United States Golf Association Greens Section Research
Annual Report, 1993

Project Title: Microbial Basis of Disease Suppression in Composts Applied to Golf Course Turf

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EXECUTIVE SUMMARY

Our goal in this project is to develop more effective biological control strategies with compost-based organic fertilizers by understanding the microbial ecology of disease-suppressive composts. In particular, we hope to understand the microbiology such that disease-suppressive properties of composts might be predicted and an assemblage of beneficial microorganisms useful in the development of microbial fungicides for turfgrass disease control might be discovered.

The objectives of our study are to 1) determine the spectrum of turfgrass pathogens suppressed by compost applications, 2) establish relationships between overall microbial activity, microbial biomass, and disease suppression in composts, 3) identify microorganisms from suppressive composts that are capable of imparting disease-suppressive properties to conducive composts or those rendered conducive by heat treatment, and 4) determine the fate of compost-derived antagonists in golf course putting greens following application of individual antagonists and composts fortified with these antagonists.

Over the past year, our efforts have been focussed on 1) further evaluating composts in the field for disease suppression; our goal has been to verify previous findings as well as expand the diseases for which composts are suppressive; 2) further developing laboratory assays to assess microbial activity; and 3) enumerating and recovering specific isolates of bacteria, fungi, and actinomycetes from suppressive composts. Much of our emphasis in 1993 was on the isolation of various microbial groups from composts and evaluating their efficacy as potential biological control agents of a number of turfgrass diseases.

During the course of this three-year study, were are able to determine that suppressiveness in composts is either of a microbiological origin or of a non-biological origin. It appears that nearly all of the poultry manure-based composts were of the latter type and were not investigated in any detail. Many, if not all, of the other composts evaluated fell into the first type. We found that any given compost was not suppressive to a wide range of diseases. Nearly all of the composts evaluated were suppressive to only a few diseases. However, a suppressive compost was found for every disease evaluated.

In our microbial analyses of composts, we found that population levels in composts of either bacteria, fungi, or actinomycetes, were not directly related to the suppression of *Pythium graminicola*. Composts low in some populations were quite suppressive while other composts high in all microbial populations were not suppressive. Rather, qualitative aspects of microbial activity appears to be more related to the suppression of *P. graminicola.* In general, the greater the microbial activity, the greater the suppressiveness. The usefulness of FDA hydrolysis as a predictive measure for disease suppressiveness was not realized during the course of this study. The principal problems were associated with getting the microbial biomass assays working properly. Whereas we could rank composts on the basis of their comparative microbial activities, we could not establish precise relationships between microbial activity per unit biomass and disease suppression.

Finally, from all composts, a large number of specific bacteria and actinomycetes were isolated that were suppressive to *P. graminicola* when tested singly in bioassays. A high frequency of both actinomycetes and bacteria were suppressive. Fungi were isolated but, due to time limitations, were not tested in bioassays. Although the results of field trials with individual microbes were disappointing, further testing will need to be pursued. This collection of bacteria and actinomycetes should provide a useful library of microbial germplasm for future studies in the biological control of turfgrass diseases.

Although we have made tremendous progress in advancing our understanding of disease suppression in composts, a more thorough understanding of the composition and activities of disease-suppressive microbial communities in composts is needed. Future work will need to be directed at understanding how disease-suppressive microbial communities develop in composting organic matter and the how the properties of composts themselves and the processes by which they were derived, regulate the activities of these communities.

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1) To determine the spectrum of turfgrass pathogens suppressed by compost applications.

2) To establish relationships between overall microbial activity, microbial biomass, and disease suppression in composts.

3) To identify microorganisms from suppressive composts that are capable of imparting disease-suppressive properties to conducive composts or those rendered conducive by heat treatment.

4) To determine the fate of compost-derived antagonists in golf course putting greens following application of individual antagonists and composts containing same antagonists.

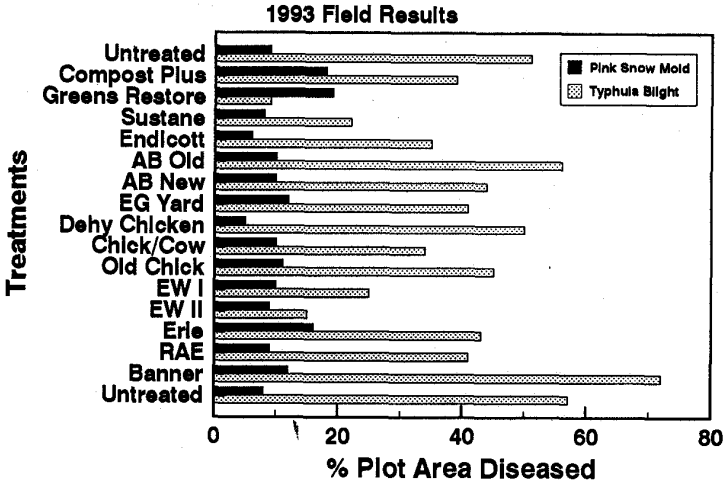
Progress in 1993:

OBJECTIVE 1: For this objective, our research in 1993 focussed on field aspects of compost evaluation; verifying previous findings and expanding the diseases examined. Due to the extremely wet conditions in the early spring, considerable snow mold development was observed as was Pythium root rot. The remainder of the summer was relatively dry with only occasional hot periods. Thus we were able to evaluate composts for Dollar spot suppression but conditions were not favorable for severe Brown patch development.

All plots were established at the Cornell University Turfgrass Field Research Laboratory. Snow mold plots were established in the spring of 1992 whereas all other plots were established in May of 1993. All disease evaluations relied on natural inoculum, with the exception of Pythium root rot evaluations, where inoculations were made on 24 May 1993 and again on 4 October 1993.

Both pink and gray snow mold diseases were extremely severe in 1993. Data for plot evaluations are shown in Figure 1.

Figure 1. Typhula Blight and Pink Snow Mold Suppression



Typhula Blight: Snow mold plots were under a heavy snow cover for much of the winter and early spring months. At one point, up to 7 ft of snow covered plot areas. Applications of compost-amended topdressings were discontinued in October of 1992. Between 55 and 60% of the plot area was covered with Typhula blight patches on April 23rd when ratings were taken. Of the treatments evaluated, Greens Restore, Sustane, and the two EW composts were significantly suppressive as compared to the untreated plots. The Banner-treated had significantly more Typhula blight than the untreated plots.

Pink Snow Mold: Only a low level of pink snow mold was observed in 1993. However, despite the low level, the dehydrated chicken manure compost was significantly suppressive as compared with untreated plots

Pythium Root Rot: Plots were inoculated with *P. graminicola* on May 24, 1993 by placing 50 cm^3 of infested wheat inoculum into the bottom of a 20-cm-diam hole cut with a cup cutter to a depth of approximately 5 cm. Ratings were made thereafter as symptoms developed. Ratings were taken on June 2, June 16, and June 30. After the June 30 rating, symptoms began to diminish rather rapidly due to warm dry conditions. Results are shown in Figure 2. Only a low level of Pythium root rot activity was observed during the course of the experiment. The disease ratings for the untreated plots remained at approximately the same levels throughout the experimental period. By the first rating, only the Plant Right (composted turkey manure) and the EW 5-4-5 (composted chicken manure) were suppressive as compared to the untreated plots. In contrast, plots treated with Technagro TF (composted yard waste) were significantly more symptomatic than the untreated plots. By the second and third ratings, nearly all treatments were suppressive, with the exception of OF-1 compost (poultry manure-based), EW 5-4-5, LG1022A,B, and C, and Plant Right. Even the Subdue treatment was ineffective at both the first and the third ratings.

Dollar Spot: Relatively low levels of Dollar spot were observed in 1993 and those symptoms that did appear, appeared quite late into the season. Ratings were not taken until September 29, 1993. Those results are presented in Figure 3. From among the materials tested, only LG1022A and Plant Right were significantly suppressive. Rubigan served as the fungicide standard, and was extremely effective.

Figure 2. Pythium Root Rot Suppression

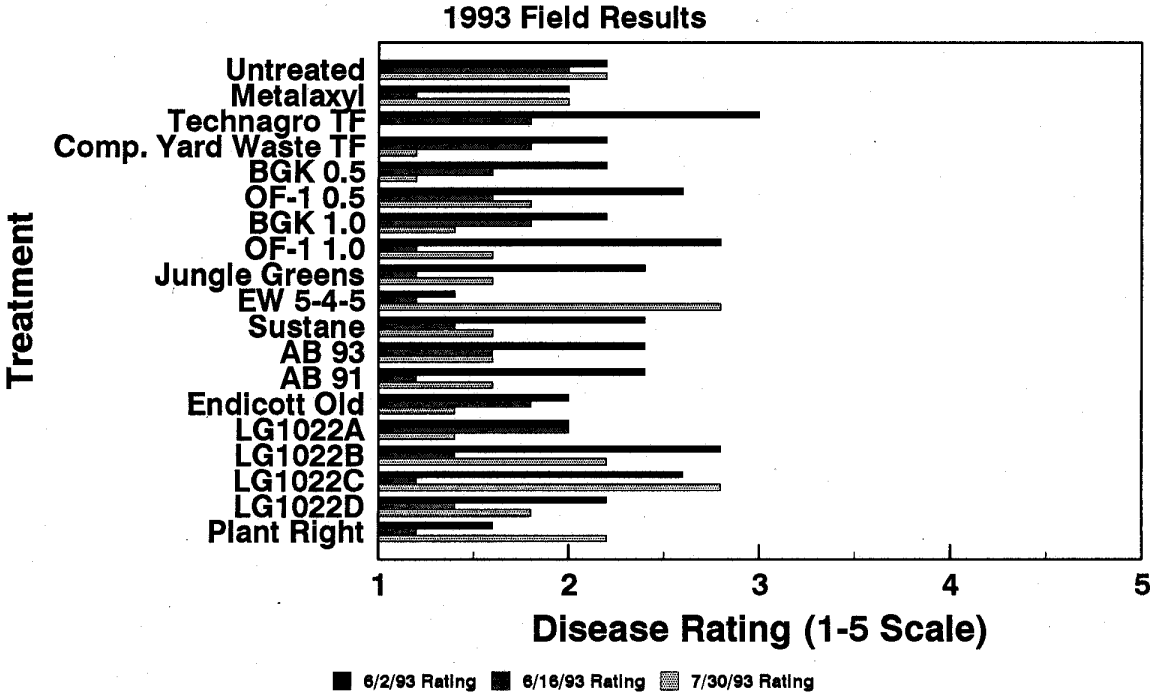
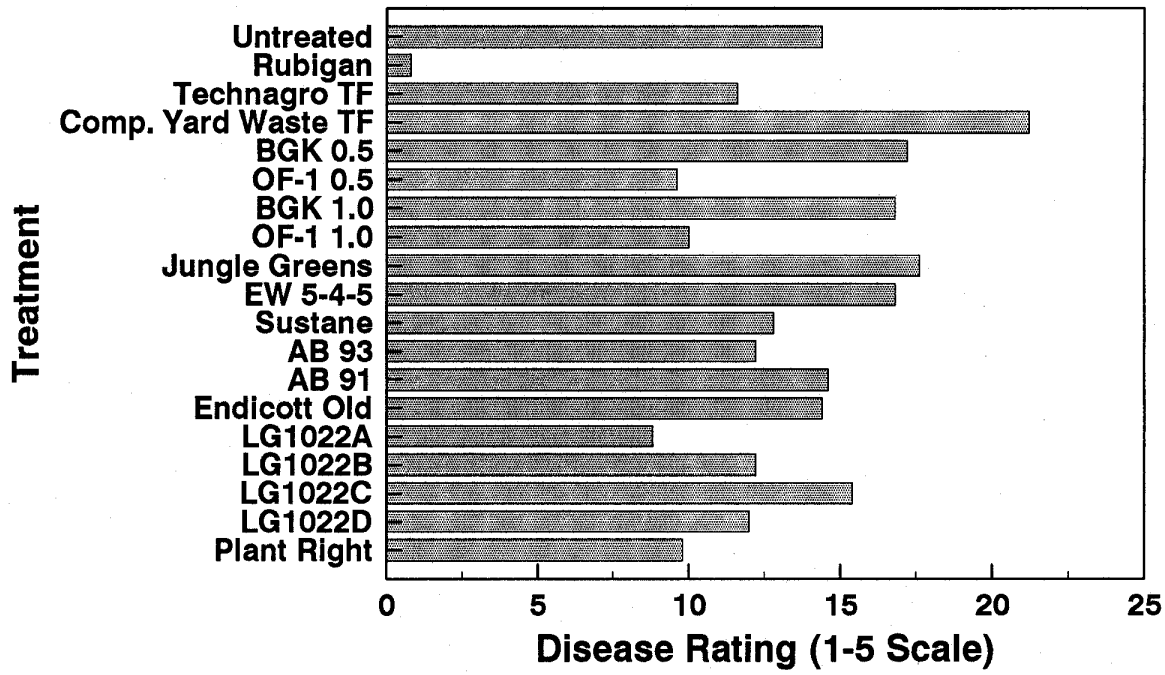


Figure 3. Dollar Spot Suppression



OBJECTIVE 2: From our previous studies, we have observed that the suppressiveness of composts falls into two broad mechanisms: those that are suppressive because of chemical properties of the compost and those that are suppressive because of the microbial communities present in the finished material. Nearly all of the poultry manure-based composts fall into the first category. All of the other composts appear to be in the microbial-based category. As part of our attempts to determine the relative contributions of microbial activity to *Pythium* suppression in these composts, we conducted a series of experiments whereby the suppressiveness of the native compost was compared with that of the same compost that had been sterilized prior to inoculation with *P. graminicola*. Further, to strengthen the evidence for a role of microbial activity in *Pythium* suppression, we added small amounts of the unsterilized material back to the sterilized compost and allowed the mixture to incubate. The results of these experiments are presented in Tables 1 and 2.

TABLE 1. Suppressiveness of various sterile and non-sterile composts to Pythium root rot of creeping bentgrass caused by *Pythium graminicola.*

	Non-sterile		Sterile	
Compost	Uninoculated	Inoculated	Uninoculated	Inoculated
Endicott Sludge 1989	2.3	2.3	2.3	4.8
Endicott Sludge 1990	1.0	1.8	2.8	4.0
Brewery Waste 1989	1.0	1.3	1.3	5.0
Brewery Waste 1991	3.8	4.5	4.0	4.5
Brewery Waste 1992	1.3	2.3	1.0	5.0
EG Leaf	1.0	4.5	1.8	4.8
EG Chicken/Cow	2.0	3.3	4.0	5.0
Sand	2.8	4.6	-	-

TABLE 2. Restoration of *P. graminicola* suppression to sterile composts with the addition of non-sterile compost

Compost	Non-sterile	Sterile	Sterile +*
Endicott Sludge 1989	1.5	3.5	3.0
Endicott Sludge 1990	1.0	3.3	2.5
Brewery Waste 1989	1.8	3.3	2.3
Brewery Waste 1991	2.3	3.0	2.3
Brewery Waste 1992	1.3	3.0	1.8
Sand	4.5	-	-

* 1% (v/v) non-sterile compost added to sterile compost and incubated for 2 days prior to inoculation

These results clearly support the contention that microbial activity is linked with the suppression of *P. graminicola* in these composts (Endicott sludge and Brewery waste).

The relationship between microbial activity and *P. graminicola* suppression can be seen more clearly when quantitative estimates of microbial activity are used. We have focussed on the hydrolysis of fluorescein diacetate (FDA) as a measure of microbial activity. The relative ranking of various composts based on microbial activity is shown in Table 3.

TABLE 3. Relative ranking of composts based on decreasing levels of microbial activity as determined by FDA hydrolysis

Compost	μg FDA hydrolysed/hr[a]
Brewery Waste 1992	1,298
Brewery Waste 1989	768
Brewery Waste 1991	768
Endicott Sludge 1989	319
Leaf/Chicken	255
EW 5-4-5	200
Endicott Sludge 1990	173
Saratoga Horse Manure	123
Technagro TF	95
EG Leaf	46
Sustane	27

[a] Determined from 0.35 g samples incubated at 30 C

OBJECTIVE 3: Isolations of microbes from suppressive and conducive composts continued in 1993. Our major focus was on species of bacteria and actinomycetes. We were able to previously isolate a high frequency of actinomycetes with a high level of antagonistic activity. Actinomycetes were isolated from composts by using a slightly modified version of the triple-agar-layer plate method of Herr (Herr, 1959). This method was described in the previous report to the USGA. Actinomycete colonies were selected based on their ability to inhibit the growth of *P. graminicola*. Isolates were transferred as necessary to eliminate *Pythium* and other contaminants and to separate mixed genotypes. A total of 104 strains were selected from 10 different composts.

In the initial screening, approximately 8 g of compost-amended sand (85.37 g oven-dried sand, 8 g oven-dried compost) was placed in 2 oz. screw-capped glass jars and autoclaved 3 times for 30 min on 3 successive days. Jars of the sand mixture were inoculated with a single plug removed with a #2 corkborer from an actinomycete culture grown on yeast maltose agar amended with 20 mg/L Polymyxin B (4 g yeast extract, 10 malt extract, 4 g dextrose, 15 g agar, 1 L distilled water). To facilitate the distribution of actinomycete propagules throughout the sand mixture, jars were shaken several times both before and after the addition of 500 ml of sterile deionized distilled water. Strips of parafilm were wrapped around the caps to further retard the moisture loss during the 3 weeks incubation at ambient temperature.

Glass rings (2.0 x 2.5 cm diameter) were placed on moistened blotting paper supported on a plexiglass sheet placed in a plastic box which served as a moist chamber. Forty glass rings, arranged in a 5 by 8 grid, were placed in each incubation chamber. There were 2 replicates per treatment and the treatments were completely randomized within each box. The bioassay units were

inoculated with *P. graminicola* by placing a mycelial disk removed with a #2 cork borer from a 2

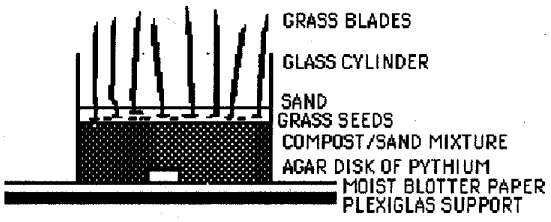


Figure 4. Illustration of individual bioassay unit

day-old culture of PRR-8 grown on TrypSoy Agar (Difco) on the filter paper in the center of the glass cylinder. Using a funnel, the rings were partially filled with approximately 4 g of the actinomycete-infested sand mixture. The mixture was tamped down, 1.5 ml of deionized distilled water was added, approximately 0.43g of creeping bentgrass cv. Putter was sprinkled on the surface of the sand mixture, and the seeds in each ring were covered with approximately 0.35g of sterile sand (Figure 4). The controls were the same as the treatments described above except that the oven-dried autoclaved sand mixture was not inoculated with an actinomycete strain nor were the uninoculated jars incubated with the addition of 500 ml of sterile water. This was done to prevent the growth of heat-resistant micro-organisms which might have survived multiple autoclaving. To determine if the actinomycete strain was phytotoxic, preliminary trials included a control in which the actinomycete-infested sand was not inoculated with *Pythium*. A second control, in which the sand mixture was not inoculated with *Pythium* or an actinomycete isolate, was included to check if additional factors were effecting grass germination.

The incubation boxes were placed in a growth chamber and incubated at approximately 25°C for 6 days. The treatments were misted with distilled water as needed to maintain adequate moisture. Germination was rated on a 0 to 2 scale (0=no germination, 1=poor germination, 2=high germination). An actinomycete strain was considered to be suppressive if the average rating was greater than 1.

Based on the initial screening, 11 actinomycete isolates were included along with an uninoculated control in a more detailed trial in which each treatment was replicated 10 times. The procedure was identical to that described for the initial screening except that approximately 4 (instead of 8) g of sand mixture was placed in each jar along with an agar disk of inoculum and 250 ml of sterile deionized distilled water. The jars were incubated at 27°C for three weeks.

Selected actinomycete strains were further tested for their ability to suppress the following pathogens of creeping bentgrass: *Microdochium nivale*, *Rhizoctonia solani*, and *Sclerotinia homoeocarpa*. The same actinomycete strains that were used in the field trial were challenged with a single pathogen in each bioassay. Positive and negative controls were included. In the case of *Rhizoctonia solani*, two replicates of each isolate were challenged by each of 2 strains of the pathogen; R6 and one isolated from cotton.

To get an initial assessment of whether antibiotics were produced by the effective actinomycetes, leachates were collected from composts in which individual strains had been grown. Fifty ml of milliQ water was added to 50 g moist weight of actinomycete-infested sand mixture prepared as in the procedure for the field inoculum. The mixture was shaken vigorously in a 250 ml Erlenmeyer flask. Approximately 0.5 h after the addition of the water, the supernatant was decanted and poured through a strainer to remove course particulates. The remaining leachate was filtered through a Whatman #1 filter to further remove particulates. Part of the filtered leachate

was then passed through a 0.2 μm filter to remove bacteria, protozoa, and fungal spores and mycelial fragments. The other part of the filtrate was autoclaved for 20 minutes. Two ml aliquots of unfiltered, filtered, or autoclaved extract were applied to each well. The bioassay was set up as usual except that sterile compost-amended sand was used to fill the glass cylinders instead of actinomycete-infested sand mixture. A disk from a 3 day old culture of PRR-8 was placed on the blotter paper at the bottom of every glass cylinder except those of the negative control. Two ml of milliQ water was added to each well in the positive and negative control treatments. The bioassay was rated after 5 days.

Of 104 isolates tested for their ability to prevent infection by isolate PRR-8 of *P. graminicola*, the majority, 84.6%, were shown to be suppressive to this pathogen. The results of the replicated bioassay are given in Table 4. The majority of the 11 isolates tested were highly suppressive to *Pythium graminicola*.

TABLE 4. Results of bioassay of 11 representative actinomycete strains to determine their suppressive to *Pythium graminicola* (PRR-8).

STRAIN	RATING[a]	STRAIN	RATING[a]
ABF91-25	2.0	FA-2	1.8
CCA-2	1.7	GRA-4	2.0
LVB-2	1.5	GRB-5	2.0
CKB-1	1.3	EN89-3	1.9
LCA-1	1.0	FB-1	1.9
CONTROL	0.0	ABF91-2	1.8

[a]The bioassay was rated 6 days after incubation at approximately 25°C on a 0 to 2 scale (0= no grass germination, 1= poor germination, and 2= good germination). A sterile compost/sand mixture was substituted for the actinomycete-infested sand mixture in the control. All treatments were inoculated with a disk of a 2 day-old culture of *Pythium graminicola* (PRR-8). There were 10 replicates per treatment arranged in a completely randomized design.

Bioassays with *Rhizoctonia solani* revealed that 5 of the 8 isolates were able to suppress disease caused by the R6 isolate of the pathogen but none were able to suppress disease caused by the "cotton" isolate (Table 5).

The suppressiveness of aqueous extracts from actinomycete-colonized compost was quite variable among experiments in the control of *P. graminicola*. Results are presented in Table 6. The unfiltered extracts of the strains tested suppressed *Pythium* in the first 2 experiments but not in the third while the filter-sterilized extract was suppressive only in the first experiment. In all 3 experiments, autoclaving the extract destroyed its suppressive activity. Figure 5 illustrates the average of the 3 experiments.

TABLE 5. Bioassay to test the effect of actinomycete isolates grown in compost/sand mixture to suppress disease caused by 2 isolates of *Rhizoctonia solani* on creeping bentgrass.

ACTINOMYCETE STRAIN	BIOASSAY RATING[a] RHIZOCTONIA SOLANI STRAIN COTTON	R6
GRA-4	1	2
CCA-2	1	1.5
GRB-5	1	2
ABF91-25	1	2
ABF91-2	1	2
FB-1	1	2
FA-2	1	2
EN89-3	1	1
-A/+R	1	1
-A/-R	1	1

[a] Bioassay rating (0=no germination of grass seed, 1=poor germination, and 2=good germination) after 7 days incubation at approximately 27°C. There were 2 replications per treatment arranged in a completely randomized design.

TABLE 6. Effect of aqueous extract of actinomycete-infested compost/sand inoculant on suppression of disease caused by *Pythium graminicola* (PRR-8) on creeping bentgrass cv 'Putter".

STRAIN	TREATMENT OF AQUEOUS EXTRACT UNFILTERED[ab]			FILTER STERILIZED[c]			AUTOCLAVED[d]		
	EXPERIMENT 1	2	3	1	2	3	1	2	3
CCA-2	1.90	1.06	0.48	1.10	0.04	0.00	0.5	0	0
EN89-31.5	1.43	0.08	1.50	0.00	0.00	0.60	0.13	0	
GRB-5	1.80	-	-	0.5	-	-	0.8	-	-
-A/+P	0.00	0.00	0.00	-	-	-	-	-	-
-A/-P	-	1.88	1.88	-	-	-	-	-	-

[a]The bioassay ratings for each of 3 experiments were the average of 4 replications per treatment. Treatments were arranged in a completely randomized design. 50 ml of water was added to 50 grams of infested or uninfested compost-amended sand mixture. The resulting suspension was briefly shaken and then immediately decanted. 2ml of the treated extract was added to each well of the bioassay. Ratings were made after 6 days incubation at 25°C. Average of bioassay rating (0=no germination of grass seed, 1=poor germination, and 2=good germination)
[b]decanted extract passed through a sieve to remove coarse organic debris
[c]extract passed through a Whatman #2 filter before being filter-sterilized with a 0.2μm filter.
[d]decanted sieved extract autoclaved for 20 min.

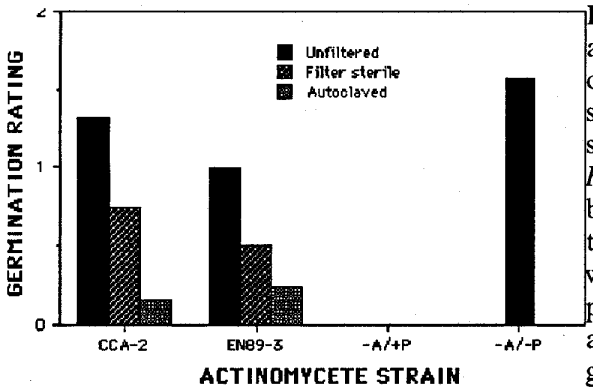


Figure 5. Bioassay to determine the effect of aqueous extract of actinomycete-infested substrate on Pythium Root Rot. Two ml of unfiltered, filter sterilized, or autoclaved extract was added to sterile compost-amended sand inoculated with *Pythium graminicola* and seeded with creeping bentgrass. The graph represents the averages of three experiments (except the -A/-P treatment which was only included twice) with 4 replications per treatment. Germination was rated 5 days after seeding on a 0 to 2 scale (0= no germination, 2= good germination).

In addition to actinomycete strains, bacterial strains were also isolated from various composts and tested for their suppressiveness to *P. graminicola* in laboratory assays. We chose to focus on these groups initially because they are more readily quantified, they are easily handled in the laboratory, and they are likely to be more tolerant of other turfgrass management practices when introduced into the field. Bacteria were isolated on 0.1X TSA using methods as described for the actinomycete isolations. Bioassays were performed as described for the actinomycete experiments. Results of the laboratory screenings are shown in Table 7. Of the 62 bacterial strains tested in bioassays, 38 (61.3%) were effective antagonists of *P. graminicola* (disease rating of 2 or less). Nearly 86% came from the 1992 brewery waste compost, with between 50 and 60 % coming from the Chicken Manure and Food composts.

TABLE 7. Laboratory re-screening of effective bacterial strains recovered from various suppressive composts

Source Compost	Isolate Number	Colony Color	Disease Rating
Brewery Waste 1992	AG1		1.5
	AG2		1.5
	AG3		2.0
	AG5		3.0
	AG6		3.0
	AG7		2.0
	AG8		2.0
EG Chicken Manure (6-4-92)	H1	Yellow	2.3
	B9-c	Cream/Yolk	1.3
	B8-c	Cream	1.3
	A2	?	1.0
	B1-y	Yellow	1.3
	B1-c	Cream	2.0

	A1		1.8
	B8	Yellow	1.5
	A12-y	Yellow	1.5
	B7	Yellow	2.3
EG Food (6-4-92)	A1-w	White	1.0
	A1-c	Cream	1.5
	B8		1.0
	A5-c	Cream	1.5
	A3		1.0
	B11		1.7
	B10	White	1.3
	A12-w	White	1.8
	B1		1.7
	B6		1.8
	A3-y2	Yellow	1.0
	B3		1.0
	B4		1.0
	A6-c	Cream	1.0
	A1		1.3
	A4-c	Cream	1.8
	B12	Cream	2.3
	A5-y	Yellow	1.8
	A4-y	Yellow	1.7
	B7		1.8

[a] Ratings based on a scale of 1-5 for which 1= completely healthy and 5= 100% unemerged or necrotic seedlings

OBJECTIVE 4: Field trials were established to evaluate both the efficacy and environmental fate of compost-derived microbes in golf course putting greens. Based on the replicated bioassay, 8 actinomycete isolates which were highly suppressive to *Pythium graminicola* were selected for inclusion in a field trial along with 8 suppressive bacterial strains also isolated from compost. The inoculum was prepared by autoclaving a compost-amended sand mixture (2305 g sand and 216 g composted brewery waste (both given in oven dry weight)) 3 times at 60 minutes, adding 30 mycelial disks of PRR-8 cut with a #2 cork borer from a 2 to 3 day-old culture on TrypSoy agar, adding 158 ml sterile distilled water (which resulted in 6% w/w moisture content), and incubating for 3 weeks at ambient air temperature (approximately 25°C) in 4L Nalgene jars. The jar lids were sealed with strips of parafilm to retard moisture loss. The jars were shaken before and after the addition of the water and were shaken weekly to redistribute the inoculum

Four hundred ml of the inoculum was applied to each turf plot of creeping bentgrass (3 X 3 feet) located on the Cornell Turf Research plots in Ithaca, NY. The inoculum was evenly distributed by rubbing it, by hand, into the turf plots. The plots were irrigated within a few hours of application of the biocontrol inoculum. There were 5 plots per treatment distributed in a completely randomized design. The trial included 2 controls: one in which uninoculated sand mixture was applied and a second in which the plots were left untreated. The inoculum was applied, at approximately, monthly intervals, 5 times throughout the growing season. The first application

of inoculum was made on May 20, 1993. The plots were rated throughout the growing season as symptoms developed for brown patch (caused by *Rhizoctonia solani*), dollar spot (attributed to *Sclerotinia homoeocarpa*), Pythium root rot (caused by *Pythium* spp.) and for snow mold (caused by *Microdochium nivale*) which will be rated the following Spring. Disease development was dependent upon natural pathogen inoculum and favorable environmental conditions.

Samples of infested compost-amended sand mixtures from each application date were included in a standard bioassay to confirm their *in vitro* efficacy. In addition, plate counts were made to determine the actinomycete population of the inocula from several of the application dates. Three g moist weight of the inocula (2.76 g oven dry weight) was homogenized with 25 ml sterile distilled H_2O for 60 sec in a blender. The homogenate was diluted serially and each plate of 0.1 X strength TSA was inoculated with four 10 ml drops of 10^{-2} through 10^{-5} dilutions of the inoculum homogenate. The plates were sealed in parafilm and incubated for 7 days at 30oC. The populations of actinomycetes in the inoculum at each of the application dates are given in Table 8.

TABLE 8 Population counts of actinomycetes and results of bioassay to test efficacy of field inoculants at each monthly inoculation date.

	APPLICATION DATE				
	5/20 (0)[c]	**6/18** (29)	**7/15** (56)	**8/20** (88)	**9/23** (126)
log cfu/g inoc[a].	8.3	n.a.	8.2	n.a.	?
suppressiveness of test strains	yes	yes	no[b]	yes	?

[a]The population is the log of the colony forming units per gram (oven dry weight) of compost-amended sand inoculant measured close to the date of field application.
[b]Inoculants included in 3 bioassays with variable results possibly due to elevated temperatures in the environmental chamber. Only isolate GRA-4 was shown to be consistently suppressive in these tests.
[c] Days after the initiation of the field trial with first application of inoculants.

Near the end of the growing season, on October 4, 1993, these plots were inoculated with *Pythium graminicola* (PRR-8) by adding 50 g of infested wheat seed into holes which were created by the removal of plugs of turf (15 cm diameter) and replacing the same plugs after the roots had been exposed by shaving off excess soil below the root zone.

In a complementary trial, two turf cores were removed with a soil probe from each plot, trimmed to fit glass rings and challenged with *Pythium graminicola* (PRR-8) by placing 5 *Pythium*-infested wheat seeds (approx. 0.54 g) beneath each turf plug. The inoculum and cores were placed in glass rings on blotter paper in incubation boxes as in the standard bioassay. The treatments were blocked by box and were completely randomized within each box (2 cores x 18 treatments x 5 replicated treatments). One ml of sterile water was added to each well and the entire box was misted with water before the boxes were closed and wrapped in plastic bags to retard moisture loss. The bioassay was incubated at 8.5°C in low light. They were rated for symptom expression after 14 days.

An additional 4 cores were taken from each plot in the field trial and included in a bioassay to assess the effect of treatment with actinomycete isolates on suppression of snow mold caused by *Microdochium nivale* and/or other pathogens. The bioassay depended on natural inoculum of the

pathogen present in the turf samples. The turf cores were arranged as in a standard bioassay except that glass rings were not used. The treatments were blocked by box and randomized within each box (4 cores x 18 treatments x 5 replicated plots). The bioassays were incubated at 8.5°C in low light for 4 days to harden-off the grass before transferring them to 5°C where they were incubated in the dark for 7 days.

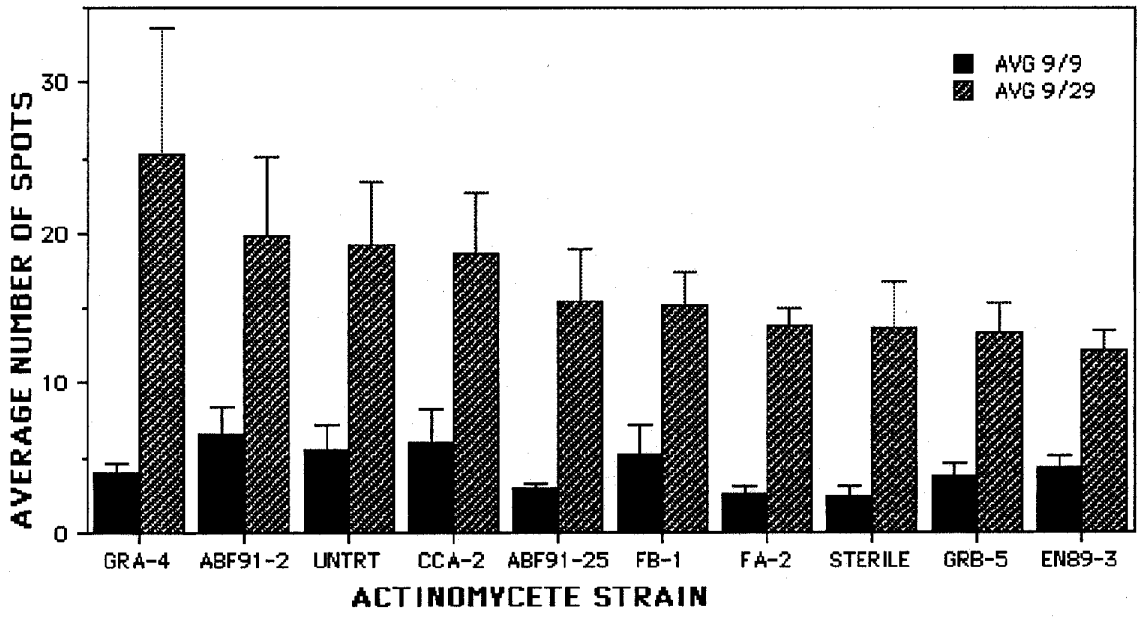


Figure 6. Average number of Dollar Spot spots, attributed to *Sclerotinia homoeocarpa*, on established creeping bentgrass turf after monthly applications of actinomycete-infested compost-amended sand mixture. Disease was caused by naturally-occurring inoculum. Each treatment was replicated 5 times and treatments were arranged in a completely randomized design. Error bars represent the standard error of the individual treatment means.

Field trial. The field disease ratings for Dollar spot, Brown patch, *Pythium* root rot, and Snow mold are given in Figures 6-8. In both the first and the second rating for Dollar spot (Figure 6), 7 treatments appeared to control this disease better than the untreated control. However, none of the treatments were significantly different from the control (UNTRT) at $\alpha=0.05$. The sterilized uninfested compost-amended sand treatment (STERILE) appears to suppress dollar spot disease more than the untreated control. It is possible that, subsequent to application in the field, this substrate became infested with some organism that is suppressive to Dollar Spot.

At the first rating of Brown Patch, 3 treatments were less diseased than the untreated control. However, by the second rating this was reduced to 2. In contrast to Dollar Spot, both Brown Patch and Pythium Root Rot were more serious in the plots treated with the uninfested compost-amended sand mixture (STERILE) than in the untreated controls. In these instances, perhaps this substrate became infested with the pathogens.

Three treatments had equal or less Pythium Root Rot than the untreated control (10/21). However, none of these treatments were significantly different (Figure 7). Treatment with the actinomycete strain FB-1 resulted in slightly less disease than the control at each of the 3 rating dates. FB-1 also slightly suppressed Dollar Spot (9/29) but not Brown Patch (9/7). Strains which

are unable to suppress Pythium Root Rot are in all cases also unable to suppress Dollar Spot but those strains that were able to suppress Dollar Spot ,namely GRB-5, FA-2, ABF91-25, EN89-3, AND FB-1, were not necessarily able to suppress Pythium Root Rot. This suggests that the causal agent of dollar spot is more able to be suppressed than *Pythium* by these actinomycetes.

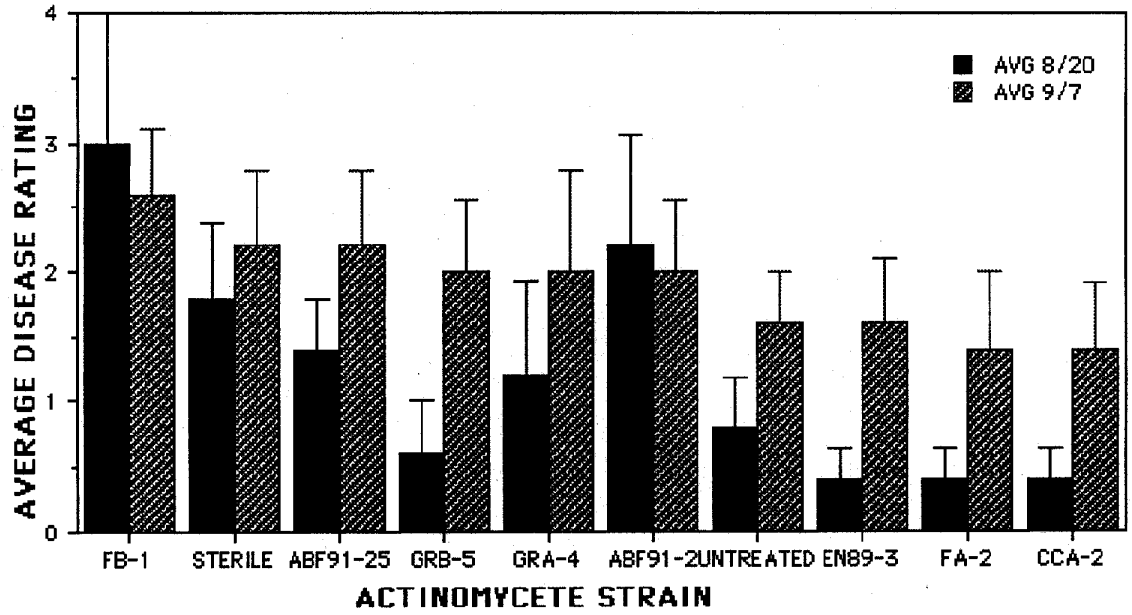


Figure 7. Average disease rating of Brown Patch caused by *Rhizoctonia solani* on established creeping bentgrass turf after monthly applications of actinomycete-infested compost-amended sand mixture. Ratings were made on a 0 to 10 scale by estimating the proportion of diseased area in each plot (0= none of the area diseased, 10= all of the area diseased). Disease was caused by naturally-occurring inoculum. The ratings are the averages of 5 replicated treatments. Error bars represent the standard error of the individual treatment means.

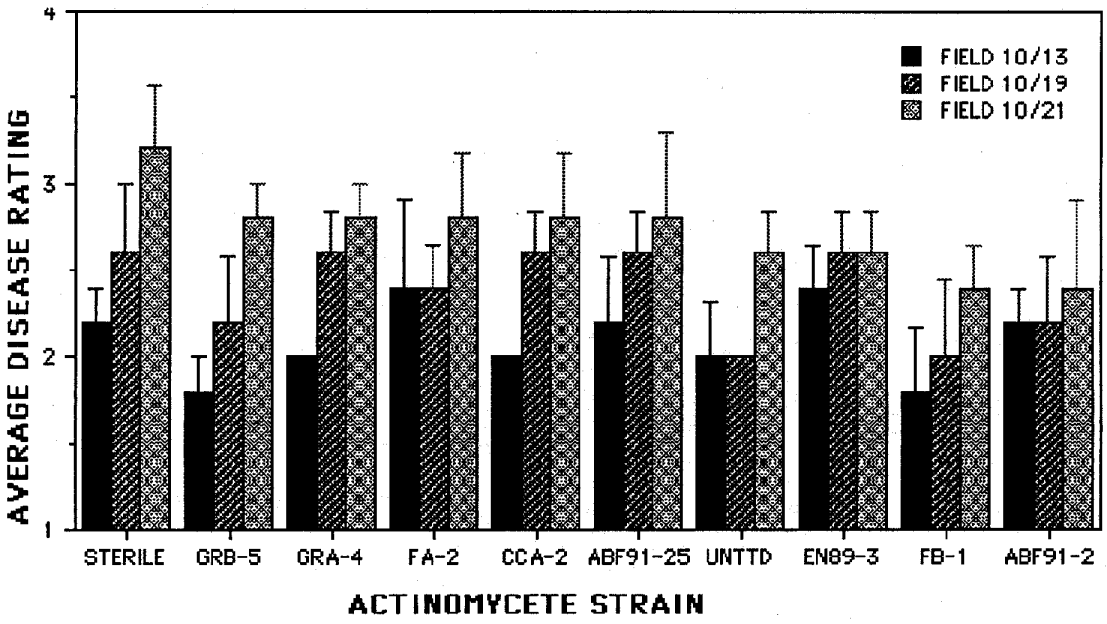


Figure 8. Average disease ratings of turf plots for Pythium root rot after 5 monthly applications of actinomycete-infested compost-amended sand mixture. Ratings were made on a 1 to 5 scale (1= healthy, 5= completely dead) 9 days after individual plots were inoculated with 50 ml of Pythium-infested wheat grain. The ratings are the averages of 5 replicated treatments. Error bars represent the standard error of the individual treatment means.

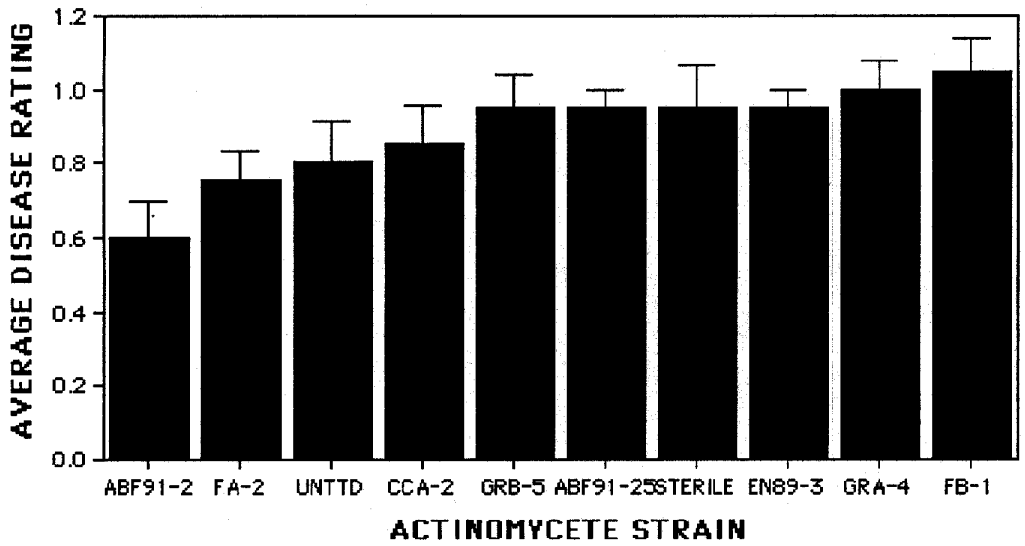


Figure 9. The average disease rating for Pythium root rot in cores of established creeping bentgrass removed from the field plots treated monthly with applications of individual actinomycete strains.

The cores were artificially inoculated with *Pythium graminicola*-infested wheat grain and included in a standard bioassay. The bioassay was incubated at 8.5°C in low light. The results are the averages of 10 cores each. Individual cores were rated on a scale of 0 to 2. The bioassay was blocked to remove the effect of individual incubation chambers.

GENERAL CONCLUSIONS

From the three years of this study we are able to make a number of conclusions:

1: Suppressiveness in composts is either of a microbiological origin or of a non-biological origin. It appears that nearly all of the poultry manure-based composts were of the latter type and were not investigated in any detail. Many, if not all, of the other composts evaluated fell into the first type.

2: Any given compost was not suppressive to a wide range of diseases. Nearly all of the composts evaluated were suppressive to only a few diseases. However, a suppressive compost was found for every disease evaluated.

3: Population levels in composts of either bacteria, fungi, or actinomycetes, were not directly related to the suppression of *P. graminicola*. Composts low in some populations were quite suppressive while other composts high in all microbial populations were not suppressive. Rather, qualitative aspects of microbial activity appears to be more related to the suppression of *P. graminicola.* In general, the greater the microbial activity, the greater the suppressiveness. The usefulness of FDA hydrolysis as a predictive measure for disease suppressiveness was not realized during the course of this study. The principal problems were associated with getting the microbial biomass assays working properly. Whereas we could rank composts on the basis of their comparative microbial activities, we could not establish precise relationships between microbial activity per unit biomass and disease suppression.

4: A large number of specific bacteria and actinomycetes were isolated from a number of composts that were suppressive to *P. graminicola* when tested singly in bioassays. A high frequency of actinomycetes were suppressive as were a moderate frequency of bacteria. Fungi were isolated but, due to time limitations, were not tested in bioassays. Although the results of field trials with individual microbes were disappointing, further testing will need to be pursued. This collection of bacteria and actinomycetes should provide a useful library of microbial germplasm for future studies in the biological control of turfgrass diseases.

Although we have made tremendous progress in advancing our understanding of disease suppression in composts, a more thorough understanding of the composition and activities of disease-suppressive microbial communities in composts is needed. Future work will need to be directed at understanding how disease-suppressive microbial communities develop in composting organic matter and the how the properties of composts themselves and the processes by which they were derived, regulate the activities of these communities. Many of the newer developments in molecular biology will facilitate these investigations.

Opportunities for the understanding of how individual microbes suppress turfgrass pathogens are also possible because of many developments in biotechnology. These new techniques offer

microbiologist an opportunity to genetically dissect biocontrol processes so that mechanisms of biological control can be adequately understood. One of the more important practical reasons pursuing research on the mechanisms of biological control is to develop approaches for the prediction of antagonist behavior. Due to the extremely close link between antagonist function and performance, one cannot readily predict the behavior of biological control microbes without an understanding of the mechanisms involved in pathogen or disease suppression. It also follows that the performance of biological control agents can be enhanced if antagonist function is more clearly understood. These types of studies are clearly the direction in which biological control of turfgrass diseases is heading and they will open the door to enhanced performance and commercialization of biological control microbes.