

Microbial Properties of Composts That Suppress Damping-Off and Root Rot of Creeping Bentgrass Caused by *Pythium graminicola*

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Composts prepared from a variety of feedstocks were tested for their ability to suppress seedling and root diseases of creeping bentgrass caused by *Pythium graminicola*. Among the most suppressive materials in laboratory experiments were different batches of a brewery sludge compost and a biosolids compost from Endicott, N.Y. Batches of these composts that were initially not suppressive to *Pythium* damping-off became more suppressive with increasing compost age. Leaf, yard waste, food, and spent mushroom composts as well as certain biosolids, cow manure, chicken-cow manure, and leaf-chicken manure composts were not suppressive to *Pythium* damping-off. In some cases, turkey litter, chicken manure, chicken-leaf, and food waste composts were inhibitory to creeping bentgrass seed germination in laboratory experiments. Microbial populations varied among all of the composts tested. Bacterial populations were high in all composts except the turkey litter compost, in which populations were 1,000- to 10,000-fold lower than in the other composts tested. Among the highest populations of heterotrophic fungi and antibiotic-producing actinomycetes were those found in all batches of the brewery sludge compost, whereas the lowest populations were found in turkey litter, chicken manure, and food waste composts. Heat treatment of suppressive composts reduced populations of bacteria, fungi, and actinomycetes in all composts tested. Disease suppressiveness was also reduced or eliminated in heated composts. Amending heated composts with small amounts of nonheated compost restored suppressive properties and partially restored microbial populations to wild-type levels. A strong negative relationship between compost microbial activity (as measured by the hydrolysis of fluorescein diacetate) and *Pythium* damping-off severity was observed. When composts were applied to creeping bentgrass in field experiments, a significant level of suppressiveness was evident with some composts when disease pressure was high (i.e., disease ratings high in uninoculated plots). A 1991 batch of turkey litter compost and the 1990 batch of Endicott biosolids were consistently suppressive to foliar symptoms of *Pythium* root rot on creeping bentgrass. This study indicates that suppression of *Pythium* diseases of creeping bentgrass in batches of brewery sludge and Endicott biosolids composts, and possibly in other suppressive composts examined in less detail in this study, is related directly to the microbial activities in the composts. On the other hand, the mechanisms of *Pythium* suppression in turkey litter and perhaps other poultry-based composts is not related directly to the compost microbial activity. Although turkey litter showed a lack of suppressiveness in laboratory bioassays and low microbial populations and activity, it resulted in a significant and consistent level of suppressiveness in field experiments. Therefore, the microbiological properties of *Pythium*-suppressive composts may differ substantially, and measurements of microbial populations and activity may not be predictive of the level of disease suppression in all composts.

Increasing interest in composting as a waste management strategy has led to increased research efforts directed toward compost utilization. Composts have been widely accepted as soil amendments or as amendments to growing media for the production of agronomic and horticultural crops (10, 13). One of the beneficial properties of compost-amended plant growth media is the microbially induced suppression of soilborne plant pathogens and diseases (24).

The effectiveness of composts in controlling soilborne plant diseases is now well known (16, 22–25, 47). In particular, the suppression of diseases caused by *Pythium* spp. has been well documented (4, 7–9, 12, 15, 27–32, 42–44). In some studies, the levels of *Pythium* disease suppression has been linked directly to elevated levels of microbial activity provided by the compost amendment (7, 9, 15, 30, 31). According to Hoitink et al. (23), “bark, sewage sludge, and yard waste composts prepared with

grass clippings are suppressive to *P. ultimum* within days after the composting process has begun and after the high temperature substrate is colonized by mesophiles.” Furthermore, container media prepared from biosolids (i.e., sewage sludge) compost have been shown to be suppressive to *Pythium* damping-off of cucumber shortly after windrow temperatures fell below 60°C (27).

In several studies, however, *Pythium* spp. have not been adequately suppressed in compost-amended substrates. For example, Phae et al. (38) examined the suppressive properties of 10 different composts to a number of soilborne fungal pathogens, including *P. ultimum*, using an agar plate bioassay. Although most pathogenic fungi were suppressed by various composts as measured by this technique, *P. ultimum* was not suppressed. Only slight suppression of *P. ultimum* was observed when sludge, bark, or cattle manure composts were inoculated with a suppressive strain of *Bacillus subtilis* (38, 39). Amending *P. ultimum*- and *Pythium aphanidermatum*-infested field soils with mature biosolids compost did not initially sup-

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TABLE 1. Compost age and storage conditions

Compost	Yr of collection and receipt	Age at time of receipt	Storage conditions ^a
Brewery sludge	1989	1 mo	CU
	1991	1 mo	O
	1992	1 mo	C4
	1993	1 mo	O
Endicott biosolids	1989	2 yr	CU
	1990	1 yr	CU
Baltimore biosolids	1989	1 yr	CU
Schenectady biosolids	1989	1 yr	CU
Turkey litter	1989	<1 yr	CU
	1990	<1 yr	CU
	1991	<1 yr	CU
Chicken-cow manure	1989	<1 yr	CU
	1991	<1 yr	C22
	1992	<1 yr	C22
Moody Hill cow manure	1989	<1 yr	CU
Saratoga horse manure	1991	<1 yr	CU
BactoPlus cow manure	1989	<1 yr	CU
	1991	<1 yr	CU
Spent mushroom compost	1989	<1 yr	CU
Chicken manure	1991	<1 yr	CU
	1992	<1 yr	C22
Leaf-chicken manure	1992	<1 yr	F
Leaves	1992	<1 yr	F
Food waste	1992	<1 yr	F
Yard waste	1992	<1 yr	F

^a Composts were stored in one of five ways: CU, in 75-liter containers in an unheated room; O, outdoors in large piles; C4, in 75-liter containers at 4°C; C22, in 75-liter containers at 22°C; and F, frozen, in 1-liter containers.

press damping-off of peas and beans (29). However, after one year, *Pythium* damping-off was suppressed in the compost-amended soil.

Diseases of turfgrasses incited by *Pythium* species are among the most damaging of all turfgrass diseases (1, 18–21, 35). Of the numerous *Pythium* species recovered from symptomatic turfgrass plants, *Pythium graminicola* is among the most virulent and destructive (1, 35). In recent years, as the biological control of turfgrass diseases has increased (34), some control strategies have employed composted organic amendments as a disease-suppressive amendment. Although the use of composts for the control of *Pythium* diseases of turfgrasses has met with some success (34, 36, 46), levels of disease control have not been predictable or consistent. Not only do composts prepared from different feedstocks vary in disease suppressiveness, but those prepared from different batches of the same feedstock are also variable (33a, 36). This variability is poorly understood, in part, because of the poor understanding of the mechanisms by which composted amendments suppress *Pythium*-incited plant diseases. Extensive comparative studies of disease suppression with composted amendments have not been conducted. The purpose of this study was to investigate the mechanisms by which a variety of composted amendments suppress *P. graminicola*-incited damping-off and root rot of creeping bentgrass and to determine the role of compost microbial populations and activity in disease suppression.

MATERIALS AND METHODS

Composts. Composts prepared from a variety of feedstocks of differing ages were used in these studies (Table 1). The brewery sludge, chicken-cow manure, and BactoPlus cow manure composts were all produced in an agitated-bed system with a 3-week retention time. The different batches of Endicott biosolids composts were produced in a silo composting reactor with an approximately 3-week retention time. All of the above composts were removed from the reactors and placed in curing windrows for various periods. The remaining composts

were produced in windrows, generally turned on 1- to 2-week intervals. Except for the leaf and yard waste composts, all sludges, biosolids, and manures were mixed with a bulking agent (straw, bedding, or wood chips) prior to the composting process. Composts prepared from brewery sludge, biosolids, cow manure, chicken manure, and food waste were bulked with wood chips. Straw bedding was used as the bulking agent for turkey litter composts.

Except for the turkey litter composts, which had been commercially milled to a uniform particle size, all composts were sieved through a 2-mm screen prior to use in experiments. For most laboratory bioassays, composts were mixed with similarly sieved, oven-dried sand at a rate of 80 mg (dry weight) of compost per cm³ (total volume) of material.

***Pythium* suppression assays.** Laboratory bioassays were set up in two different ways. For early experiments in this study (conducted in 1991 and 1992), bioassays were set up as follows. The wells of 24-well tissue culture plates were first filled with 0.5 g of fine sand. A 4-mm agar disk from a 48-h-old culture of *P. graminicola* PRR-8 (35) was placed on the sand surface, covered with 2 cm³ of a compost-sand mixture, and moistened with 0.7 ml of sterile distilled water (SDW). Next, creeping bentgrass (*Agrostis palustris* Huds) seeds were sown in the wells by sprinkling seeds over the surface of the wells so that the surface of the compost-sand mix was completely covered with seeds. Finally, seeds were covered with an additional 0.3 cm³ of compost-sand mix. Each treatment was repeated in four wells. Covers were placed on the tissue culture plates until seedlings emerged (usually 4 to 5 days). Tissue culture plates were placed in a clear plastic box to reduce moisture loss and maintain high relative humidities.

For assays conducted later in this study (after 1992), glass cylinders (2.0 by 2.5-cm diameter) were arranged on a sheet of moistened blotting paper (20 by 28 cm) supported on a Plexiglas sheet. This assembly was also placed in a clear plastic box as described above to reduce moisture loss during the experiment. Forty glass rings were arranged in a grid (4-cm centers) on the surface of the blotter. Next, a 5-mm-diameter mycelial disk taken from a 48-h culture of *P. graminicola* PRR-8 was placed on the filter paper in the center of each cylinder. The sand used in these assays was sieved through nested 1-mm and 500- μ m mesh sieves. The particles retained on the 500- μ m mesh were used in compost-sand mixtures. Cylinders were filled with 3 cm³ of compost-sand mixture and then seeded with 0.2 cm³ of creeping bentgrass. Seeds on the surface of each cylinder were covered with 0.2 cm³ of sand, and additional water was applied with an alcohol-rinsed plant mister filled with SDW until the sand surface just glistened. Three to five replicate cylinders were prepared for each treatment.

For both types of assays, controls consisted of a set of noninoculated wells for each compost treatment and a set of inoculated and noninoculated sand-filled wells. The tissue culture plate assays and the cylinders were incubated in a 28°C growth chamber with a 16-h photoperiod. Assays were watered with the plant mister as necessary to keep the compost-sand mixture surfaces moist. Assays were rated after 5 days of incubation on a scale from 1 to 5, where 1 is asymptomatic turf, 2 is from 1 to \leq 25% of the turf nonemerged or necrotic, 3 is from 26 to \leq 50% of the turf nonemerged or necrotic, 4 is from 51 to \leq 75% of the turf nonemerged or necrotic, and 5 is from 76 to \leq 100% of the turf nonemerged or necrotic. The results obtained with each of the two bioassay configurations were similar. However, variation between replicates was reduced with the latter configuration. Prior to applying composts in field experiments (see below), compost-sand mixtures were tested for *Pythium* suppression in laboratory assays.

To determine whether compost suppressiveness to *Pythium* damping-off could be correlated with populations of compost-inhabiting microbes, experiments comparing autoclaved and nonautoclaved batches of brewery sludge and Endicott biosolids composts were conducted. Glass ring assays were conducted as described above. For autoclaved treatments, compost-sand mixes were treated at 121°C for 1 h on two consecutive days. To ensure that observed losses in disease suppression were due to the removal of the microbiota rather than to biochemical changes in materials due to heat treatment, an additional treatment in which autoclaved compost-sand mixes were amended with either 1 or 2% (vol/vol) nonautoclaved material was included. On the day of the assay, 1 or 2 cm³ of nonsterile compost-sand mix was added to 100 cm³ of twice-autoclaved mix, and the container was shaken vigorously before use. All laboratory bioassays were conducted at least two times. Some composts were tested as many as four times in each of the 3 years of the study. Microbial populations in the compost-sand mixtures were determined as described below.

Microbial isolations. To isolate and enumerate populations of bacteria, fungi, and actinomycetes from composts, 1 g of compost or compost-sand mixture was placed in 99 ml of 0.1% water agar (WA; 1 g of Bacto agar [Difco] per liter). The mixture was blended for 45 s on high speed in a Waring blender. Tenfold dilution series were prepared with SDW, and 0.1 ml of the suspensions was aliquoted onto four replicate plates of appropriate culture media. For fungi, suspensions were plated onto 0.3 \times potato dextrose agar (Difco) amended with 50 μ g/ml each of rifampin and penicillin G. Populations of heterotrophic bacteria and actinomycetes were isolated and enumerated by plating compost dilutions on 1/10- and 1/50-strength Trypticase soy agar (BBL), respectively. On the latter medium, actinomycete colonies were readily discerned from oligotrophic bacteria by examining colonies under a dissecting microscope. All plates were incubated for 72 h at 24°C. Colonies were enumerated, and populations were expressed as CFU per gram (dry weight) of compost.

In some experiments, actinomycetes were isolated and selected based on their antibiotic-producing abilities by a triple-layer agar technique (17). These were

designated antibiotic-producing actinomycetes. Petri plates (9-cm diameter) were first filled with 10 ml of 1.5% WA. The following day, 5 ml of 1% WA containing a compost or compost-sand suspension was poured over the solidified 1.5% WA layer. After 48 h, 5 ml of 2% WA containing macerated mycelium of *P. graminicola* was added to the surface of the previously poured agar layers. Actinomycete colonies surrounded by cleared or cloudy zones where the *P. graminicola* mycelium was absent or less dense were enumerated after a 4-day incubation at 24°C. Populations were expressed as CFU per gram (dry weight) of compost or compost-sand mixture.

Microbial activity. General levels of microbial activity in composts were determined by measuring the rate of fluorescein diacetate (FDA) hydrolysis by a modification of the technique described by Schnürer and Rosswall (41) and used previously to study microbial activity in composts (3, 7, 9, 26). The following composts were tested: the 1989, 1991, 1992, and 1993 batches of brewery sludge; the 1989 and 1990 batches of Endicott biosolids; the 1992 batch of leaf-chicken manure; the 1991 batch of Saratoga horse manure; the 1992 batch of leaves; and the 1990 and 1991 batches of turkey litter. The fresh weight of a compost sample equivalent to 0.5 g (dry weight) was placed in duplicate 125-ml Erlenmeyer flasks. Then 20 ml of sodium phosphate buffer (60 mM, pH 7.6) was added, followed by 400 µg of FDA (2 mg/ml in acetone). The 400 µg of added FDA provided excess substrate, so that the reaction rate was not limited by the lack of available substrate. The mixture was incubated on a rotary shaker (90 rpm) at 25°C for 1 h and then filtered through a Whatman no. 1 filter with a light vacuum. Two 0.6-ml subsamples were transferred to microcentrifuge tubes and mixed with 0.6 ml of acetone to stop the reaction. Samples were centrifuged for 2 min at 4,000 rpm to remove particulates. The fluorescein concentration was determined by measuring the A500. To compensate for background absorbance from soluble sample components, absorbance blanks that consisted of a buffer extract from each compost type prepared as described above but without the addition of FDA were used. Any samples with an absorbance reading out of the linear range were diluted and remeasured. A standard curve was prepared for each compost to which 0, 40, 80, 100, 160, and 200 µg of FDA was added in triplicate to screw-on cap tubes with 5 ml of buffer. A maximum of 200 µg was added because larger amounts gave rise to fluorescein levels that could not be determined spectrophotometrically without dilution. Tubes were capped and placed in a boiling water bath for 60 min. After cooling for 10 min, hydrolyzed FDA was removed with Pasteur pipettes to flasks containing 0.5 g (dry weight) of compost and 15 ml of phosphate buffer and treated as described above.

Field studies. Field plots were established at the Cornell University Turfgrass Field Research Laboratory (CUTFRL) in Ithaca, N.Y., to evaluate composts for their ability to suppress foliar chlorosis and necrosis resulting from root infection by *P. graminicola*. In 1991 and 1992, experiments were conducted on a creeping bentgrass (*A. palustris* Huds) sand-based putting green (pH 8.0), which was 3 years old in 1991. In 1993, plots were established on a 2-year-old creeping bentgrass green built on a native Arkport loam soil (pH 6.6). All greens were mowed three times a week at a 5-mm cutting height. In 1991 and 1992, total amounts of nitrogen applied were 178 and 215 kg/ha, respectively. In 1993, a total of 225 kg of nitrogen per ha was applied. No pesticides were applied at any time during the 3-year experimental period.

Plots were inoculated with *P. graminicola*-infested wheat seed as described previously (35) to ensure symptom development. In 1991 and 1992, a mixture of four geographically distinct strains was used for inoculations. In 1993, PRR-8, an isolate from a site at CUTFRL, was used alone. In 1991 and 1993, 50 cm³ of inoculum was used per plot. In 1992, 100 cm³ was used. Spring inoculations were made on 19 June 1991, 23 April 1992, and 24 May 1993. Fall inoculations were made on 20 October 1992 and 30 September 1993. Plots were inoculated by removing 15-cm-diameter cores to a depth of 4 to 5 cm with a golf course cup cutter. A small amount of soil was scraped from the bottom of the cores to expose roots. Infested wheat was then placed in the holes, and the sod was replaced and tamped to prevent scalping injury. Cores were monitored weekly for foliar responses to root infection. Controls consisted of noninoculated cores.

Compost treatments were applied to five replicate plots per treatment on the day of the spring inoculation and at monthly intervals thereafter. For the 1991 field study, composts were mixed with sand in the proportions of 30% compost and 70% sand (vol/vol). This amount was chosen to represent the maximum amount of organic matter that would be applied to a golf course putting green during normal topdressing applications. In 1992 and 1993, a field application rate of 48.8 g (dry weight)/m² was desired. Therefore, in 1992, the corresponding fresh weight of compost was mixed with sand to obtain a total application volume of 500 cm³/m². In 1993, the total volume of material applied varied slightly because the appropriate fresh weight of compost was simply mixed with sand for a total application volume of 400 cm³/m² per treatment.

Treatment applications were made to plots measuring 0.3 by 0.3 m in 1991 and 1 by 1 m in 1992 and 1993. In 1991, only one monthly application was made. In 1992 and 1993, plots received seven and five treatments, respectively. The entire core surface area was rated for foliar symptoms (chlorosis and browning) of *Pythium* infection on a scale of 1 to 5, where 1 is asymptomatic turf, 2 is from 1 to ≤25% of the core area symptomatic, 3 is from 26 to ≤50% of the core area symptomatic, 4 is from 51 to ≤75% of the core area symptomatic, and 5 is from 76 to ≤100% of the core area necrotic.

Statistical analysis. All plant bioassays were arranged as completely randomized designs with three to five replications. Laboratory bioassays had three or

TABLE 2. Suppression of *P. graminicola* damping-off of creeping bentgrass in various compost-sand mixtures

Compost	Yr of receipt	Disease severity rating ^a	
		Noninoculated	Inoculated
Brewery sludge	1989	1.2	1.7*
	1991	1.0	2.5*
	1992	1.0	1.3*
Endicott biosolids	1989	1.7	2.3*
	1990	1.7	2.2*
Baltimore biosolids	1989	2.4	2.2*
Schenectady biosolids	1989	1.1	3.8
Turkey litter	1989	5.0*	4.7
	1990	5.0*	4.8
Chicken-cow manure	1989	3.0*	3.4*
	1991	3.3*	4.1
Moody Hill cow manure	1989	1.0	4.9
Saratoga horse manure	1991	1.0	3.4*
BactoPlus cow manure	1989	1.0	2.7*
Spent mushroom compost	1989	1.0	3.8
Chicken manure	1992	2.8*	3.4*
Leaf-chicken manure	1992	2.0	4.3
Leaves	1992	3.1*	4.5
Food waste	1992	2.7*	3.5
Yard waste	1992	1.5	4.3
Sand (control)		1.2	4.9
Kruskal-Wallis statistic (<i>H</i>)		58.95 (<i>P</i> < 0.001)	24.85 (<i>P</i> = 0.038)

^a Disease severity was rated 5 days after sowing on a scale of 1 to 5 (see text for details). Means represent results of at least two separate experiments. Means followed by an asterisk have Kruskal-Wallis rankings that are significantly (*P* = 0.05) different from that of the sand control according to the LSD test.

four replications per treatment, whereas field experiments all had five replications per treatment. Disease ratings were subjected to the Kruskal-Wallis test, a nonparametric ranking procedure. When significant treatment effects were observed (*P* ≤ 0.05), rankings were subjected to analysis of variance, and mean ranking values were separated using a least significant difference (LSD) test. Microbial population data were analyzed by analysis of variance. Means were separated by using Duncan's new multiple range test or the LSD test. For FDA hydrolysis experiments, data were analyzed by linear regression analysis in which levels of hydrolyzed FDA were regressed over mean disease ratings in laboratory disease suppression bioassays.

RESULTS

Suppressiveness of composts to *Pythium* damping-off. Different composted materials were evaluated for the suppression of *P. graminicola* seed rot and damping-off of creeping bentgrass (Table 2). Several composts were consistently suppressive to *Pythium* damping-off in laboratory bioassays. The most notably suppressive materials were different batches of brewery sludge and Endicott biosolids. However, the following composts were also suppressive: Baltimore biosolids, a 1989 batch of chicken-cow manure, Saratoga horse manure, BactoPlus cow manure, and a 1992 batch of chicken manure compost. Some composts were inhibitory to creeping bentgrass seed germination. For example, amending sand with certain batches of turkey litter, chicken manure, chicken-cow manure, leaf, and food waste composts significantly reduced seed germination from that in 100% sand medium (Table 2). The leaf, food waste, yard waste, Schenectady biosolids, 1991 chicken-cow manure, Moody Hill cow manure, leaf-chicken, and spent mushroom composts were not suppressive to *Pythium* damping-off in any of the experiments. Batches of the brewery sludge and the Endicott biosolids composts were selected for further laboratory studies.

Certain batches of the Endicott biosolids and brewery waste compost that were initially not suppressive to *Pythium* damp-

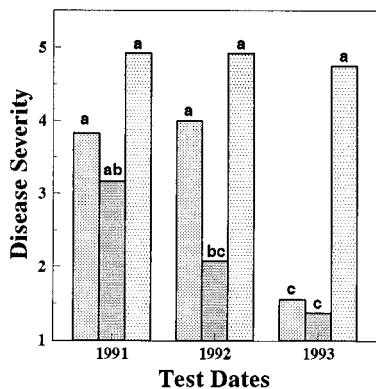


FIG. 1. Increase in suppressiveness of the 1990 batch of Endicott biosolids (second bar of each group of three bars) and the 1991 batch of brewery waste (first bar) composts to *Pythium* damping-off of creeping bentgrass with increasing compost age. Disease severity was rated 5 days after sowing on a scale of 1 to 5, where 1 is asymptomatic turf and 5 is 100% nonemerged or necrotic seedlings. (Scale details are given in the text.) Ratings represent the mean of at least three separate bioassay experiments conducted in each of the 3 years. Means followed by the same letter are not significantly different ($P = 0.05$), based on the analysis of variance and LSD test of mean Kruskal-Wallis ranking values (Kruskal-Wallis statistic [H] = 80.63; $P < 0.001$). Sand (third bar) was used as a control.

ing-off became suppressive as the material aged (Fig. 1). For example, in laboratory assays performed over a 3-year period from 1991 to 1993, mean disease ratings for the 1991 batch of brewery waste compost decreased from 3.83 in 1991 to 1.56 in 1993. Similarly, mean disease ratings for the 1990 batch of Endicott biosolids decreased from 3.17 to 1.38 over the same time period. Compost batches that were suppressive shortly after collection (e.g., the 1989 and 1992 batches of brewery waste compost) changed little in the level of *Pythium* damping-off suppression over the 3-year period.

Compost microbial populations. Populations of heterotrophic bacteria and fungi and antibiotic-producing actinomycetes varied among all of the composts tested (Table 3). Populations of heterotrophic bacteria ranged from 5.46 to 9.85 \log_{10} CFU/g (dry weight) of compost. Populations were highest in the 1991 and 1992 batches of brewery waste, the 1989 batch of Endicott biosolids, and the 1992 batch of yard waste compost. With the

exception of the yard waste compost, each of these composts was suppressive to *Pythium* damping-off. Bacterial populations were lowest in the turkey litter composts, in which populations ranged from 5.46 to 5.51 \log_{10} CFU/g of compost.

Populations of heterotrophic fungi ranged from 3.65 to 7.53 \log_{10} CFU/g, whereas populations of antibiotic-producing actinomycetes ranged from 2.26 to 9.86 \log_{10} CFU/g. The highest populations of heterotrophic fungi and antibiotic-producing actinomycetes were found in the 1989 and 1992 batches of brewery sludge compost. Of all the composts tested in our study, these were the most suppressive to *Pythium* damping-off. The lowest populations of heterotrophic fungi and antibiotic-producing actinomycetes were found in the nonsuppressive chicken manure and food waste composts and in the turkey litter composts.

Heat treatment of composts. Populations of heterotrophic bacteria, fungi, and actinomycetes were significantly reduced in most autoclaved composts and generally below detectable levels (populations of ≤ 1.0 to 1.9 \log_{10} CFU/g) (Table 4). After the addition of 1% (data not shown) or 2% (vol/vol) nonautoclaved compost to autoclaved composts, populations of all microbial groups were 100-fold below original population levels at the time that the disease suppression assays were initiated. Populations at the termination of the disease suppression assays were not determined.

Heat treatment of suppressive brewery sludge and Endicott biosolids composts eliminated suppressiveness to *P. graminicola* damping-off (Table 4). Addition of 1% (data not shown) or 2% nonautoclaved compost to the autoclaved material restored suppressiveness to all of the compost batches tested except the 1989 batch of Endicott biosolids compost.

Relationship between microbial activity and disease suppression in laboratory assays. The relationships between levels of microbial activity in various composts and the suppression of *Pythium* damping-off were determined by examining levels of FDA hydrolysis in different composts and compost batches. As the rate of FDA hydrolysis increased, the levels of *Pythium* damping-off of creeping bentgrass seedlings growing in compost-amended mixes decreased (Fig. 2). Disease severity was significantly and negatively correlated with microbial activity ($y = -0.79 + 4.5$; $r^2 = 0.64$). The composts with the highest rates of FDA hydrolysis were the 1989, 1991, 1992, and 1993

TABLE 3. Populations of heterotrophic bacteria, fungi, and antibiotic-producing actinomycetes in *Pythium*-suppressive and nonsuppressive composts

Compost	Yr of receipt	Disease suppression ^a	Microbial population ^b (\log_{10} CFU/g [dry wt])		
			Bacteria	Fungi	Antibiotic-producing actinomycetes
Brewery sludge	1989	++	8.65 d	7.53 b	9.86 a
	1991	+	9.85 a	5.73 d	8.00 b
	1992	++	9.78 ab	8.26 a	9.83 a
Endicott biosolids	1989	++	9.65 abc	6.54 c	6.43 e
Chicken manure	1992	+	8.80 d	3.65 f	2.26 i
Leaves	1992	-	8.61 d	5.59 d	6.79 cd
Leaf-chicken manure	1992	-	9.23 c	5.23 e	6.91 c
Yard waste	1992	-	9.43 bc	5.04 e	6.68 d
Food waste	1992	-	8.70 d	3.83 f	5.23 f
Turkey litter	1989	NA	5.51 e	1.76 g	4.06 h
	1990	NA	5.46 e	3.76 f	4.85 g

^a ++, highly suppressive to *P. graminicola* damping-off of creeping bentgrass seedlings in laboratory assays (ratings of 1.0 to 2.3); +, moderately suppressive (ratings of 2.4 to 3.4); -, nonsuppressive (ratings of 3.5 to 5.0). NA, not applicable (no seedlings emerged).

^b Heterotrophic bacterial and actinomycete populations were determined by plating on Trypticase soy agar. Heterotrophic fungal populations were determined by plating on potato dextrose agar. *Pythium*-suppressive antibiotic-producing actinomycetes were determined by the triple-layer agar plating procedure described by Herr (17). Population means in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

TABLE 4. Populations of heterotrophic bacteria, fungi, and actinomycetes and *Pythium* damping-off suppression in various compost-sand mixes

Compost-sand mix ^a	Population ^b (log ₁₀ CFU/g [dry wt] of compost)			Disease severity rating ^c	
	Bacteria	Fungi	Heterotrophic actinomycetes	Noninoculated	Inoculated
Nonautoclaved mixes					
Brewery sludge 1989	7.6 d	6.1 c	6.1 c	1.0	1.8*
Brewery sludge 1991	7.6 d	5.3 d	6.2 c	2.0	2.0*
Brewery sludge 1992	9.6 a	6.7 a	8.1 a	1.0	1.0*
Endicott biosolids 1989	7.9 c	6.0 c	5.8 c	1.0	1.0*
Endicott biosolids 1990	8.3 b	6.4 b	6.0 c	1.0	1.0*
Autoclaved mixes					
Brewery sludge 1989	1.9 k	<1.0 i	1.9 i	1.5	4.3
Brewery sludge 1991	2.2 j	<1.0 i	2.4 h	1.0	4.0
Brewery sludge 1992	<1.9 k	<1.0 i	<1.9 i	1.0	5.0
Endicott biosolids 1989	<1.9 k	<1.0 i	<1.9 i	2.0	3.5
Endicott biosolids 1990	<1.9 k	<1.0 i	<1.9 i	1.5	3.0
Autoclaved + 2% nonautoclaved mixes					
Brewery sludge 1989	6.3 f	4.1 g	3.9 e	2.0	2.3*
Brewery sludge 1991	4.9 i	3.9 h	3.7 f	1.3	2.3*
Brewery sludge 1992	7.1 e	4.8 e	6.5 b	1.0	1.8*
Endicott biosolids 1989	5.6 h	4.4 f	3.0 g	2.3	3.0
Endicott biosolids 1990	6.0 g	4.9 e	4.5 d	2.3	2.5*
Sand (control)	ND	ND	ND	1.0	5.0
Kruskal-Wallis statistic (<i>H</i>)				NS	17.0 (<i>P</i> = 0.031)

^a Composts were autoclaved for 1 h on two consecutive days. Two percent nonautoclaved compost-sand mix was added to autoclaved compost-sand mix, and the disease suppression assay was performed immediately.

^b See Table 3, footnote b. ND, not determined. Population means in each column followed by the same letter are not significantly (*P* = 0.05) different according to Duncan's new multiple range test.

^c See Table 2, footnote a. NS, no significant treatment effects according to the Kruskal-Wallis test.

batches of brewery sludge compost. The compost with the lowest level of microbial activity in the regression analysis was the 1992 leaf compost. However, batches of turkey litter compost which were tested but not included in the regression analysis had the lowest levels of microbial activity (<1 µg of FDA hydrolyzed per min per g) of any of the composts tested in our study.

Suppression of *Pythium*-incited foliar chlorosis and necrosis on established creeping bentgrass. Various compost amendments were evaluated for the control of *Pythium* suppression in field trials over a 3-year period. Results for 1991 and 1992 are presented in Table 5. Only very low levels of disease development were observed in 1993, masking any treatment effects. The variability in symptom expression from year to year made these field evaluations problematic, since all treatment effects were relative to the level of symptom expression in noninoculated plots. Another complicating factor was the limited availability of some batches of compost, which made longer-term evaluations impossible. However, some important trends are worth noting.

In 1991, the 1989 batch of Endicott biosolids and the 1991 batch of turkey litter compost were suppressive for up to 26 days after application. The only other composts that showed some level of disease suppression in 1991 were the 1991 batch of brewery waste and the 1990 batch of Endicott biosolids compost. Both were suppressive 26 days after application. In 1992 field experiments, all composts were suppressive at the first rating 6 days after application. However, because of a general turfgrass recovery (due to weather conditions unfavorable for disease development), no significant treatment effects were observed thereafter. Metalaxyl, a standard fungicide for

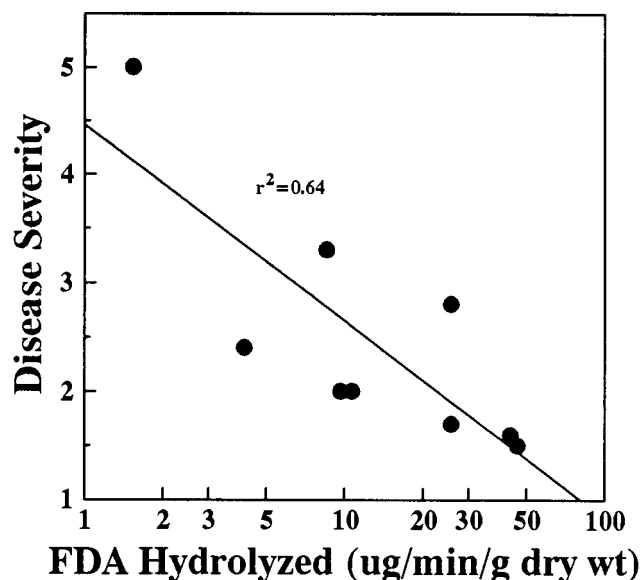


FIG. 2. Relationship between microbial activity (as measured by FDA hydrolysis) and the severity of *Pythium* damping-off of creeping bentgrass seedlings grown in different composts ($r^2 = 0.64$; $y = -0.79 + 4.5$). Disease severity was rated 5 days after sowing on a scale of 1 to 5, where 1 is asymptomatic turf and 5 is 100% nonemerged or necrotic seedlings (see text for rating details). Each point represents the mean of at least eight replicates for disease severity and three replicates for FDA hydrolysis. The composts used were the 1989, 1991, 1992, and 1993 batches of brewery sludge; the 1989 and 1990 batches of Endicott biosolids; the 1992 batch of leaf-chicken manure; the 1991 batch of Saratoga horse manure; and the 1992 batch of leaves. The x axis is represented by a log scale.

TABLE 5. Suppression of foliar symptoms of *P. graminicola*-incited root rot of creeping bentgrass in 1991 and 1992 field experiments

Treatment ^a	Disease severity rating ^b on day postapplication:						
	1991				1992		
	5	12	16	26	6	13	18
Brewery sludge 1989	1.4	2.2	2.4	2.2	—	—	—
Brewery sludge 1991	1.4	2.4	3.2	1.8*	1.2*	1.0	1.0
Brewery sludge 1992	—	—	—	—	1.0*	1.2	1.0
Endicott biosolids 1989	1.0*	2.0	1.6	1.6*	—	—	—
Endicott biosolids 1990	1.2	1.8	2.2	1.6*	1.2*	1.0	1.2
Turkey litter 1991	2.6*	1.6	1.8	1.2*	1.0*	1.0	1.0
Chicken manure 1992	—	—	—	—	1.0*	1.4	1.6
Chicken-cow manure 1992	—	—	—	—	1.0*	1.0	1.4
BactoPlus cow manure 1991	1.4	2.2	3.6	2.4	—	—	—
Saratoga horse manure 1991	1.6	2.0	4.2	2.2	—	—	—
Yard waste 1992	—	—	—	—	1.2*	1.0	1.2
Metalaxyl	1.8	2.0	2.2	2.4	1.2*	1.2	1.0
None	1.8	2.4	3.8	3.2	2.2	1.4	1.6

Kruskal-Wallis statistic (*H*) 17.7 (*P* = 0.040) NS (*P* = 0.431) NS (*P* = 0.368) 15.9 (*P* = 0.070) 16.2 (*P* = 0.040) NS (*P* = 0.253) NS (*P* = 0.393)

^a Sand-compost mixtures were applied on 19 June in 1991 and on 23 April and 22 May in 1992. Inoculations with *P. graminicola* PRR-8 were made on 19 June 1991, 23 April 1992, and 24 May 1993.

^b Disease severity was rated on a scale of 1 to 5 (see text for scale). Means followed by an asterisk (*) have Kruskal-Wallis rankings that are significantly (*P* = 0.10) greater or less than the rankings of the untreated control according to LSD tests. NS, no significant treatment effects according to the Kruskal-Wallis test. —, not tested.

control of *Pythium* diseases, was effective only at the first rating date in 1992 and not effective in 1991.

DISCUSSION

Our results have shown that composts prepared from a number of different feedstocks may be suppressive to *P. graminicola* diseases on creeping bentgrass. Although the absolute levels and consistency with which these composts suppressed *Pythium* diseases varied, in general, composts prepared from brewery sludge, Endicott biosolids, and some animal manures were the most suppressive in laboratory experiments and were also suppressive in field experiments.

We conclude from our study that, with the exception of turkey litter and perhaps other poultry manure composts, the microbial properties of the composts themselves are the major factors influencing the suppression of *Pythium* diseases on creeping bentgrass. This confirms other reports of *Pythium* suppression in composts (3, 4, 7–9, 15, 27, 29–32, 42–44) and is supported by several lines of evidence.

First, we observed that the level of suppression of *Pythium* damping-off in laboratory assays increased in some compost batches with increasing compost age. This trend was particularly apparent in the 1991 batch of brewery sludge and the 1990 batch of Endicott biosolids compost. Similar trends have been observed with municipal biosolids composts suppressive to *P. ultimum* (27–29), where microbial activity was shown to increase as the level of decomposition increased.

Second, recoverable microbial populations, particularly of fungi and actinomycetes, were generally higher in suppressive composts than in nonsuppressive composts. This was particularly true for batches of the brewery sludge and Endicott biosolids composts. Even though no clear relationship between bacterial populations and disease suppression was observed, we have found in other studies that many of the bacteria and actinomycetes recovered from these suppressive composts are also suppressive to *P. graminicola* when tested in laboratory bioassays (45; unpublished data).

Third, heat treatment of batches of the brewery sludge and the Endicott biosolids compost-sand mixtures reduced bacte-

rial, fungal, and actinomycete populations and eliminated suppression of *Pythium* damping-off. Other studies have also shown that composts that are heated, irradiated with gamma radiation, or taken from the high-temperature centers of compost windrows are generally not suppressive to diseases caused by *Pythium* species (8, 9, 15, 27). Chen et al. (9) related the low level of suppression in hardwood bark composts taken from the high-temperature centers of windrows to reduced populations of oligotrophic and copiotrophic bacteria and actinomycetes and to the correspondingly low levels of microbial activity. Addition of small amounts of nonheated compost-sand mixtures to those that had been heated allowed microbial populations to recolonize the substrate, resulting in elevated populations of each of the microbial groups studied and in restoration of the suppressive properties of the brewery waste composts and the 1990 batch of Endicott biosolids compost. Addition of nonheated compost-sand mixtures, however, failed to fully restore suppressive properties to the 1989 batch of Endicott biosolids compost. This may be related to the relatively low level of actinomycete recolonization in this compost batch.

Fourth, as the level of microbial activity (as measured by FDA hydrolysis) increased among different batches of composts, the level of *Pythium* damping-off suppression, as determined from laboratory bioassays, also increased. This also confirms previous studies in which similar relationships between microbial activity and suppression of *Pythium* diseases of various plant species with composted amendments were established (3, 7, 9, 26, 28, 30). It is possible that the elevated levels of microbial activity result in increased competition between compost-inhabiting microbial populations and *P. graminicola* for root exudate components essential for the germination of *Pythium* propagules and tactic mycelial growth (9, 15, 33, 48). This could account for the decreased activity of *P. graminicola* in both laboratory and field experiments. It therefore seems clear that suppression of *Pythium* diseases of creeping bentgrass, at least in batches of brewery sludge and Endicott biosolids composts and possibly in other suppressive composts examined in less detail in this study, was related directly to the microbiological properties of the composts.

Although the microbial nature of *P. graminicola*-incited damping-off and root disease suppression in the brewery sludge and Endicott biosolids is clear, our conclusions concerning the mechanisms of *Pythium* suppression with turkey litter composts differ substantially, based primarily on the following observations: (i) an apparent phytotoxicity of turkey litter composts in laboratory bioassays, (ii) observations of a significant level of suppressiveness of the turkey litter compost in field experiments, and (iii) low microbial populations (particularly of fungi and actinomycetes) and activity, as determined by plate counts and FDA hydrolysis levels, respectively.

In laboratory experiments, we could not determine whether batches of turkey litter compost were suppressive to *Pythium* damping-off, since seed germination was completely inhibited. These composts were inhibitory to the germination of creeping bentgrass seed even at compost application rates lower than the 80 mg/cm³ used in this study (unpublished data). Other reductions in seed germination were observed with other composts (e.g., chicken-cow and chicken manure composts) containing poultry manures. Although it was not determined directly in our studies, it is likely that this apparent phytotoxicity is due to elevated levels of ammonia. Ammonia is known to be toxic to a wide variety of plant species (14) and is commonly volatilized from poultry manure amendments (5). The conditions under which the laboratory bioassays were conducted would not have permitted the free dissipation of ammonia from the bioassay wells, and this could very well explain the observed inhibition of germination. Furthermore, of all of the composts examined in this study, the batches of turkey litter had the lowest levels of microbial activity. Since ammonia is also an effective inhibitor of microbial activity (2, 6, 11, 37, 40), it is not surprising to find these low levels of microbial activity if ammonia is indeed biologically active in these composts.

When placed on established turf in the field, however, turkey litter compost was suppressive and has been consistently suppressive to other turfgrass diseases (34). Additionally, no evidence of phytotoxicity has ever been observed in field experiments. It is possible that, in the field, ammonia may be rapidly volatilized and dissipated, eliminating its phytotoxicity and the suppressed microbial activity, leaving the substrate available for microbial colonization. Assuming that turkey litter compost serves as a suitable food source for soil microorganisms, soil microbial activity could be enhanced following application of such amendments to turfgrass soils in the field. Such increases in microbial activity, particularly among soil bacteria that are members of the family *Enterobacteriaceae*, have been observed in field experiments with these turkey litter composts (unpublished data).

Alternatively, it is possible that the level of disease suppression observed with the turkey litter composts is due to enhanced turfgrass nutrition allowing for more rapid recovery from disease. However, even though the amount of nitrogen in turkey litter composts (5% total nitrogen and 0.6% water soluble nitrogen) is slightly higher than that in other composts tested (1 to 2% total nitrogen), it is unlikely that the low level of water-soluble nitrogen in the turkey litter compost could rapidly increase turfgrass growth and mask disease symptoms within 5 days of application without the added stimulation of microbial activity, resulting in a release of nitrogen from an insoluble to soluble form. Further work is needed to more clearly elucidate the mechanisms of disease suppression in turkey litter and other poultry-based composts.

We conclude, therefore, that the suppression of *Pythium* diseases of creeping bentgrass with compost amendments is dependent on the microbial properties of the amendment and the soil microbial responses following application of the

amendment. We have found that the microbiological properties of *Pythium*-suppressive composts differ substantially and that even though measurements of compost microbial populations or activity may be predictive of *Pythium* suppression in some composts, these measurements may not be predictive, in all cases, of disease suppression. This is particularly true for turkey litter and perhaps other poultry composts for which, although compost microbial populations and activity are relatively low, *Pythium* suppression may be a result of stimulation of soil microbial activity.

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