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

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United States Golf Association Greens Section Research
Final Report, 1991

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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.

We have now established the suppressiveness of various composts to turfgrass diseases caused by two different *Pythium* species and *Typhula incarnata*. This extends the range of turfgrass pathogens already known to be suppressed by compost applications. In field studies we have shown that some composts are as effective as standard fungicides in suppressing Pythium root rot and Typhula blight on creeping bentgrass putting greens.

Our laboratory studies have focussed on *Pythium*-incited diseases of creeping bentgrass. We have shown that disease suppression in some composts is a result of microbial activity whereas suppression in other composts is due to non-microbiological factors. In general, immature composts (i.e. less than 1 yr old) are less suppressive to *Pythium* than mature composts (i.e. greater than 1.5 yr old). These results further indicate a microbiological nature to disease suppression in these composts. Sterilization of some composts eliminates disease-suppressive properties. In examining a number of suppressive and conducive composts, we have shown direct relationships between microbial activity and disease suppression.

In preliminary experiments with a poultry manure compost, populations of fungi and actinomycetes were quite low whereas populations of bacteria ranged from 4.4 - 7.5 million cells per gram of compost. Current studies are focussing on the qualitative microbiological differences between suppressive and conducive composts and the interactions of specific microorganisms with turfgrass pathogens. Our goal is to determine the key microorganisms inhabiting composts so that their physiology and ecology might be better understood. This information will be important in being able to predict whether particular composts at particular stages of maturity will be suppressive at particular sites and under particular environmental conditions.

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The objectives of our study are as follows:

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.

During the 1991 granting period, several aspects of the study were initiated and progress was made toward objectives 1, 2, and 3. A summary of the progress related to each project objective is outlined below.

Objective 1) Both laboratory and field experiments were performed to evaluate the suppressiveness of various composts to different turfgrass pathogens. Major emphasis in laboratory experiments was on root rotting *Pythium* species, particularly *P. graminicola*. Previous work from other funding sources has established the suppressiveness of selected composts to Dollar spot, Brown patch, Red thread and Pythium blight. Field trials established in 1991 were targeted for Pythium root rot and Typhula blight. The origin and selected chemical properties of composts and organic fertilizers studied are listed in Table 1. Immature composts ranged from 3 wk-old to 7 mo-old whereas mature composts ranged from 1.5 yr-old to 3.5 yr-old.

Field plots were established in 1990 on a creeping bentgrass (*Agrostis palustris* Huds.)/annual bluegrass (*Poa annua* L.) putting green at the Country Club of Rochester, Rochester, NY to evaluate season-long applications of various composts and organic fertilizers for their ability to carry over and suppress gray snow mold the following spring. The putting green was over 60 yr-old and constructed from the native alkaline clay loam soil (pH 7.2) in the area. The green contained a mixture of bentgrasses and annual bluegrass and was naturally infested with pathogenic *Typhula* species. The green was mowed at a 5 mm cutting height and aerified (0.6 cm diameter tynes) one day prior to application of treatments.

Experiments to evaluate composts for Typhula blight control were established the previous season (1990) on a creeping bentgrass/annual bluegrass putting green at the Country Club of Rochester, Rochester, NY. Organic components were mixed with fine quarry sand (pH 7.5) in the proportions of 30% organic component and 70% sand (v:v). This mixture was chosen to represent the maximum amount of organic matter that would typically be applied to a golf course putting green during commercial top-dressing applications. Top-dressings were placed in small styrofoam cups or plastic bags and stored at room temperature prior to application to plot areas.

TABLE 1. Composition and nutrient availability in composts and organic fertilizers used in this study.

Material	Composition	pH[b]	Available Nutrients (μg/g material)[a]								
			NO_3^{-}[c]	P[d]	K[e]	Ca[e]	Mg[e]	Fe[e]	Mn[e]	Zn[e]	Cu[e]	Al[e]
Organic Fertilizer CP[f]	Plant/animal meals	6.6	81	3845	5010	2805	802	49.0	35.0	3.00	1.0	26.0
Organic Fertilizer GR[f]	Plant/animal meals	6.5	24	2889	26627	1577	1578	40.4	50.2	2.95	0.9	0.9
Turkey Litter Compost 89	Turkey litter	6.2	9	3685	13358	2671	2608	24.8	75.6	2.54	0.6	0.6
Turkey Litter Compost 90	Turkey litter	6.2	9	3685	13358	2671	2608	24.8	75.6	2.54	0.6	0.6
EG Manure Compost	Chicken/cow manure	6.9	4178	5065	14805	19923	2876	3.5	57.1	1.02	1.1	15.0
Paygro Manure Compost	Cow manure	5.0	853	2375	2629	4151	739	3.0	47.8	1.01	2.1	21.3
IPS Manure Compost	Poultry/cow manure	7.1	4164	8337	14867	18743	3965	12.5	83.0	0.94	1.1	45.3
Saratoga Compost	Horse Manure	6.1	1689	886	1305	15007	627	3.6	45.0	2.54	0.8	13.4
MH Manure Compost	Horse manure	6.7	1316	858	5020	4900	1422	6.6	44.6	0.39	0.6	8.1
Spent Mushroom Compost	Horse manure	7.3	1134	1147	6993	17377	1481	4.8	21.8	0.53	0.9	15.0
AB Brewery Compost-immature	Brewery waste	6.5	1294	2923	7671	6980	2590	3.1	46.8	0.69	0.8	8.9
AB Brewery Compost-mature	Brewery waste	4.9	1603	2134	2322	6833	877	2.8	45.8	0.62	2.1	22.7
Endicott Sludge Compost-immature	Sewage sludge	5.3	3220	723	1545	16454	1006	12.4	129.5	11.90	4.5	26.8
Endicott Sludge Compost-mature	Sewage sludge	5.6	1343	832	124	792	108	1.6	6.6	0.16	1.6	7.0
Schenectady Sludge Compost	Sewage sludge	5.9	2864	1111	1553	13910	1677	13.2	76.2	3.35	5.9	21.0
Baltimore Sludge Compost	Sewage sludge	4.9	1689	886	1088	14495	2236	76.5	93.9	12.95	1.1	40.5

[a] Extracted with Morgan's solution, 10% sodium acetate in 3% acetic acid buffered to pH 4.8, using a 1:5 (v:v) soil:solution ratio.
[b] Determined in a 1:1 (v:v) soil:water suspension
[c] Determined by an automated hydrazine reduction method.
[d] Determined by a stannous chloride reduction method.
[e] Determined by atomic absorption.
[f] These materials were not composted.

Treatments were applied at roughly monthly intervals on 5 Jun, 5 Jul, 14 Aug, 6 Sep and 26 Oct to 0.9 m X 1.5 m plots at the rate of 500 cm^3 of top-dressing formulation per m^2. Top-dressings were distributed by hand as uniformly as possible over the plot area then lightly rubbed-in to distribute the material into the turf canopy. Propiconazole (Banner) was applied at the rate of 173 mg a.i./m^2 as a fungicide standard. Controls consisted of plots to which no top-dressing was applied. All plots were then watered by applying 0.6 cm of irrigation. Plots were evaluated for Typhula blight damage on 18 Apr 91 on a scale of 0 to 10 where 0 = no disease and 10 = 100% of plot area diseased.

Topdressing applications of a mature sludge compost from Endicott, NY, a horse manure compost from Moody Hill Farms and an immature brewery waste compost from Baldwinsville, NY significantly reduced the incidence of Typhula blight on a creeping bentgrass/annual bluegrass putting green as compared with untreated plots (Table 2). Levels of control among these composts ranged from 54 - 70%. The levels of control provided by the sludge compost and the brewery compost were significantly (P=0.05) better than the fungicide, Banner.

TABLE 2. Effect of selected composts and organic fertilizers on the suppression of Typhula blight on a creeping bentgrass/ annual bluegrass putting green.

Treatment[a]	Disease Rating[b]	% Control
Untreated	3.3	-
Organic Fertilizer CP	5.5	-66.7[c]
Organic Fertilizer GR	4.5	-36.4
Paygro Manure Compost	2.8	15.2
Turkey Litter Compost	2.8	15.2
EG Manure Compost	2.3	30.3
MH Manure Compost	1.5	54.5
AB Brewery Compost-immature	1.0	69.7
AB Brewery Compost-mature	2.3	30.0
Endicott Sludge Compost-immature	1.0	69.7
Endicott Sludge Compost-mature	1.8	45.5
Propiconazole	2.8	15.2
LSD (P=0.05)	1.7	

[a] Composts and organic fertilizers mixed with sand (30:70 compost:sand; v:v) and applied as a topdressing at monthly intervals.

[b] Determined on 18 Apr 91 on a scale of 0 - 10 for which 0 = none of the plot area diseased and 10 = 100% of the plot area diseased.

[c] Negative numbers indicate increases in disease severity as compared with the untreated control.

Experiments to evaluate composts for Pythium root rot control were performed under both laboratory and field conditions. In laboratory experiments, wells of a 24-well tissue culture plate were filled with a sand:compost mixture (80 mg compost/ml sand) (4 wells/treatment) and inoculated with either *Pythium graminicola* or *P. ultimum* by placing a 2-mm-diameter agar disk colonized by the test pathogen. Wells were then seeded with creeping bentgrass (*Agrostis palustris* 'Providence') or, in some experiments, planted with 10 seeds of perennial ryegrass (*Lolium perenne* 'Palmer'). Distilled deionized water (0.75 ml) was added to each well and cultures were allowed to incubate for 5-7 days. Each well was then rated for disease development

on a scale of 1 - 5 for which 1 = no disease or 100% seed germination and 5 = 100% disease or 0% seed germination. In some experiments, the compost:sand mixture was autoclaved prior to filling wells.

In the absence of pathogenic *Pythium* spp., creeping bentgrass germinated poorly in the presence of turkey litter composts, IPS manure compost, and EG manure compost (Table 3, Table 4). Little or no change in the disease rating was obtained upon inoculation with *P. graminicola*. Of those composts not inhibitory to seed germination, only the mature AB brewery compost, the Paygro manure compost and the mature Endicott sludge compost were suppressive (Table 3). The level of suppression ranged from 54 to 74%. Immature batches of the AB brewery compost and the Endicott sludge compost were not suppressive. The turkey litter composts remained highly inhibitory to germination of creeping bentgrass seed.

TABLE 3. Suppression of Pythium (*P. graminicola*) seed and root rot of creeping bentgrass with various composts

Compost	Disease Rating (5 da)		
	Uninoc.	Inoc.	% Control
AB Brewery Compost-immature	1.0	3.3	34.0
AB Brewery Compost-mature	1.0	1.3	74.0
IPS Manure Compost	3.0*	3.3	-
MH Manure Compost	1.0	5.0	0.0
EG Manure Compost	3.3*	3.5*	-
Saratoga Manure Compost	1.0	3.3	34.0
Turkey Litter Compost 89	5.0**	5.0**	-
Turkey Litter Compost 90	5.0**	5.0**	-
Paygro Manure Compost	1.0	2.3	54.0
Spent Mushroom Compost	1.0	3.0	40.0
Endicott Sludge Compost-immature	2.0*	3.0	40.0
Endicott Sludge Compost-mature	1.0	2.3	54.0
Baltimore Sludge Compost	2.0	4.0	20.0
Schenectady Sludge Compost	1.0	4.0	20.0
Loon Lake Soil	1.0	4.3	14.0
Sand (Control)	1.0	5.0	-
LSD (P=0.05)	0.5	0.9	

Scale: 1=Healthy, 5=100% Disease; determined 5 days after inoculation
* = Delayed Germination; ** = No germination

Similar results were obtained when wells were inoculated with *Pythium ultimum* (Table 4). Both the mature AB brewery waste compost and the mature Endicott sludge compost were suppressive to *P. ultimum*. In addition, the immature Endicott sludge and the Schenectady sludge compost were suppressive to *P. ultimum*. The level of suppression among these composts ranged from 37 - 52%.

When wells were inoculated with *P. graminicola* and planted to perennial ryegrass, little reduction in seedling stands was observed with most composts. The notable exception was the sterilized mature Endicott sludge compost. Seedling stands were reduced from 9.8

in the unsterilized Endicott compost to 1.5 in the sterilized batch. The turkey litter composts, regardless of sterilization, were suppressive to perennial ryegrass seed germination. Plant heights were reduced in the EG manure compost and the sand control (Table 5).

TABLE 4. Suppression of Pythium (*P. ultimum*) seed and root rot of creeping bentgrass with various composts

Compost	Disease Rating (5 da)		
	Uninoc.	Inoc.	% Control
AB Brewery Compost-immature	1.0	3.8	20.8
AB Brewery Compost-mature	1.3	2.3	52.1
IPS Manure Compost	3.0*	3.8	20.8
MH Manure Compost	1.0	4.8	0.0
EG Manure Compost	3.3*	4.3*	10.4
Saratoga Manure Compost	1.0	3.0	37.5
Turkey Litter Compost 89	5.0**	5.0**	-
Turkey Litter Compost 90	5.0**	5.0**	-
Paygro Manure Compost	1.0	3.0	37.5
Spent Mushroom Compost	1.0	3.8	20.8
Endicott Sludge Compost-immature	1.8	3.0	37.5
Endicott Sludge Compost-mature	1.3	2.3	52.1
Baltimore Sludge Compost	3.3*	3.8	20.8
Schenectady Sludge Compost	1.3	2.5	47.9
Loon Lake Soil	1.5	4.0	16.7
Sand (Control)	2.0	4.8	-
LSD (*P*=0.05)	0.6	1.2	

Scale: 1=Healthy, 5=100% Disease; determined 5 days after inoculation
* = Delayed Germination; ** = No germination

TABLE 5. Suppression of Pythium seed and root rot (*P. graminicola*) of perennial ryegrass with various composts[a]

Treatment	Uninoculated		Inoculated	
	Seedling Stand	Avg. Plant Height	Seedling Stand	Avg. Plant Height
Endicott Sludge Compost-mature	8.8	4.0	9.8	4.0
Endicott Sludge Compost-immature	6.3	4.0	9.3	4.5
Sterile Endicott Sludge Compost-mature	4.5	4.0	1.5	-
MH Manure Compost	8.8	5.0	8.5	4.5
Paygro Manure Compost	9.0	5.0	8.8	5.5
Loon Lake Soil	9.0	4.5	7.3	3.0
EG Manure Compost	3.5*	2.5	5.0	2.0
Turkey Litter Compost 89	0.0	-	0.0	-
Turkey Litter Compost 90	0.0	-	0.0	-
Sterile Turkey Litter Compost 89	0.0	-	0.0	-
Sterile Turkey Litter Compost 90	0.0	-	0.0	-
Sand (Control)	9.3	4.0	6.0	2.0
Means	5.9	4.1	5.6	3.6
LSD (P=0.05)	1.9		2.5	

[a] Determined 5 days after inoculation

Field experiments were established in 1991 on a creeping bentgrass sand putting green at the Cornell University Turfgrass Field Research Laboratory (CUTFRL). Composts were prepared and applied as described above to plots inoculated with *Pythium graminicola*. Approximately 2 cm of irrigation was applied daily through the duration of the experiment. Plots were rated weekly after application on a scale of 0-10 for which 0= none of the inoculated area symptomatic whereas 10 = 100% of the inoculated area chlorotic or necrotic. Results from 2-wk and 4-wk ratings are presented in Table 6. On July 5 (16 days after application), only the turkey litter compost and the 2.5 yr-old Endicott sludge compost provided significant levels of disease control. Metalaxyl (Subdue) was ineffective in suppressing Pythium root rot. By 26 days after application, both the turkey litter compost and the 2.5 yr-old Endicott sludge compost remained suppressive. Additionally, the EG manure compost, a 3-wk Endicott sludge compost, and a 3-wk AB brewery compost were significantly suppressive as well as the uncomposted organic fertilizers CP and GR. Metalaxyl remained ineffective in suppressing Pythium root rot.

TABLE 6. Suppression of Pythium Root Rot of Creeping Bentgrass with Field Applications of Various Composts

Treatment	Rating 1[a]	% Control	Rating 2[b]	% Control
Sand	3.8	-	3.2	-
Turkey Litter Compost 90	1.8	52.6	0.2	93.8
EG Manure Compost	2.8	26.3	1.2	62.5
Endicott Sludge Compost-mature	1.6	57.9	1.6	50.0
Endicott Sludge Compost-immature	2.2	42.1	1.6	50.0
AB Brewery Compost-mature	2.4	36.8	2.2	31.3
AB Brewery Compost-immature	3.2	15.8	1.8	43.8
Paygro Manure Compost	3.6	5.3	2.4	25.0
Saratoga Manure Compost	4.2	-10.5	2.2	31.3
Organic Fertilizer CP	2.2	42.1	1.4	56.3
Organic Fertilizer GR	2.0	47.4	1.4	31.3
Chicken Manure Compost	2.8	26.3	2.2	31.3
Metalaxyl (Subdue)	2.2	42.1	2.4	25.0
LSD (P=0.05)	2.4		1.5	

[a] Plots evaluated on 5 Jul 91 (16 days after application) on a scale of 0 - 10 for which 0 = no disease, 10 = 100% of inoc. area chlorotic or dead.

[b] Plots evaluated on 15 Jul 91 (26 days after application) on a scale of 0 - 10 for which 0 = no disease, 10 = 100% of inoc. area chlorotic or dead.

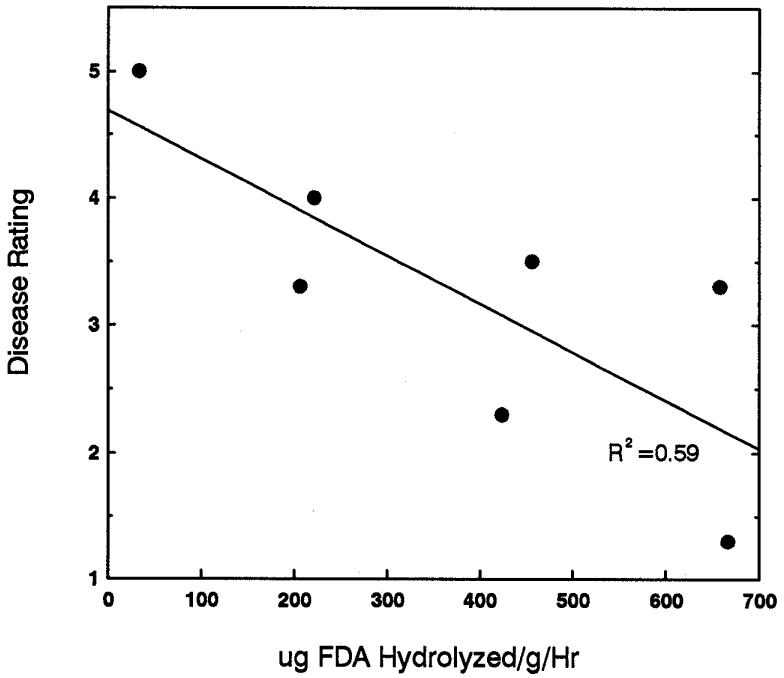
Objective 2) Experiments were established to determine the relationship between microbial activity and disease suppression. Total mesophilic microbial activity was assessed in a number of different compost samples (both suppressive and non-suppressive) by monitoring the hydrolysis of fluorescein diacetate (FDA) in aqueous compost extracts. Extensive preliminary work was required in order to establish more appropriate experimental parameters such as sample size, sample dilution, appropriate amount of FDA to add to each sample, filtration parameters, etc. These data will not be presented in this report but are available on request. FDA, which can be degraded by enzymes unique to actively growing organisms to yield a fluorescent compound easily detected spectrophotometrically, has been used previously to assess microbial activity in composts. We have concentrated our initial experiments on *Pythium* diseases of creeping bentgrass in order to adequately develop the method. Seven composts and one soil have been examined thus far for both FDA hydrolysis and *Pythium* suppression. Levels of FDA hydrolyzed are listed in Table 7. The level of microbial activity was quite low in the turkey litter composts but 10 to 100 times higher in the other composts. The relationship between microbial activity in these composts and Pythium seed and root rot suppression caused by *P. graminicola* is illustrated in Figure 1.

Table 7. Microbial Activity in Composts Determined with FDA Assays

Compost	μg FDA hydrolyzed/g/hr[a]
Turkey litter compost 89	4.9
Turkey litter compost 90	32.8
Saratoga Manure Compost	206.1
Loon Lake Soil	221.1
Endicott Sludge Compost-mature	423.5
EG Manure Compost	455.9
AB Brewery Compost-immature	657.8
AB Brewery Compost-mature	666.4

[a] Determined using 0.5 g compost samples

Figure 1.



In general, as microbial activity increases, the level of *Pythium* suppression increases. Composts tested that do not fit this model are the turkey litter composts which, in field experiments, are quite suppressive. However, they possess extremely low levels of microbial activity and are also suppressive to creeping bentgrass seed germination. Our speculation is that ammonia levels in these composts might be responsible for the suppression of seed germination and possibly *Pythium* suppression in the field.

Additionally, experiments have been performed to assess total microbial biomass in both suppressive and non-suppressive composts. A number of technical difficulties have prevented us from being able to rely on this technique for the determination of microbial biomass in compost samples. The technique is dependent on the ability of malachite green, a dye, to interact with the phosphate groups of chloroform-extracted lipids. Repeated attempts to react the dye with standard phosphates have failed. Although we are using standard protocols developed by others, the technique has not been reliable in our hands. We will continue to try additional ways of determining microbial biomass in these composts.

Objective 3) We have just begun experiments to examine the specific microflora of both suppressive and non-suppressive composts. Microbial groups on which we are focussing include suppressive compost samples, mesophilic bacteria, fungi and actinomycetes. These organisms have been enumerated using conventional microbiological techniques with selective media. To isolate these microorganisms, 10 g of compost was placed in 90 ml of distilled water and placed on a shaker for 10 min. at room temperature. A dilution series was prepared and 0.1 ml aliquots plated onto appropriate culture media. For bacterial isolations, suspensions were plated onto 1/3-strength trypticase soy agar. For actinomycetes, suspensions were plated onto 2% water agar amended with nystatin and polymyxin B to inhibit fungal and bacterial growth. For fungi, suspensions were plated onto 1/3-strength potato dextrose agar amended with rifampicin to inhibit unwanted bacterial growth. After incubation of isolation media at 27C, developing colonies of each respective group of microorganisms were then be enumerated and expressed as colony forming units (CFU) per gram dry wt of compost. We are in the process of performing isolations from the following composts: EG Manure Compost, mature and immature Endicott Sludge Compost, and both mature and immature AB Brewery Compost. The AB Brewery composts and the Endicott composts represent our most consistently suppressive composts whereas the EG manure compost is our only relatively "non-toxic" poultry manure compost. The following is an example of the population levels found in three batches of the EG manure compost:

Batch 1:	Fungi	270 CFU (colony-forming units)/g
	Bacteria	7,500,000 CFU/g
	Actinomycetes	<100 CFU/g
Batch 2:	Fungi	3,150 CFU/g
	Bacteria	4,600,000 CFU/g
	Actinomycetes	<100 CFU/g
Batch 3:	Fungi	180 CFU/g
	Bacteria	4,400,000 CFU/g
	Actinomycetes	<100 CFU/g

Predominant fungal genera in all three samples were *Penicillium* spp. Bacterial genera were not characterized.

Objective 4) No progress to date.

Proposed Research Schedule:

Objective 1. Of the turfgrass pathogens examined to date in this laboratory, composts have been suppressive to *Pythium graminicola, P. aphanidermatum, P. ultimum, Rhizoctonia solani, Sclerotinia homoeocarpa, Typhula incarnata* and *Laetisaria fuciformis*. Future studies will continue to emphasize *Pythium* spp., particularly others involved in the Pythium root rot complex. However, studies are planned to determine the suppressiveness of composts to *Microdochium nivale* (pink snow mold), *Colletotrichum graminicola* (anthracnose) and *Bipolaris sorokiniana* (leaf spot). These will be examined, where practical, in the field as well as in the laboratory. We expect to find composts that will be suppressive to some degree to all of these pathogens.

Objective 2. Our goal with this objective is to develop predictive assays for disease suppression in composts. Our focus thus far has been on FDA hydrolysis as an indicator of microbial activity. We plan to look further at specific activity of the entire compost microbial community. Specifically, we plan to examine single source carbohydrate utilization by the intact microbial community as a means of distinguishing predominant microbial activities in suppressive and conducive composts.

In order to define a number of the technical aspects of microbial activity assays based on FDA hydrolysis, we have focussed on *Pythium* spp. We plan to develop such relationships with *R. solani* (brown patch) and *M. nivale* (pink snow mold). We also plan to enhance our efforts in developing a suitable technique for the determination of microbial biomass in composts. We feel that comparisons can be made among composts of different types and stages of decomposition only if microbial activity can be standardized per unit biomass.

Objective 3. After colonies have developed on isolation plates, individual colonies will then be transferred to test tubes containing an appropriate growth medium and stored until positive identifications can be made. Identification of bacteria and fungi will be made to the genus level according to standard taxonomic criteria and every attempt will be made to identify effective antagonists to the species level. Comparisons of microbial diversity and population levels will be made among suppressive and non-suppressive composts.

Individual organisms will be tested in plant assays to identify those that are able to restore suppressiveness in heat-treated compost to levels found in the naturally recolonized suppressive compost. Suppressive samples will be made conducive by heating to 60 C for five days prior to inoculation (the extended heating period was chosen as representative of temperature/time exposures in the center of a typical compost windrow). Composts will then be inoculated by culturing candidate organisms in the laboratory on an appropriate liquid culture medium for two to five days. The suspension (containing cells, spores, etc.) will then be poured into a mixture containing 80 mg compost/ml sand and incubated at room temperature for 24 hr. The inoculated mixture will be placed in wells of a tissue culture plate, seeded with creeping bentgrass and allowed to grow for 7 days. Wells will then be inoculated with the target pathogens listed above.

As we proceed with isolations from suppressive and conducive composts, we plan to give particular emphasis to bacteria in our initial screenings for disease suppression since we feel that our best candidates suitable for integration into greens and fairway management practices will come from this group. Although the activity of fungi and actinomycetes will be studied in some detail, our selection of strains for field testing will not initially emphasize these groups due to handling and monitoring problems as well as compatibility with turf fungicides.

Objective 4. Our final year of funding on the current USGA grant will conclude with a field test examining the fate of selected antagonists in golf course putting greens. Emphasis will be on sand-based putting greens. To determine how well select antagonists establish on plants and in soil following field applications of an antagonist-amended compost, we will inoculate both suppressive and non-suppressive compost samples with cultures of known antagonists recovered from suppressive compost samples. Individual antagonists shown to be effective in laboratory experiments will be cultured in the laboratory on an appropriate culture medium for two to five days. The suspension of the culture (containing cells, spores, etc.) will be poured into a mixture containing 80 mg compost/ml sand and allowed to incubate at room temperature for 24 hr prior to application. Plots will be treated and monitored for disease development as in previous experiments. Untreated and fungicide-treated plots as well as plots treated with composts not fortified with antagonists will serve as

controls.

Population dynamics of select bacterial antagonists will be monitored weekly from the time of application through the end of the season by removing 20 random cores (1 cm-diameter) from each replicate plot. Cores for each treatment will be pooled and 10 g of core samples placed in 90 ml of distilled water and comminuted in a blender for 1 min. A dilution series will then be prepared and 0.1 ml aliquots plated onto selective media. Rifampicin-resistant derivatives of parental strains will be selected from the wild-type population and used to monitor populations. After incubation at 27C, colonies of the target organism will be enumerated and expressed as CFU/g dry wt of sample. Soil temperature (3 cm depth) and moisture will be monitored throughout the experiment and soil analyses will be performed before and after applications are made. Cultural inputs such as mowing heights, aerification, fertilization, pesticide applications, etc. will also be recorded.

Additional Comment:

During this granting period we feel we have made considerable progress in refining techniques and generating some baseline information useful for subsequent aspects of this study. During this funding period we were somewhat hampered by a late starting date for the project. Although the project was formally approved by Cornell and USGA on May 23, monies were not present in accounts until June 25. Since funding was requested to support technical personnel, searches could not begin until monies were deposited. A person was finally hired September 1. The progress made thus far has been primarily a result of part-time efforts by several of my program personnel. We look forward to more substantial progress in the coming years.