Nontarget Effects of Turfgrass Fungicides on Microbial Communities in USGA Putting Green Profiles

Gary E. Harman, Eric B. Nelson, and Kristen L. Ondik

Executive Summary

This research is examining in detail the nontarget effects of fungicides commonly used for disease control on golf course putting greens. Our goal is to understand the scope and magnitude of microbial responses to fungicide applications so that potentially detrimental side effects may be avoided. We established plots on peat-based bentgrass greens constructed using USGA specifications, and similar greens to which brewery compost was added during construction and to which the biocontrol fungus *Trichoderma harzianum* was added at the beginning of the experiment. These green structures were used because they were expected to contain different microbial populations and so fungicides may have dissimilar nontarget effects on these different microbial communities. The fungicides chosen for these experiments were Daconil Ultrex (chlorothalonil), Chipco 26019 Flo (iprodione), Subdue Maxx (mefenoxam), Banner Maxx (propiconazole), Bayleton 25W (triadimefon), Prostar 50WP (benzamide), and Sentinel (cyproconazole).

Surprisingly, the first preliminary data suggests that the various fungicides, even when multiple applications at their maximum legal rates were made, had little effect upon microbial communities. Numbers even of organisms known to be highly sensitive to the fungicides being applied were little affected by the treatments used in this experiment. These data suggest that these fungicides are not present at the fungitoxic concentrations below about 1 inch below the soil surface, since the samples to this depth into the turf soil profile. Several reasons for this lack of efficacy may be possible, including binding to soil particles and rapid microbial degradation.

This lack of efficacy suggests, first, that the fungicides tested may be less disruptive to normal soil microflora than originally expected. Second, the data suggest that the fungicides should largely be effective only on leaf diseases and have little effect upon subterranean fungal populations and root health.

Augmentation of plots with the compost + *T. harzianum* addition had two noticeable effects. First, levels of *T. harzianum* increased about 1000-fold with the addition of this biocontrol product and remained at a consistent level over the sampling times. Second, levels of actinomycetes were lower in augmented than in nonaugmented plots at the second sampling time.

These results are preliminary and will be followed by additional tests on other microflora with measures of both soil microfloral activity and further measures of microbial diversity.

Budgetary Items

<u>% time spent on project since 2/1/96</u>

G. E. Harman	5%
E. B. Nelson	5%
K. Ondik	50%
C-T Lo	10%

<u>Expenditures</u>

Salaries	$11,325
Fringe benefits	3,300
Supplies	475
Total Direct	15,298
Indirect costs (16%)	2,447
Total	$17,425

Note: Other funds are available to continue work over the next several months.

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Objectives

This research is examining in detail the nontarget effects of fungicides commonly used for disease control on golf course putting greens. Our goal is to understand the scope and magnitude of microbial responses to fungicide applications so that potentially detrimental side effects may be avoided. During the past several months we have:

1) Established and microbially characterized standard and biologically-augmented root zones on USGA and soil-based putting greens.

2) Determined comparative responses of native and constructed microbial communities to fungicide applications on USGA and soil-based putting greens.

3) Assessed sensitivities of important groups of turf-associated microbes to common turfgrass fungicides.

4) Started to evaluate impacts of fungicide applications on levels of biological control in native and microbially-augmented USGA and soil-based putting greens.

This study should provide the first comprehensive information on nontarget effects of fungicides on microbial communities and be of practical importance to golf course managers.

Experimental Plan

Establishment and Monitoring of Experimental Turfgrass Plots

Ten eight-foot diameter swimming pools constructed in 1995 at the Cornell University Turf Research Farm in Ithaca, NY, are used as the experimental microplots. Five of the pools are the standard USGA sand/peat profile (nonaugmented), and the other five are the standard USGA profile augmented with Anhauser-Busch brewery-waste compost, and at the start of the experiment, *Trichoderma harzianum* as a commercial biocontrol product. Prior to addition of this organism, the plots had no detectable *T. harzianum* (although it contained high levels of the related organism *T. virens*). The addition of this organism resulted in a stable population of this fungus in soils. The compost additions were expected to enhance microbial activity and addition of *T. harzianum* provided a known fungus with biocontrol capability. Further, the fungicide tolerances of this biocontrol fungus largely are known, and so the presence of this organism provides a specific marker for activity of fungicides known to be toxic to this organism. For example, it is resistant to Chipco 26019 but very susceptible to Banner.

In all of the data presented below, the compost + *T. harzianum* amended plots will be labelled as augmented plots, while the standard plots prepared using standard USGA construction are labelled

as nonaugmented. The plots should provide a difference in microbial activity and provide useful information on the effects of fungicides on different microbial communities.

Subplots consist of an untreated plot and the seven fungicide treatments. Each subplot is three square feet and each treatment is represented on each pool. The fungicides selected represent different classes of fungicidal activity. For example, Daconil Ultrex (chlorothalonil) is a contact fungicide with a relatively non-specific mode of action against most classes of fungi. Chipco 26019 Flo (iprodione) selectively damages energy-producing organelles in select fungi. Banner Maxx (propiconazole) and Bayleton (triadimefon) are systemic in plants and have a very specific mode of action, inhibiting a specific enzyme necessary for fungal cell integrity. In all cases, if alternative rates are registered, we always used the maximum legal rate of the fungicide. The treatments, active ingredients, rates, and application schedules are as follows:

Trt	Fungicide	Active Ingredient	Rate	Application Interval
1	Untreated	----------	--------	--------
2	Daconil Ultrex	chlorothalonil	10 lb/A	14 days
3	Chipco 26019 Flo	iprodione	8 oz/1000 sq. ft.	21 days
4	Subdue Maxx	mefenoxam	1 oz/100 sq. ft.	21 days
5	Banner Maxx	propiconazole	4 oz/1000 sq. ft.	21 days
6	Bayleton 25W	triadimefon	4 oz/1000 sq. ft.	21 days
7	Prostar 50WP	benzamide	3 oz/1000 sq. ft.	14 days
8	Sentinel	cyproconazole	0.167 oz/1000 sq. ft.	21 days

Subdue Maxx is an experimental fungicide but is expected by Ciba Geigy to replace the standard formulation of Subdue by next year. The active ingredient is the same in Subdue and Subdue Maxx. Two hundred milliliters of the appropriate rate was applied to each plot using a hydraulic CO_2 sprayer. All subplots were replicated five times on each soil profile. The first application was applied on June 10, 1996.

Granules colonized with *Trichoderma harzianum* strain 1295-22 were added to the augmented pools on May 31, June 27, and August 27 at a rate of 1.5 lb/1000 sq. ft.

Samples were taken on May 22 (T_0- before any fungicide application), June 6 (T_{0T}- after *Trichoderma* was applied but before any fungicide application) and monthly thereafter until September 30, 1996. Since no fungicides were yet applied at T_{0T}, representative cores within each pool were combined to give ten samples (one for each pool). Thereafter, each subplot is represented in each sample set, i.e., 8 separate samples per pool. Nine to twelve 1.0 cm-diameter cores were taken from each subplot at a depth of 3 cm and transported to the laboratory for microbial assays. For purposes of simplicity, only T_{0T} values are shown in the following data.

Characterization of Microbial Communities in Putting Green Profiles

Microbial plate counts were determined by performing a serial dilution in phosphate-buffered saline (PBS) and plating appropriate dilutions on solid media. Major microbial groups were plated on the following media:

Total Fungi	(API)	Acidified Potato Dextrose Agar + Igepal
Trichoderma harzianum		
T. virens		
Total Bacteria	(1/10 TSA)	1/10-strength Trypticase Soy Agar
Pseudomonads	(PsSM)	Pseudomonad Selective Medium

Actinomycetes	(1/50 TSA + PMB)	1/50-strength TSA + 20 mg/L Polymixin B Sulfate
Pythium	(PySM)	Pythium Selective Medium

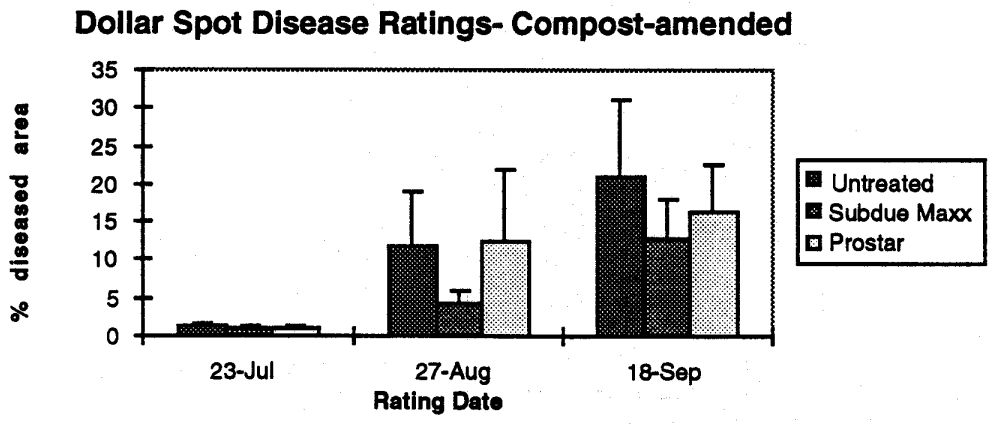
BIOLOG GN plates were used to assess functional diversity by means of metabolic profiles. General levels of microbial activity were determined by the rate of hydrolysis of fluorescein diacetate. However, the results of these procedures have not yet been evaluated.

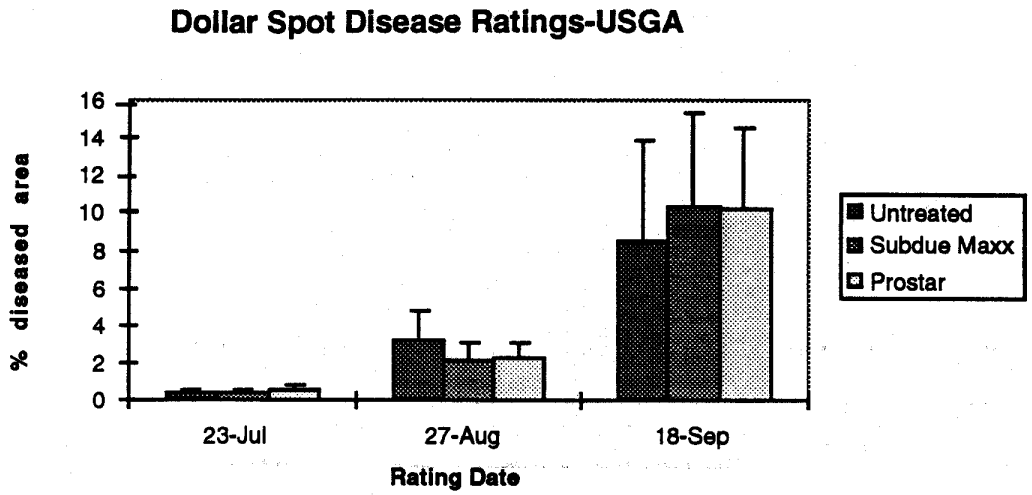
Phospholipid fatty acid profiles used to assess taxonomic diversity of microbial communities will be prepared this winter.

Results

Dollar Spot Disease Ratings

Dollar Spot is a common foliar disease of turfgrasses and is caused by *Sclerotinia homoeocarpa.* Ratings were taken three times over the course of the season: July 23, August 27, and September 18. On every plot, the number of lesions (incidence) and the diameter of each lesion (severity) were quantified. The percent diseased area was then calculated for each plot. The average and standard error for each treatment were calculated for both the augmented and the nonaugmented pools. Control of dollar spot was achieved with the expected fungicides, i.e., those that claim to control dollar spot. Presented below in Fig. 1 are the percent diseased areas for the untreated and those fungicides that do not control dollar spot for both augmented and nonagumented plots. In all cases, the fungicides registered and expected to control dollar spot gave near-perfect control. The two fungicides expected to be ineffective against dollar spot, Prostar and Subdue Maxx, had no consistent effect upon dollar spot disease ratings.





Microbial Enumeration by means of Dilution Plating

Microbial populations were enumerated on five different types of media. Numbers from sample sets T_{OT}, T1, and T2 are presented below for most of the microbes sampled. Sample Sets T3 and T4 are still being processed. *Bacillus* and total bacterial numbers also are still in process.

Samples were taken for T1 and T2 at different times for augmented and nonaugmented plots. Sampling had to be staggered to permit samples to be processed in a timely manner. For the nonaugmented plots, T1 was June 25, and these plots were treated with either two or three fungicide treatments, depending on whether the recommended interval between sprays was 14 or 21 days, and T2 was July 31. These plots received 6 fungicide applications for 14-day interval materials and 4 applications for 21 day materials. For augmented plots, T1 was July 17, and these received 3 or 2 fungicide applications for 14- and 21-day materials, respectively, and T2 was August 23 when 6 and 4 applications had been made.

Microbial populations of the various microbes assessed are presented in Figures 2-13, attached. These figures are labelled as to the microbe whose population was assessed and as to whether the data came from augmented or nonaugmented plots.

In most cases, fungicide applications had little effect upon microbial populations, even when the microbe being assessed was quite sensitive to the fungicide in question. For example, numbers of *Pythium* spp. were little affected in either augmented or nonaugmented plots by the applications of Subdue Maxx (treatment 4 in Figs. 2 and 3). Similarly, numbers of *T. harzianum* were little affected by application of Banner (treatment 5 in Fig. 6) even though this fungus is highly sensitive to this chemical.

Differences in microbial populations between augmented and nonaugmented sites were smaller than expected. The primary difference between the two was a large increase in the level of *T. harzianum*, but levels of most of the other microbes were similar. In fact, there appeared to be somewhat fewer Actinomycetes in the augmented than the nonaugmented plots at the second sampling time.

Anticipated Results

Over the next several months, we will continue to analyze the summer's data and we have several additional data types to collect. These additional experiments include (1) fatty acid analyses from the various soils. This information provides information on the presence of specific microbes, particularly bacteria, including fastidious organisms that are difficult to culture; (2) fluorescin diacetate hydrolysis in soil samples, which is a measure of total microbial activity. Other measures provide information on presence of microbes, but this test indicates their microbial activity, which is a very different factor. In addition, we have completed tests another measure of microbial species diversity based on the physiological profiles of the microbes present after different treatments, but this data is not yet analyzed.

We anticipate that it will take us into early 1997 for all data collection and analyses from the 1996 trials to be completed. It may well be that the conclusions when all data is available will be quite different from that presented below. We anticipate that the 1997 field trials will be similar to those done this year, but it may be useful to consider some modifications based on the final results from 1996. If so, we will be in contact with the USGA to discuss any modifications we may want to make in the experimental protocol.

In addition, we expect to assess the direct effects of the test fungicides on selected components of the turf microbial community; this will be initiated once we have a more complete understanding of which microbes are the most abundant in the communities we are examining. We also will do tests to determine the level of disease suppression of turf following various fungicide treatments in augmented and nonaugmented sites.

Preliminary Conclusions

Surprisingly, the various fungicides, even when multiple applications at their maximum legal rates were made, had little effect upon microbial communities. Numbers even of organisms known to be highly sensitive to the fungicides being applied were little affected by the treatments used in this experiment. These data suggest that these fungicides are not present at the fungitoxic concentrations below about 1 inch below the soil surface, since the samples were taken only about this depth into the turf soil profile. Several reasons for this lack of efficacy may be possible, including binding to soil particles and rapid microbial degradation.

This lack of efficacy suggests, first, that the fungicides tested may be less disruptive to normal soil microflora than originally expected. Second, the data suggest that the fungicides should largely be effective only on leaf diseases and have little effect upon subterranean fungal populations and root health.

Augmentation of plots with the compost + *T. harzianum* addition had two noticeable effects. First, levels of *T. harzianum* increased about 1000-fold with the addition of this biocontrol product and remained at a consistent level over the sampling times. Second, levels of actinomycetes were lower in augmented than in nonaugmented plots at the second sampling time.

It should be emphasized, however, that these data are preliminary and that substantial more data also will be collected and analyzed. In particular, these data indicate only levels of microbes and do not indicate microbial growth or activity. The results of the other tests being analyzed and conducted now will provide additional information.

Fig. 2. *Pythium*
Augmented

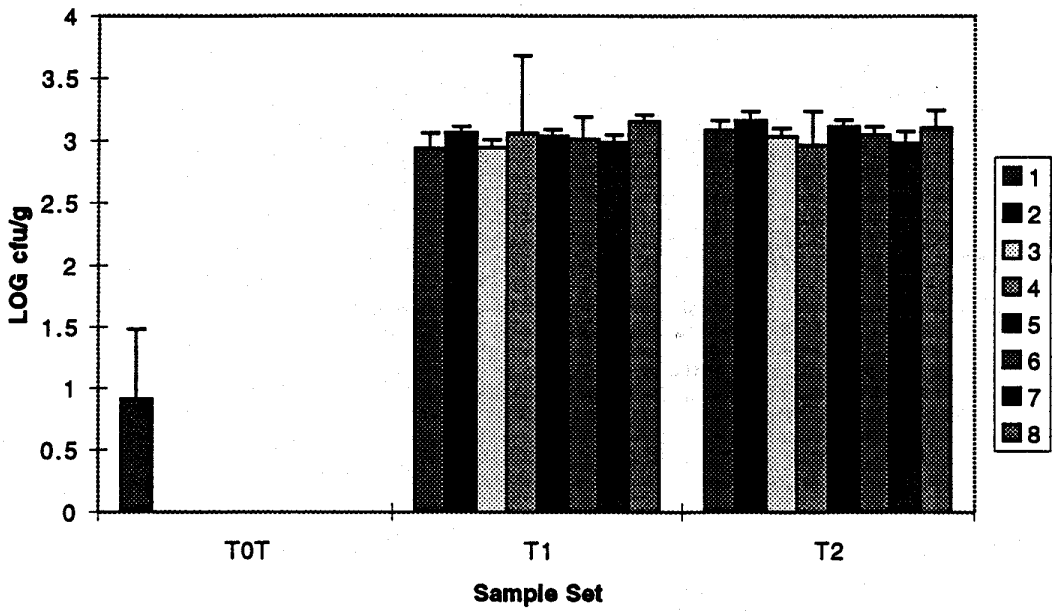
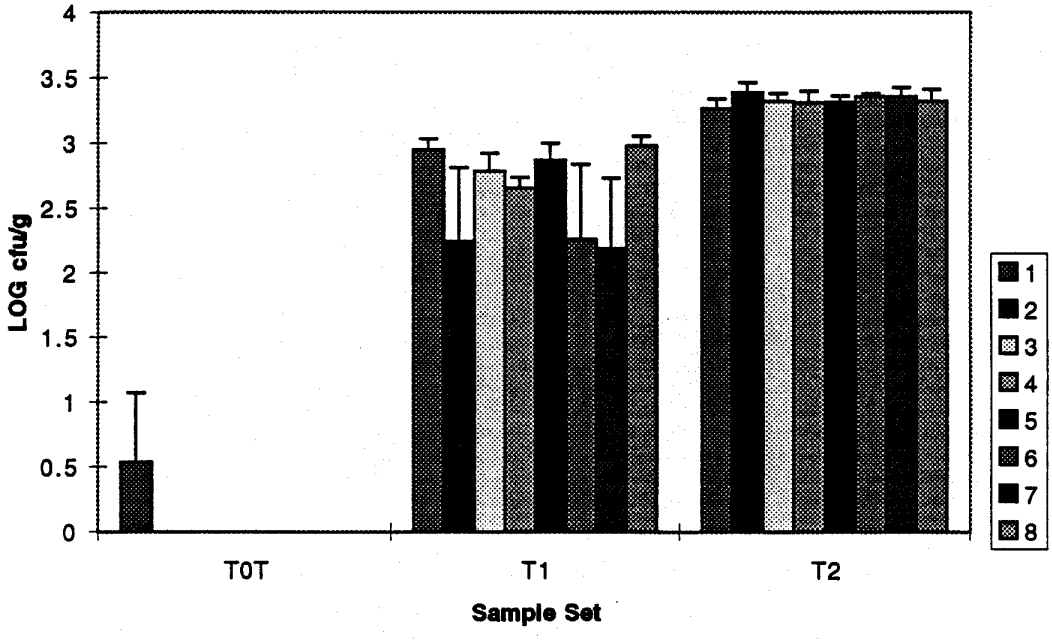


Fig. 3. *Pythium*
Nonaugmented



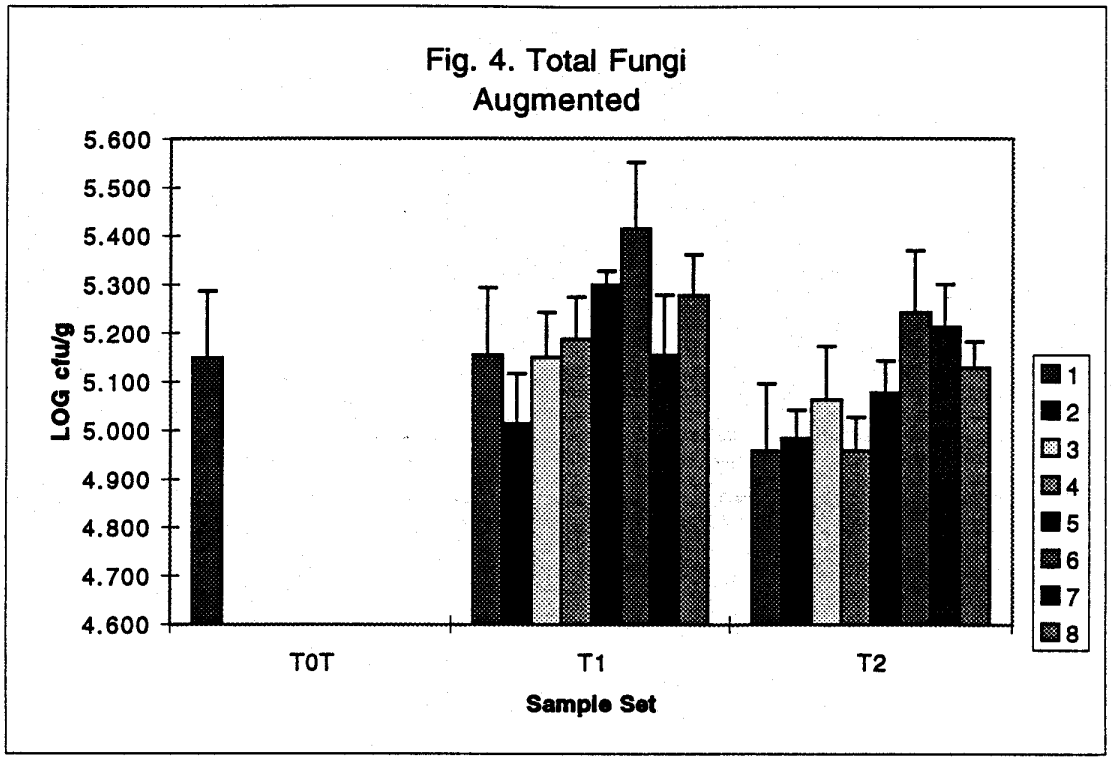


Fig. 4. Total Fungi
Augmented
5.600
5.500
5.400
5.300
5.200
5.100
5.000
4.900
4.800
4.700
4.600
LOG cfu/g
T0T
T1
T2
Sample Set
1
2
3
4
5
6
7
8

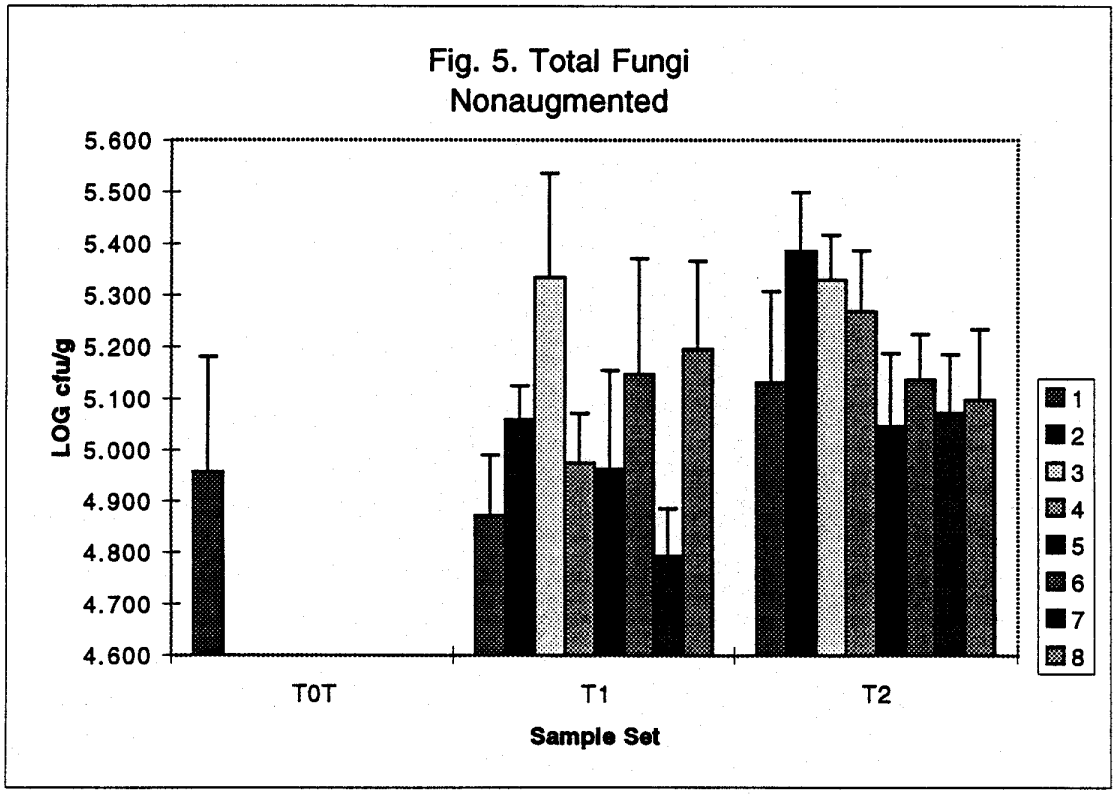


Fig. 5. Total Fungi
Nonaugmented
5.600
5.500
5.400
5.300
5.200
5.100
5.000
4.900
4.800
4.700
4.600
LOG cfu/g
T0T
T1
T2
Sample Set
1
2
3
4
5
6
7
8

Fig. 6. *T. harzianum*
Augmented

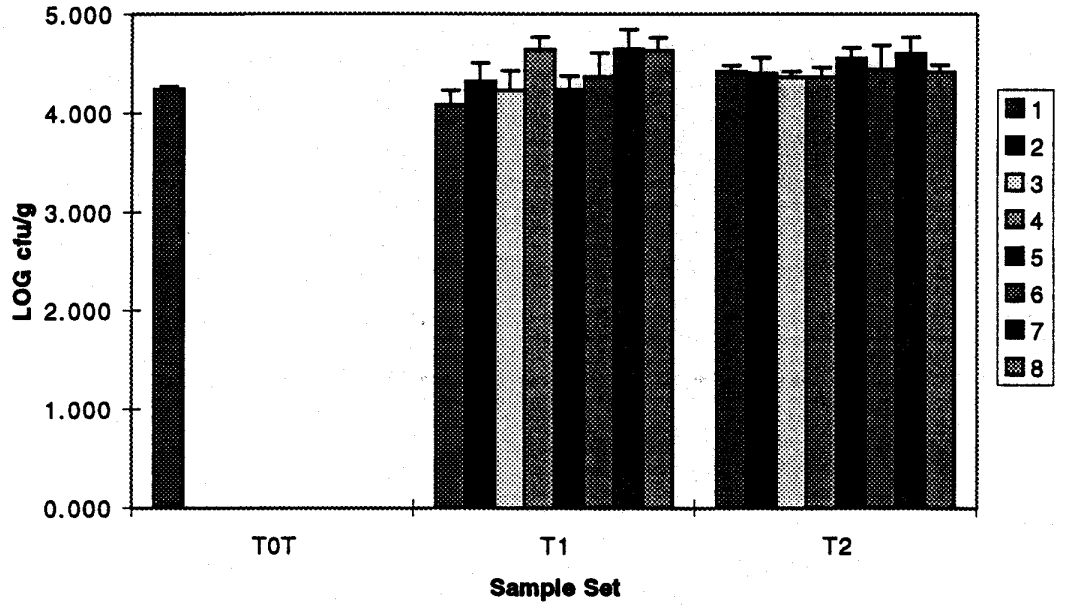


Fig. 7. *T. harzainum*
Nonaugmented

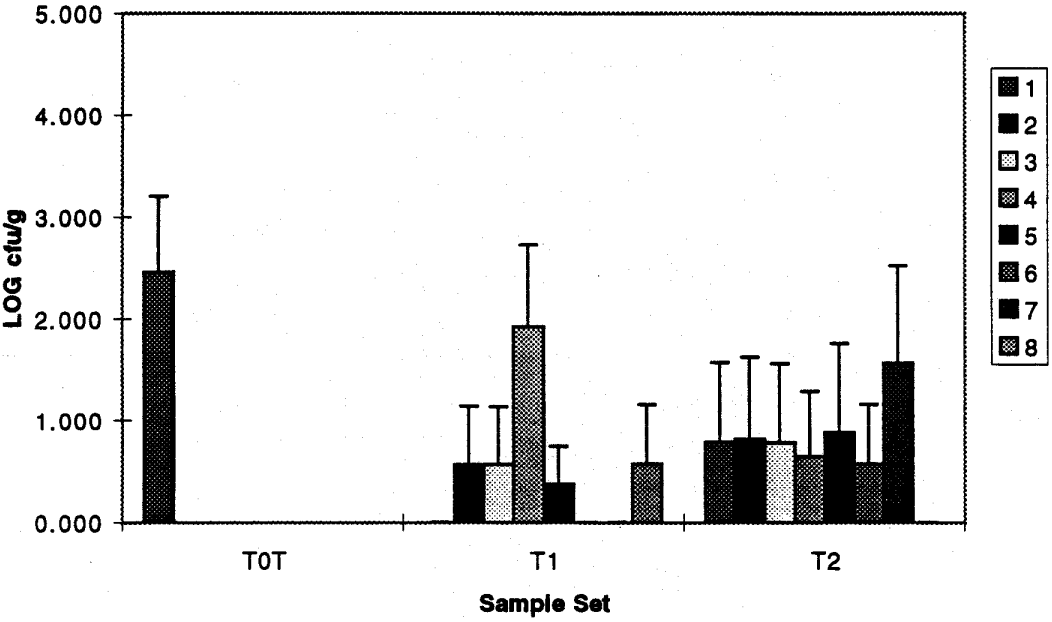


Fig. 8. *T. virens*
Augmented

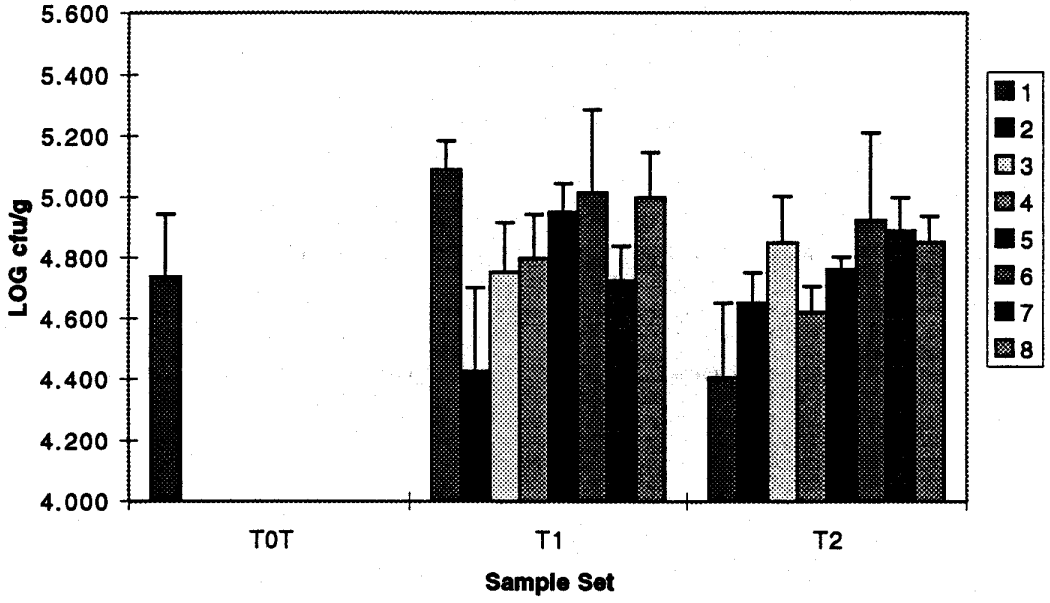


Fig. 9. *T. virens*
Nonaugmented

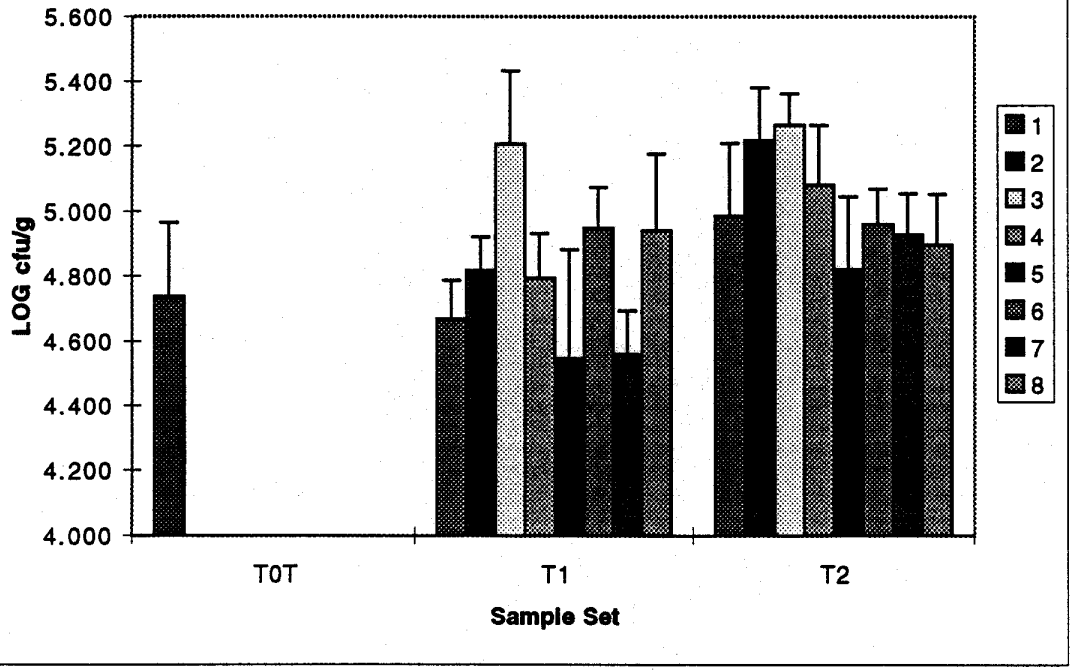


Fig. 10. Pseudomonads
Augmented

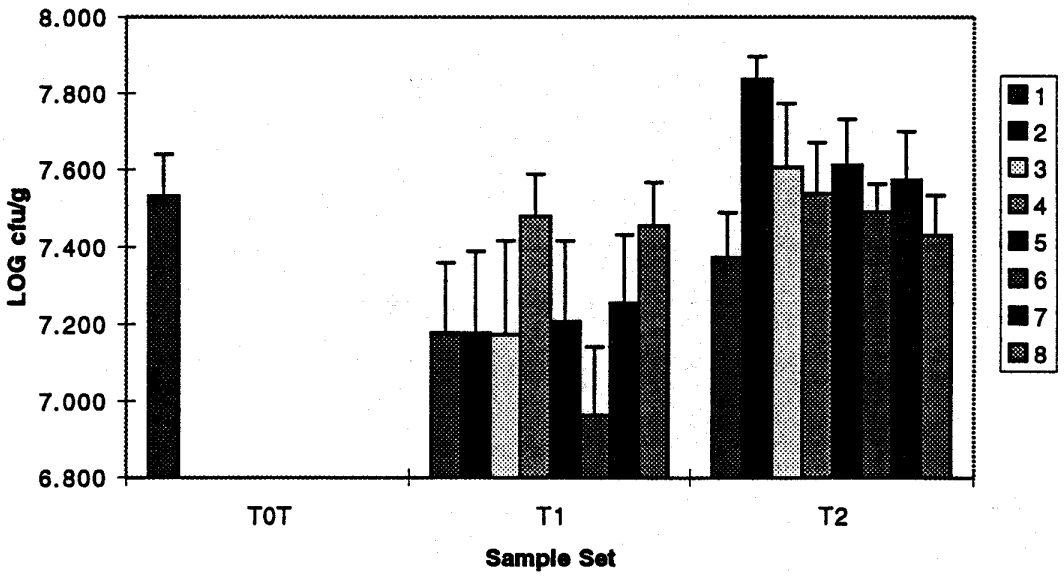


Fig. 11. Pseudomonads
Nonaugmented

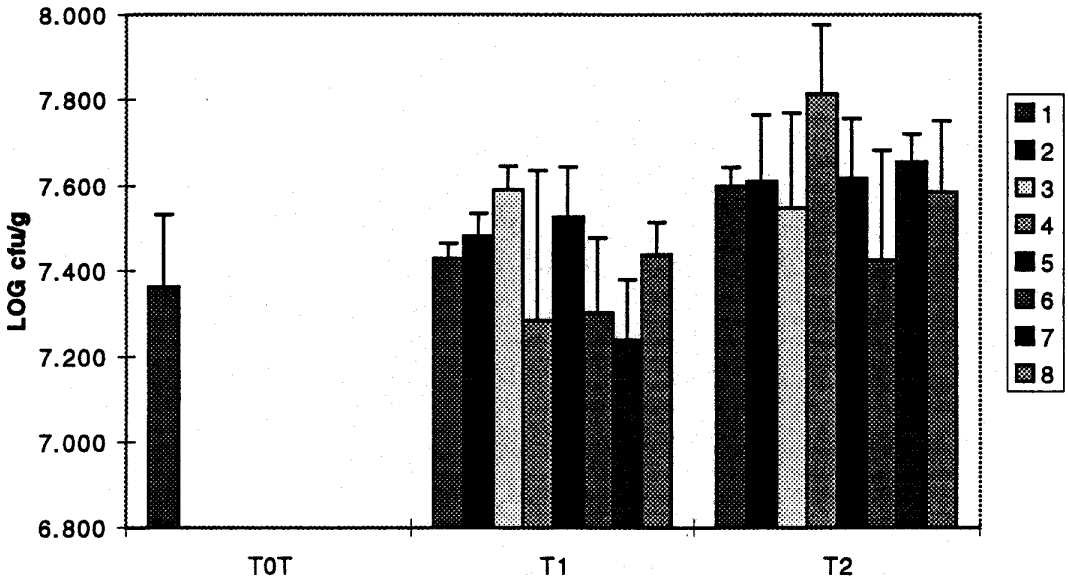


Fig. 12. Actinomycetes
Augmented

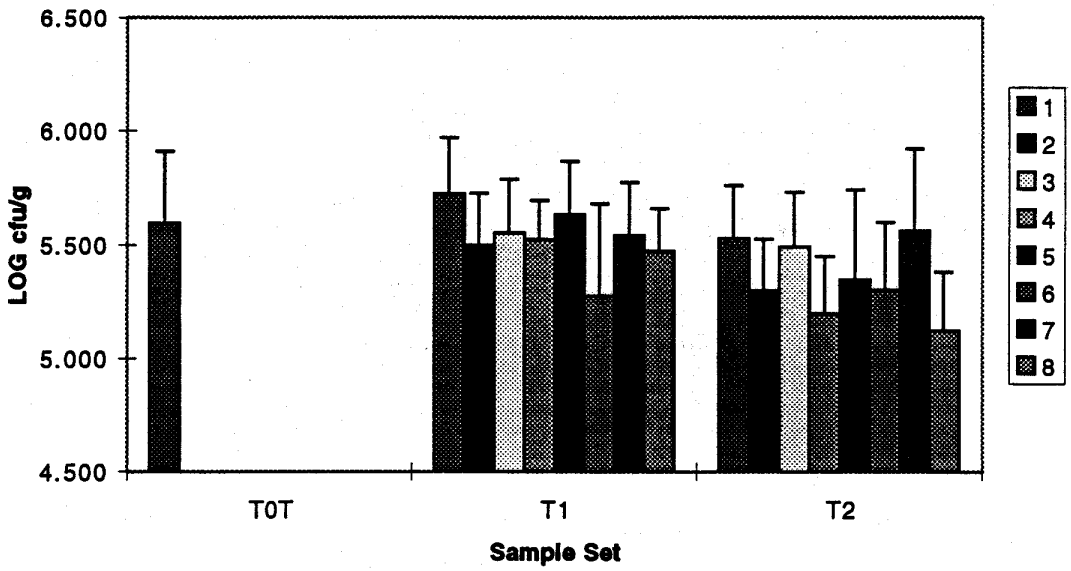


Fig. 13. Actinomycetes
Nonaugmented