USGA Annual Report, November 1998

Nontarget Effects of Turfgrass Fungicides on Microbial Communities in USGA Putting Greens

Gary E. Harman[1]
Eric B. Nelson[2]
Kristen L. Ondik[1]

[1]Department of Horticultural Sciences, Cornell University, Geneva, NY 14456
[2]Department of Plant Pathology, Cornell University, Ithaca, NY 14853

Executive Summary

This research has been designed primarily to focus on the following objective:

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

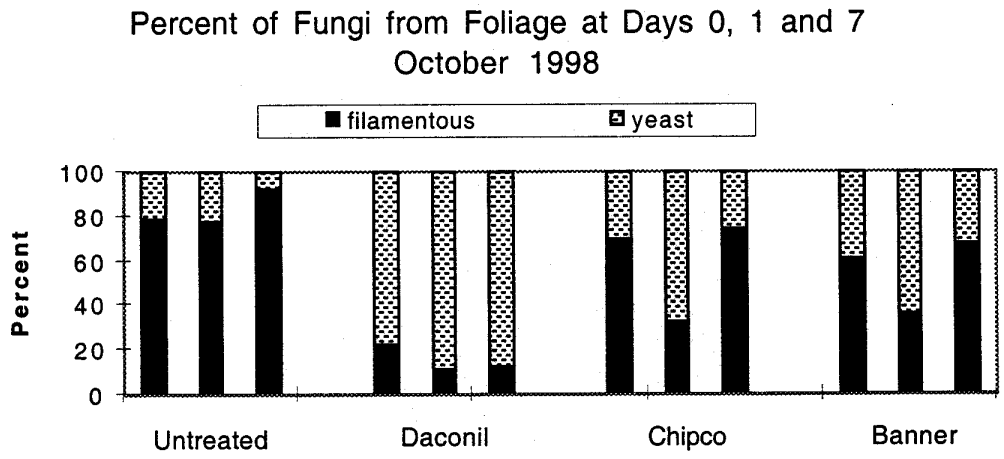
We originally expected the following:

- Substantial changes to the microflora would be found in the soil ecosystem.
- A strong correlation between both microbial composition and diversity to turf health and vigor would be evident.
- Dramatic shifts in microbial composition in soil and on foliage would also be shown.

Extensive research was conducted over the last three years to investigate the above objective and ideas. Dilution plating of different microbial groups, BIOLOG analysis, fluorescein diacetate (FDA) hydrolysis, and phosholipid analysis were among the tools utilized to derive the following conclusions:

- We found no effects whatsoever of even prolonged and extensive fungicide applications on nontarget soil microbes by any method that we employed. Our current thinking is that most fungicides do not penetrate soil in amounts sufficient to alter the established microbial communities. In plating experiments, the only effect we saw was a modest reduction in *Pythium* levels by use of Subdue, which is a target effect. We still have some measurements to complete, but effects on soil microflora are small, if present at all. This is in stark contrast with our expectations and widely held assumptions by most researchers.
- Similarly, we found no effects of repeated applications of diverse fungicides on total numbers of foliar microflora.
- We originally reported an apparent effect of fungicides applications on total microbial activity, but this year, based on more extensive experiments, suggests that there is either no difference in microbial activity or else that variation between measurements is so large that differences cannot be detected. Additional tests are underway.

- Given the surprisingly (to us at least) small results that we have discovered, this year we focused on short-term (just before, one day after, and seven days after) effects on foliage.
- Again, there was no detectable effect on total numbers of leaf microflora even short-term.
- There is, however, an effect of fungicides on composition of total fungi on leaf surfaces. In particular, filamentous fungi decrease and yeasts become much more numerous.
- This effect is dependent upon the type of fungicide used. Daconil caused a persistent increase in the numbers of yeasts relative to filamentous fungi, while application of Chipco or Banner gave rise to a transitory shift (see figure below).



Experimental Plan

1. Trials were established on a soil green at the Cornell University Turfgrass Research Facility. The experiment consisted of 8 ft^2 split-plots where one half received Bio-Trek 22G (a.i. *Trichoderma harzianum*) and the other half did not. The following fungicides were then applied at the maximum label concentration and minimum labeled time interval: chlorothalonil (Daconil Ultrex), iprodione (Chipco 26019 Flo), mefenoxam (Subdue Maxx), propiconazole (Banner Maxx), triadimefon (Bayleton), flusolazil (Prostar 50WP), and cyproconazole (Sentinel). Chlorothalonil and flusolazil were applied on 14-day schedules and the rest of the treatments were on 21-day schedules. Plots were in a randomized pattern with 5 replications.

2. Cores (1 X 5 cm) were taken in May before any fungicides were applied (T_0), in June after Bio-Trek 22 was applied to half of the plots (T_0T) and again in

October after all applications were complete. Applications began on June 17 and concluded on October 5.

3. Laboratory assays including FDA hydrolysis, BIOLOG, dilution plating and phospholipid extraction and esterification of fatty acids have been done or will be done this winter.

Changes in Year Three

1. We concentrated on the foliar microbial populations this year because of the absence of effects seen in the soil. We also deemed microbial respiration (fluorescein diacetate hydrolysis) as our most effective way to assess total microbial activity.

2. The most significant change in 1998 was the focus on the timing of sampling after application. We hypothesized that within 24 hours after application the microorganisms on the foliage should be most affected by the fungicide application.

3. We also performed a mini-experiment in September and again in October where we focused on the timing of sampling after application of fungicides. We sampled the plots before we made the scheduled application (day 0), one day after the application (day 1) and again seven days after the application (day 7). FDA hydrolysis analyses and fungal enumerations were performed at each sample time (i.e., days 0, 1 and 7) for four different treatments: untreated, Daconil Ultrex, Chipco 26019 Flo and Banner Maxx. Three repetitions of each treatment were sampled. For the final sample set, all treatments were sampled one day after the final fungicide application.

Results

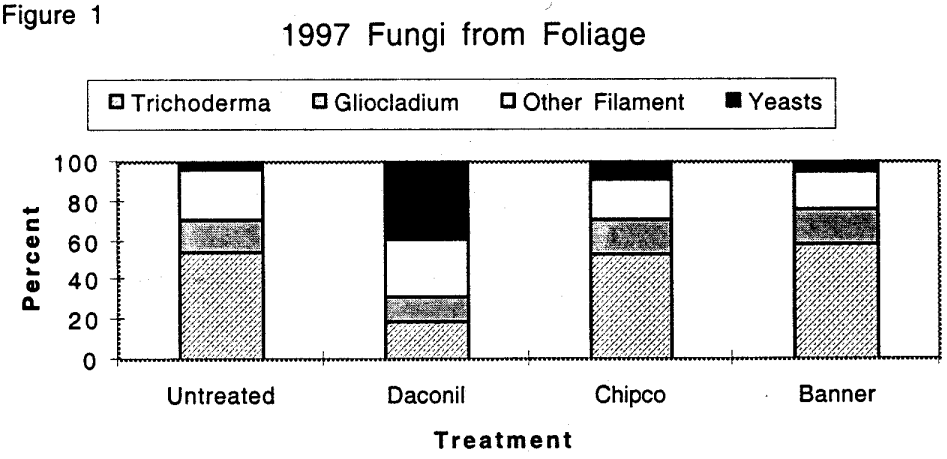
1. Addition of inundative applications of fungicides produced more subtle effects on microbial populations than what we originally expected. We originally expected the following:

- Substantial changes in the microflora in the soil ecosystem
- Strong correlation between both microbial composition and diversity to turf health and vigor.
- Dramatic shifts in microbial composition in soil and on foliage

Instead, we have not seen any noticeable change in the soil ecosystem. We are just now starting to see if any correlations exist between microflora and turf quality (see #6 below). We did not see dramatic shifts in microflora on foliage (see below).

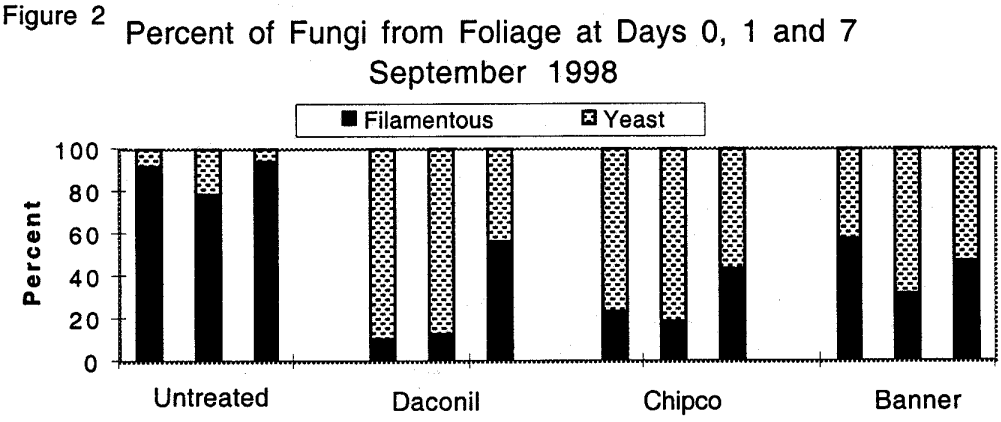
2. We saw subtle changes in some foliar microbial populations in 1997 and 1998. We stated in 1997 that we saw an increase in yeast populations and a decrease in filamentous fungal populations following application of Daconil Ultrex compared with the untreated (Fig. 1). This was again shown in our 1998 data (Figs. 2 and 3).

Figure 1



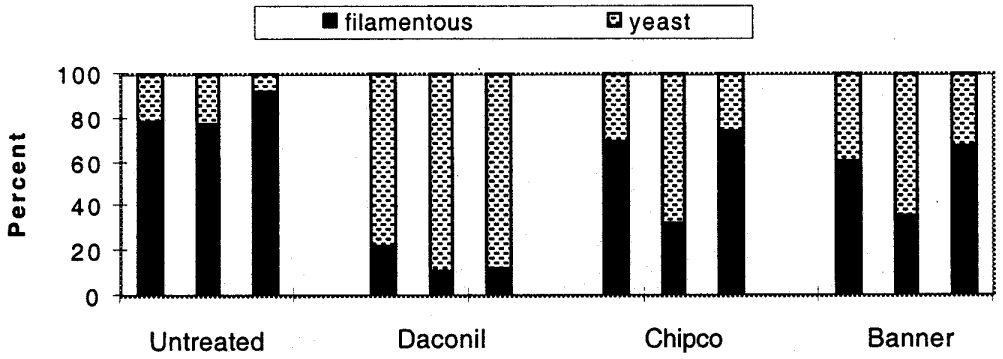
3. In the September experiment, we saw large shifts in the dominant population with respect to the untreated. Greater numbers of yeasts were present at all three sampling times compared to the untreated (Fig. 2).

Figure 2



4. The October experiment again showed qualitatively similar population shifts. For Chipco 26019 Flo and Banner Maxx, it is interesting to note that the numbers of yeasts as a percentage of the total increase at day 1 but then rebound to the original (day 0) status on day 7 (Fig. 3).

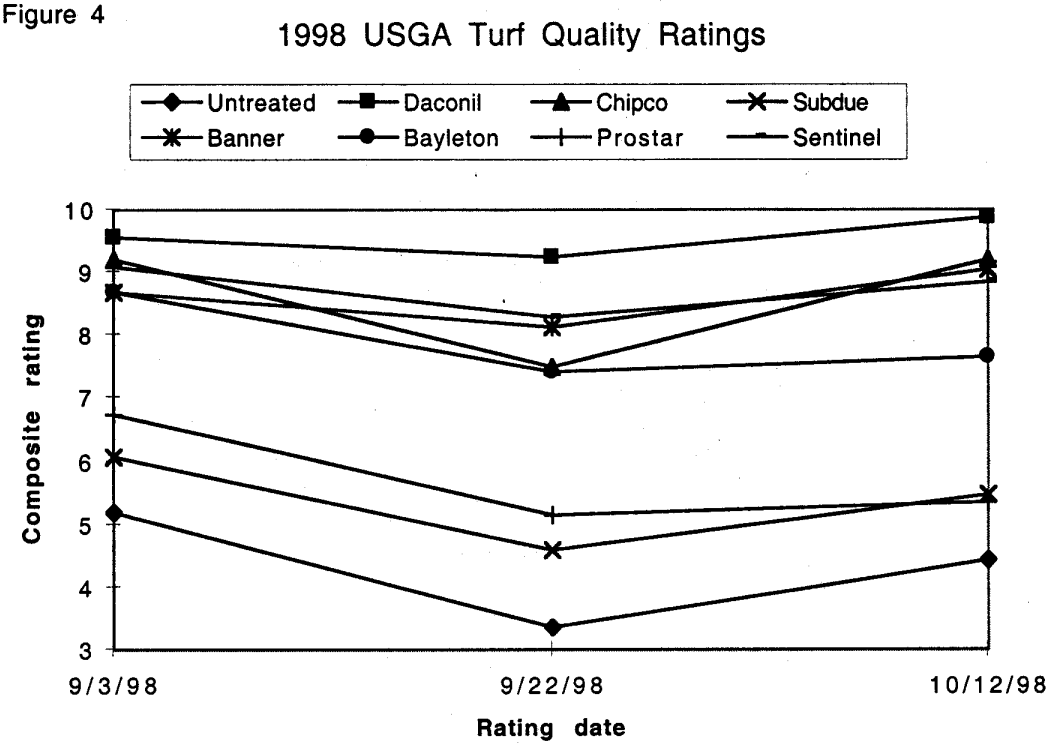
Figure 3 Percent of Fungi from Foliage at Days 0, 1 and 7
October 1998



5. For the time being, we may see only short-term effects on microbial communities, but long-term effects should still be considered. We have shown in our mini-experiments in September and October that application of certain fungicides can alter the microbial composition on the foliage (see Figs. 1-3). For Chipco 26019 Flo and Banner Maxx, we saw compositions of microflora similar to the untreated at days 0 and 7. At day 1, however, we saw moderate increases in yeast and decreases in filamentous fungi compared to the untreated. Interestingly, with Daconil Ultrex, the same population percentages are evident at day 0, day 1 and day 7 late in the season after repeated applications. It seems that the population shift is persistent with Daconil but not with Chipco or Banner. It would be very useful to examine the microbial composition of the Daconil-treated plots in the spring before the fungicide applications begin. We could then determine if the shift is due to repeated applications of Daconil Ultrex.

6. Turf quality ratings were taken this year once disease appeared. These ratings are an average of four different parameters: greenness, texture, density and disease. The higher the rating (out of 10) the better the quality of turf. We reported above that we saw shifts in fungal populations especially with Daconil Ultrex.

Figure 4



7. Fluorescein diacetate (FDA) hydrolyses were performed at days 0, 1 and 7 on the samples from the mini-experiments in September and October. We found no clear patterns or differences between treatments (Figs. 5 and 6).

Figure 5

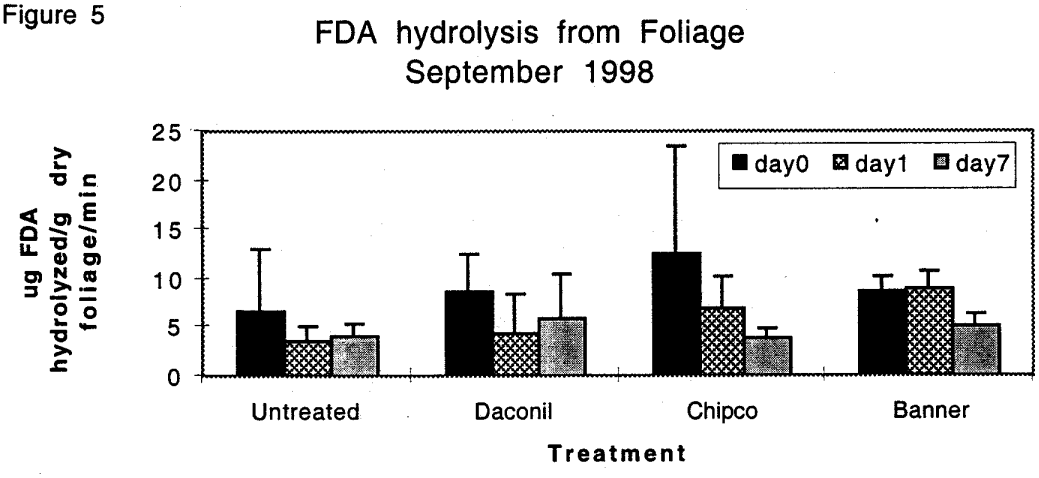
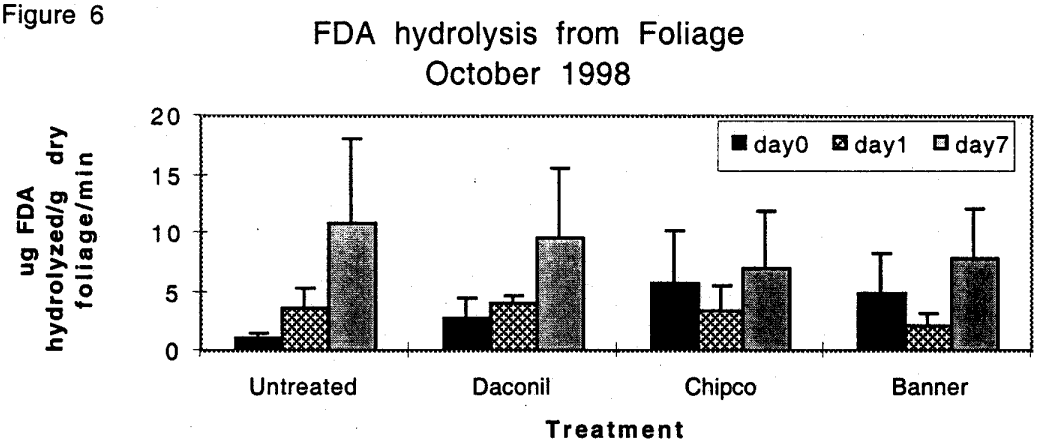


Figure 6



8. We could detect NO effect of repeated fungicide applications on gross numbers of microorganisms from subterranean microbial communities. This has been shown in previous years and we are still analyzing the data for 1998.

Upcoming Work

This fall and winter we will do the following:

1. Complete the lab assays on the turf samples this fall.

2. Isolate yeasts to see if they are resistant to certain fungicides. We will determine if they possess any biocontrol properties and then attempt to correlate their presence with overall plant health, if applicable.

3. Isolate filamentous fungi including but not limited to *Trichoderma*, *Gliocladium* and *Penicillium* spp. to see if they are NOT resistant to certain fungicides, especially Daconil Ultrex, which could explain their decrease in gross numbers from dilution plating.