of *Trichoderma* spp. In long-matured (5 months) composts, less than 1% of the organic particles

Fig. 2. The influence of adding cellulose and compost to potting soil on the growth of *Rhizoctonia solani* in a cucumber bioassay. The distance of pathogen growth was determined on the basis of the length of a row of cucumber seedlings that became infected when the first seedling in a row was inoculated. Pathogen growth is expressed as a percentage of the distance of growth in the corresponding nonamended potting soil. Composts differed in origin, P2 and C2, and in duration of additional curing; **A**, 1-month matured, and **B**, 5-months matured. Bars with the same letter are not significantly different ($P = 0.05$, LSD_{interaction}). F probabilities of main effects and interactions are given in Table 2.

Fig. 3. Inhibition of in vitro mycelial growth of *Rhizoctonia solani* on peat amended with 20% compost as determined by means of the agar-ring method. The relative parameter of mycelial growth (growth on nonsterilized substrate divided by that on sterile substrate) on nonamended peat is set at 100%. Additional maturation period of compost batches is presented in months, except for the first bar with P1 (1 week). Values on top of the bars are F probabilities of significance of difference with nonamended peat. The difference between 4-months-matured P1 and C1 was not significant ($P = 0.3$), neither was that between 2-months-matured P2 and C2 ($P = 0.6$).

TABLE 2. Significances (F probabilities) in analysis of variance of the distances of growth of *Rhizoctonia solani* in the different bioassays performed

Source of variation ^a	Composts tested in the different bioassays ^b							
	P1 (5 m) ^c	P1, C1 (7 m)	P2, C2 (0)	P2, C2 (1 m)	P2, C2 (5 m)	C4 (0)	C4 (1 m)	C4 (3 m)
Compost amendment	<0.001	0.005	0.014	0.036	<0.001	<0.001	0.181	0.160
Compost $\times$ type ^d		0.692	0.637	<0.001	0.121			
Cellulose amendment ^e				0.281	0.153			
Cellulose $\times$ compost				0.780	0.272			
Cellulose $\times$ compost $\times$ type				<0.001	0.304			

^a The main effect of compost amendment is tested, a difference between composts within one experiment occurs when compost $\times$ type interaction is significant ($P < 0.05$).

^b Bioassays are indicated by the codes of the composts tested in each experiment (peat amended with 20% compost, vol/vol, compared with nonamended peat). P and C represent two different commercial composting facilities. The number after P or C indicates different batches of compost.

^c Duration of additional curing (months [m]) in the greenhouse after collection of the fresh compost.

^d Type represents the composting facility.

^e Amendment of 2% cellulose (wt/wt on a dry weight basis) to the substrate mixtures. The cellulose treatment was included in two experiments.

were colonized by mycelium of *Trichoderma*, whereas in peat and peat-based mixtures, 2.5 and 4 to 12% were colonized (Table 4). In a batch of fresh C compost, introduced *T. harzianum* readily established itself. One week and seventeen weeks after inoculation, 55 (± 7) and 72 (± 10)%, respectively, of the organic particles were colonized by the fungus. *T. harzianum* or other *Trichoderma* species were not detected in the control treatment in which sterilized inoculum had been added to the compost.

Stability of the compost based on general microbial activity. Microbial activity of the freshly delivered composts was higher than that of compost after additional maturation (Table 5). This was apparent from the maximum temperature in self-heating. Class V (20 to 30°C) was reached after short curing periods. The classification based on the maximum temperature reached in self-heating was too broad to distinguish between short-matured and long-matured compost.

The relative activity determined by FDA hydrolysis differentiated between composts of different maturation periods, but underestimated the actual activity of freshly delivered composts that were stored cool for 2 to 4 weeks before analysis. Avoiding storage of fresh, immature compost or longer preincubation at room temperature might overcome this problem.

DISCUSSION

Mature VFG compost conferred suppressiveness to *R. solani* to a peat-perlite potting soil. In mixtures with long-matured (5 to 7

months) compost, growth of the pathogen was reduced by 30 to 70%. However, short-matured (1 month) compost even stimulated pathogen growth. This stage was preceded by an initial suppressiveness of the freshly delivered compost. The results with long-matured VFG compost are in line with those of Schöler et al. (42). In their experiments, compost was added to pasteurized sand, whereas in our experiments, a nonsterile commercial peat mixture was used. Development of suppressiveness to *R. solani* with increasing age of the compost was also found with hardwood bark compost (34) and municipal sludge compost (27). However, the change from suppressive to conducive and, subsequently, back to suppressive was not observed before. Additional maturation does not necessarily lead to suppressiveness; composted yard trimmings did not turn suppressive in 4 months (18).

Suppressiveness to *R. solani* was established using a dose of 20% (vol/vol) compost. With hardwood bark compost, it was recorded at 50% (vol/vol) (32,33) and with municipal sludge compost at 25% (27). The use of increasing amounts of composted hardwood bark (14, 25, 36, and 50% wt/wt) resulted in a proportional increase in suppressiveness (32). Commercial applications of the current VFG compost can generally not exceed 20%, because the salt content affects the growth of woody ornamentals (A. A. Pronk and T. G. L. Aendekerk, unpublished data).

We used the mixtures of VFG compost and peat 1 day after mixing, while another 4 days elapsed until the cucumber seedlings had emerged and inoculum of *R. solani* was introduced into the substrate. Kuter et al. (27) found that conducive mixtures of com-

Fig. 4. Population densities of different groups of microorganisms in compost and peat. Values are back-transformed mean log values. **A and B,** The pure (unmixed) substrate components (compost batches and peat); and **C and D,** the substrate mixtures with 20% compost or 0% compost (peat) sampled during or after the bioassay. **A and C,** Oligotrophic and cellulolytic actinomycetes and fungi; **B and D,** Copiotrophic, oligotrophic, and cellulolytic bacteria; *Bacillus* spp.; and fluorescent *Pseudomonas* spp. Population densities in 0-, 1-, and 3-month-matured C4 (0-C4, 1-C4, and 3-C4, respectively) were significantly different, as were those in pure and in substrate mixtures (Table 3). In 5-months-matured compost (5-C2, 5-P2), densities of all microbial populations differed from those in peat, except for the fungi in substrate mixtures. Except for *Bacillus* spp., densities of microorganisms in batch P2 did not differ from those in C2 (Table 3).

posted sewage sludge and peat turned suppressive when they had been stored 3 to 4 weeks before use. We did not assess the effect of preincubation of the mixtures of VFG compost and peat on the level of suppressiveness.

The suppressiveness of the freshly delivered VFG compost to *R. solani* was unprecedented. The smell and the high temperature of self-heating of the products indicated that compost delivered straight from the composting installation was unstable and not marketable. The high microbial activity in the fresh compost probably induced suppressiveness in the immature product. Abiotic factors should not be discounted at this stage. In unstable compost, am-

monia or organic acids might be present at levels toxic to the pathogen. In conclusion, the level of maturation or stability of VFG compost determines its suppressiveness to *R. solani*.

It is well known that compost or other organic amendments can affect disease caused by *R. solani* differently (7–9,36). *R. solani* can utilize cellulose, but its ability to do so in soil depends on the resident microflora and the accessibility of cellulose, which, in its turn, depends on the degree of decomposition of the cellulose-containing organic matter. In lignified substrates, a large part of the cellulose is protected from decomposition because it is enclosed in lignin, which is relatively resistant to microbial decomposition.

TABLE 3. Significances (*F* probabilities) in analysis of variance of the population densities (log-transformed) and of ratios of specific population densities in compost of different maturity and in peat

Parameter	Peat and two 5-months-matured composts, P2 and C2 ^a				Compost C4, three different maturation periods ^b		
	Pure (base substrates) ^c		Bioassay mixtures ^d		Pure vs. bioassay (condition)	Maturation period	Condition × maturation period
	Compost vs. peat ^e	P2 vs. C2	Compost vs. peat	P2 vs. C2			
Population densities							
Copiotrophic bacteria	<0.001	0.795	<0.001	0.656	<0.001	<0.001	<0.001
Oligotrophic bacteria	0.041	0.664	<0.001	0.388	<0.001	<0.001	<0.001
Cellulolytic bacteria	<0.001	0.441	<0.001	0.341	<0.001	<0.001	<0.001
<i>Bacillus</i>	<0.001	0.001	<0.001	0.008	<0.001	<0.001	<0.001
Fluorescent <i>Pseudomonas</i>			<0.001	0.575			
Oligotrophic actinomycetes	0.004	0.664	<0.001	0.112	<0.001	<0.001	0.008
Cellulolytic actinomycetes	<0.001	0.154	<0.001	0.168	<0.001	<0.001	0.020
Fungi	0.029	0.648	0.341	0.327	<0.001	<0.001	<0.001
Ratios of population densities							
Oligotrophic actinomycetes/bacteria	0.003	0.007	0.001	0.030	0.289	0.151	<0.001
Cellulolytic actinomycetes/bacteria	0.009	0.207	<0.001	0.047	0.166	0.052	0.010
Oligotrophic/copiotrophic bacteria	<0.001	<0.001	0.122	0.433	0.117	0.067	0.011
Cellulolytic/copiotrophic bacteria	0.012	0.305	<0.001	0.470	0.020	0.069	<0.001

^a P and C represent two different commercial composting facilities. The number after P or C indicates different batches of compost. Statistical analysis was performed separately for pure substrates and for mixtures used in the bioassay.

^b Statistical analysis of the data of pure compost and bioassay mixtures using fresh, short-matured (1 month), and long-matured (3 months) compost, three replicates per substrate. Nonamended peat was not included.

^c Analysis of two replicate samples per substrate.

^d Four replicate samples were taken from substrates after use in the bioassay.

^e Effects and linear contrasts tested.

Fig. 5. Ratio of population densities of specific groups of microorganisms in compost and peat. Composts differed in origin (P and C) and in duration of additional curing (0, 1, 3, and 5 months [m]). **A**, Cellulolytic actinomycetes versus bacteria; **B**, oligotrophic actinomycetes versus bacteria; **C**, cellulolytic versus copiotrophic bacteria; and **D**, oligotrophic versus copiotrophic bacteria. **A** and **B**, The ratios for 0-C4, 1-C4, and 3-C4 are ≤ 0.01 . Bars with the same letter, for each appropriate comparison (a-c, p-r, and x-z) and protected by a significant *F* probability (Table 3), are not significantly different (LSD, *P* = 0.05).

In our experiments, the effect of the addition of extra cellulose on the growth of *R. solani* depended on the presence, the type, and the age of VFG compost in the potting soil. The amount of the extra cellulose added (2% wt/wt on a dry weight basis) was based on the content of cellulose in 20% of the fresh compost. Freshly delivered compost contained 9.3% cellulose and 13.5% lignin on a dry weight basis (G. Tuitert, unpublished data). *R. solani* was suppressed in cellulose-amended peat, which corresponds with results of Kundu and Nandi (26) and Rouse and Baker (40), who found suppression of *Rhizoctonia* damping-off using amendments of 1 or 2.5% cellulose to soil, respectively.

In combination with short-matured compost, in itself conducive to *R. solani*, the addition of cellulose either led to the enhancement of conduciveness or to suppressiveness, depending on the type of compost. The difference in response might be related to differences in density or composition of the cellulolytic microflora or to differences in levels of nitrogen or other nutrients. Nitrogen content was about 15% higher in compost P2 than in C2, but the C/N ratio differed only slightly. Microbial activity, as measured by CO₂ production, was increased after the amendment of cellulose to mixtures of 20% short-matured compost and peat, and more strongly with compost P2 than C2 (G. Tuitert, unpublished data). This difference in CO₂ production following cellulose amendment perhaps reflected a difference in microflora composition. Apparently, there is a delicate balance between availability of cellulose and suppressiveness or conduciveness of the growing medium.

In growing medium with long-matured suppressive compost, the addition of cellulose did not significantly affect the suppressiveness of the two composts. Apparently, microbial antagonism in long-matured composts prevents *R. solani* from profiting from the extra cellulose. The natural cellulose content of long-matured VFG compost (4.9% on a dry weight basis) was about half that of fresh compost, whereas the lignin content was not different from fresh compost (G. Tuitert, unpublished data). In media with long-matured composted bark, the addition of 5% extra cellulose either decreased (7) or increased disease severity (8).

Suppression of *R. solani* by organic amendments has been correlated with increased antagonistic soil microbial activity (21). From bark compost, antagonistic microorganisms were isolated that were able to induce suppressiveness in conducive bark media. Nelson et al. (34) identified *Trichoderma* spp. as the most effective fungal antagonists. Kwok et al. (29) identified different bacteria, such as *Bacillus cereus*, *Pseudomonas* spp., and *Flavobacterium balustinum*, that induced suppression to *R. solani* alone or in synergistic interaction with *Trichoderma* spp. No relationship between population levels (as determined by plating out) of *Trichoderma* and suppression was observed; induction of suppression by this antagonist was influenced by the age of the compost used in the potting soil (34). In the two types of VFG compost, we failed to detect mycelium of *Trichoderma* spp. using soil-washing.

TABLE 4. Colonization of organic particles (100 per sample) by mycelium of *Trichoderma* spp. in long-matured compost and potting mixtures (20% compost) as determined by the soil-washing method

Substrate ^a	Colonization (%) of organic particles with <i>Trichoderma</i> ^b	
	Pure substrates	Mixtures after bioassay ^c
Compost P2	0	4 (3–5) ^d
Compost C2	0	5 (4–6)
Compost P3	0	...
Compost C3	0	...
Peat-perlite	2.5 (0–5)	12 (4–21)

^a P and C represent two different commercial composting facilities. The number after P or C indicates different batches of compost. The composts were analyzed after 5 months of additional curing in the greenhouse.

^b Two replicates per substrate were analyzed.

^c Samples of potting mixtures were taken at the end of the bioassay.

^d Range of the colonization percentage.

^e These composts were not used in a bioassay; therefore, data are lacking.

Apparently, in the suppression of *R. solani* by VFG compost and in the interaction with additional cellulose, *Trichoderma* does not play the major role that has been attributed to it in bark compost (20). However, since it was shown that, upon introduction of a biocontrol preparation, *T. harzianum* colonized fresh VFG compost, this antagonist might have potential for production of a fortified VFG compost.

Suppression of *R. solani* can be caused by antagonism induced by various types of microflora such as cellulolytic microorganisms, *Bacillus* spp., or *Pseudomonas* spp. Long-matured compost suppressive to *Rhizoctonia* contained higher densities of actinomycetes and lower densities of other bacteria than did conducive short-matured compost. The ratio of the number of cellulolytic actinomycetes to that of cellulolytic bacteria was a distinct separator of mature suppressive compost from the other substrates and might be a useful parameter for that, regardless of possible differences in recovery between actinomycetes and other bacteria due to the plating procedure. The role of cellulolytic actinomycetes in suppression merits further study. Workneh and van Bruggen (48) recorded positive correlations between total numbers of actinomycetes and the proportion of cellulolytic ones with suppressiveness of soil to *Pyrenochaeta lycopersici*. The densities of oligotrophic microorganisms in the different compost samples reflected those of the cellulolytic ones, which might support the suggestion of Ohta and Hattori (35) that oligotrophs live in association with cellulose decomposers on organic debris.

Densities of copiotrophic, oligotrophic, and cellulolytic bacteria (not including the actinomycetes) were high in young compost, and lower in long-matured compost. The decrease in bacterial density, associated with an increase in metabolic diversity, during maturation is well known (2). Because densities of oligotrophic bacteria were 10-fold higher in the mixtures with suppressive long-matured compost than in the pure compost, these bacteria might be involved in suppression. In the compost-peat mixtures sampled after 10 or 20 days of use in the bioassay, differences between populations reflected those found for the pure composts. Only the fluorescent pseudomonads, hardly detected in pure compost and peat, had increased to high densities in all compost-amended mixtures, and, therefore, are not expected to play an important role in suppression. This observation shows the necessity of performing in vivo bioassays to draw relevant conclusions on suppression. When recording an increase in the number of antagonistic bacterial isolates in the rhizosphere after the amendment of compost to soil

TABLE 5. Characterization of the microbial activity in batches of vegetable, fruit, and garden waste (VFG) composts after different times of additional maturation in the greenhouse

		Classification of stability (I–V) according to self-heating ^b		Relative enzyme activity in FDA hydrolysis (%) ^c			
Compost ^a	Fresh	Short- matured (2 wk.)	Short- matured (1 mo.)	Fresh	Short- matured (2 wk.)	Short- matured (1 mo.)	Long- matured (5 mo.)
P1	II (52) ^d	V (21)		100		76	
C1	I (71)	IV–V (30)	V (21)	100		87	
P2	II (55)	V (29)	V (20)	67 ^e	92	51	24
C2	IV–V (31)	V (22)	V (21)	18 ^e	69	29	19
C4	V (26)	V (20)	V (20)	21 ^e		83	34 ^f

^a P and C represent two different commercial composting facilities. The number after P or C indicates different batches of compost. The composts were analyzed by measuring the maximum temperature (°C) attained during self-heating and the relative enzyme activity in fluorescein diacetate (FDA) hydrolysis.

^b Range of maximum temperatures (°C) per stability class, from unstable to stable: 60 to 70 (I), 50 to 60 (II), 40 to 50 (III), 30 to 40 (IV), and 20 to 30 (V).

^c Activity as percentage of the maximum amount of hydrolyzed FDA per unit weight and time, determined after 20 min.

^d Maximum temperature (°C).

^e Samples were moistened and then stored at 4°C for 2 weeks (P2 and C2) or 4 weeks (C4). They were incubated for 4 h at room temperature before analysis.

^f Analysis after 3 months of additional maturation.

(10), data for the nonamended soil and on in vivo suppressiveness of compost-amended compared with nonamended soil should be presented. With fresh compost, the number of fungal propagules was increased 100-fold after mixing, so intensive fungal growth might have been involved in suppressiveness of fresh compost.

For control treatments, we did not include amendments of sterilized compost to peat, because the addition of sterile compost, rich in nutrients released from the dead biomass, to a nonsterile substrate will lead to rapid recolonization of the compost. Thus, instead of excluding the initial biological factors, new ones are introduced. Perhaps, this phenomenon was not recognized in a study on Fusarium wilt in which suppression in compost-amended soil was attributed to the zymogenous soil microflora and stated that the compost microflora had no significant influence (13). Serra-Wittling et al. (43) recorded that, in soil amended with sterilized compost, bacterial population density and total production of CO₂ were higher than in soil amended with nonsterile compost for up to 60 days. In the agar-ring assay, we found higher antagonistic activity towards *R. solani* in peat amended with matured compost than in non-amended peat. Thus, competition, antibiosis, or both contributed to the suppressiveness exerted by mature compost, as spatial separation of pathogen and substrate excluded parasitism. The effect in this in vitro test was smaller than that in the bioassay. In vivo, parasitism might have been involved or the antagonistic potential based on the other interactions is not fully expressed in the agar-ring method.

Several biological and chemical methods for the characterization of maturity or stability of compost have been described (4,6,14, 22), but not all have prospects for correlation with *Rhizoctonia*-suppressive properties. The maximum temperature of self-heating did not discriminate between short-matured conducive and long-matured suppressive VFG compost. Low relative microbial activity as measured in an assay on the rate of FDA hydrolysis might indicate that the compost is sufficiently matured. Care should be taken when samples of unstable compost have been stored at 4°C until analysis. In these samples, the level of microbial activity is low, probably due to a decrease of the population and not because of a lack of easily decomposable nutrients, and the actual stability of the compost is, therefore, underestimated. Also, the rate of FDA hydrolysis might not be sufficiently discriminative, as the relative activity for conducive short-matured C2 compost corresponded with that for suppressive long-matured P2 compost. Probably, the ratio of the population densities of cellulolytic actinomycetes to cellulolytic bacteria is an adequate parameter to distinguish suppressive long-matured compost or compost mixtures from conducive substrates. The influence of available cellulose on suppressiveness indicates that cellulolytic activity in compost, e.g., measured as the CO₂ response to cellulose amendment, can be considered as an indicator of compost maturity and suppressiveness to *R. solani*. The content of cellulose, as determined by the laborious methods of Goering and Van Soest (17), could discriminate long-matured VFG compost from freshly delivered compost (49 versus 93 g kg⁻¹ dry matter [dm]), but not from wood-enriched long-matured compost (75 g kg⁻¹ dm) (G. Tuitert, unpublished data). As mentioned before, in a lignified substrate, part of the cellulose is enclosed in lignin, therefore, the ratio of cellulose and lignin content will better reflect the availability of cellulose. This ratio gave a distinct value for fresh VFG compost (0.7) compared with long-matured VFG compost (0.3 to 0.4) and wood-enriched compost (0.3) (G. Tuitert, unpublished data). However, this laborious method is not very practicable for routine use (6) and, moreover, it might need modification because humic acids can interfere. In conclusion, although the ratio of cellulose to lignin seems to be a promising indicator of suppressiveness, a more direct relationship may exist between suppressiveness and activity and composition of the compost microflora. A study of the population of cellulolytic actinomycetes merits special attention. In further investigations to unravel the biological causes of suppression of *R. solani* in mature VFG compost, the use of

temperature-gradient gel electrophoresis (12,39) shows good prospects for characterizing the microbial composition and diversity and for identifying specific groups and determining their activity.

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