Research *You Can Use*

Non-Target Effects of Fungicide Applications on Microbial Populations of Putting Greens

Surprisingly, research at Cornell University demonstrates that fungicides have little effect on long-term populations of putting green microbes.

BY G. E. HARMAN, E. B. NELSON, AND K. L. ONDIK

Currently there are between 20 and 30 million acres of turfgrass in the United States, consisting of lawns, parks, golf courses, athletic fields, sod farms, industrial and institutional grounds, rights-of-way, and other recreation areas. The turfgrass industry continues to grow rapidly.

The management of turfgrasses, especially on golf course greens, represents perhaps the highest level of plant management practiced on any agricultural or horticultural commodity known today. Proper turfgrass management involves a number of rather complicated mechanical, physical, chemical, and biological manipulations that result in the desired product of a blemish-free carpet of green grass.

Highly maintained turfgrass sites characteristically use high inputs in the form of fuel, fertilizers, pesticides, and water for irrigation. Pesticide use, in particular, can be substantial. The use of fungicides is a major tactic for controlling diseases on high-quality turfgrasses. This is particularly true on putting greens. Short cutting heights, the ever-increasing amount of traffic on putting greens, and low nutrient inputs have placed unprecedented stresses on turfgrass plants, making them highly susceptible to damage from many different diseases, some of which were previously considered relatively unimportant.

The majority of fungicide applications are made to putting greens and tees, making the amount of fungicide applied per unit area quite high. Since many high-maintenance turfgrass sites are found in close proximity to surface waters and within groundwater recharge areas, and primarily in and around urban areas, questions have been raised as to the impact of such a land use on water quality, wildlife, and human health, particularly as it relates to pesticide exposure.

Further, there have been a number of non-target effects of fungicides in turfgrass management systems. These have included selection of fungicide-resistant biotypes of pathogens, promotion of non-target diseases, enhanced thatch buildup, decreased root or stem biomass, and rapid disease resurgence following fungicide applications.[5]

Given the high levels of fungicides applied to turfgrass, we considered it likely that high levels of applications of frequently applied fungicides would alter soil and foliar microbial communities. This perturbation would be expected to have significant consequences, including the promotion of non-target diseases and rapid disease resurgence, because of the destruction of natural antagonists of turf pathogens. This article summarizes three years of extensive sampling of turf microbial communities in the presence and absence of fungicide applications.

HOW THE RESEARCH WAS CONDUCTED

In 1996, five eight-foot diameter "swimming pool" greens constructed in 1995 at the Cornell University Turf Research Farm in Ithaca, N.Y., were used as the experimental microplots. The pools contained the standard USGA sand/peat rootzone profile.

Subplots consisted of an untreated plot and the seven fungicide treatments. Each subplot was three square feet and each treatment was represented on each pool. The fungicides selected represent different classes with different modes of action. For example, Daconil Ultrex (chlorothalonil) is a contact fungicide with a relatively non-specific mode of action against most classes of fungi.

Table 1

Cornell University scientists tested various turfgrass fungicides shown below to see whether their repeated use would have significant effects on either foliar or soil-borne microbial populations of putting greens.

Treatment	Active Ingredient	Rate	Application Interval
Untreated	— —	— —	— —
Daconil Ultrex	chlorothalonil	3.6 oz./1,000 sq. ft.	14 days
Chipco 26019 Flo	iprodione	8 oz./1,000 sq. ft.	21 days
Subdue Maxx	mefenoxam	1 oz./1,000 sq. ft.	21 days
Banner Maxx	propiconazole	4 oz./1,000 sq. ft.	21 days
Bayleton 25W	triadimefon	4 oz./1,000 sq. ft.	21 days
Prostar 50WP	flutolanil	3 oz./1,000 sq. ft.	14 days
Sentinel	cyproconazole	0.167 oz./1,000 sq. ft.	21 days

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.[3] 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 shown in Table 1.

Two hundred milliliters of the appropriate rate was applied to each plot using a hydraulic CO_2 sprayer. Samples were taken from the plots starting in May, before any fungicide application, and monthly thereafter through September. Nine to 12 1.0cm-diameter cores were taken from each subplot at a depth of 3cm and transported to the laboratory for microbial assays.

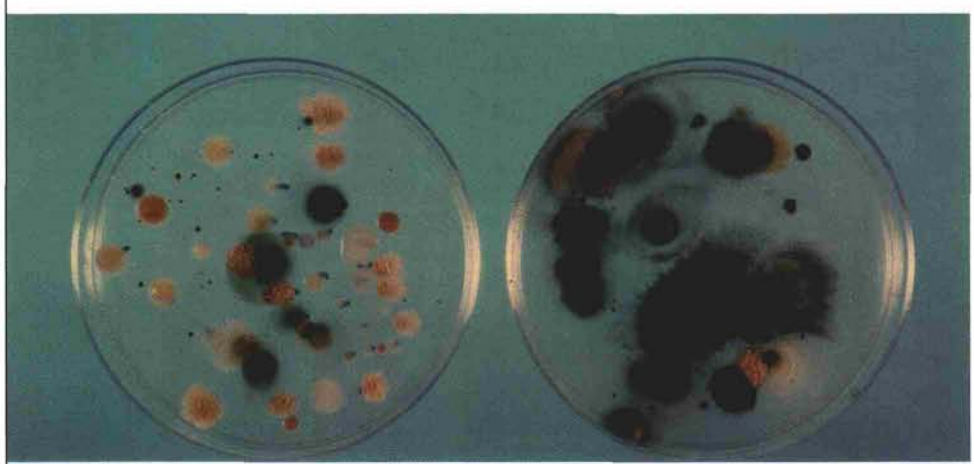
Microbial plate counts were determined by performing a serial dilution in phosphate-buffered saline (PBS) and plating appropriate dilutions on solid media. Acidified potato dextrose agar plus a microbial colony restrictor[4] was used to enumerate total culturable fungi. This medium eliminates growth of bacteria and permits characterization of colonies based on colony morphology. Some of the most common fungi encountered on this medium were *Trichoderma* and *Penicillium spp.* and yeasts. These fungi are very common in soil and on roots and usually have either few effects on plant growth or else have beneficial ones, including biocontrol abilities. Total culturable bacterial population numbers were estimated by plating on tryptic soy agar (10% strength). This is a differential medium favored by bacteria, and fungi grow poorly on it.

We also examined specific microbial groups. For Actinomycetes, which are filamentous bacteria, we used 0.02% tripticase soy agar plus the antibiotic polymixin B sulfate. This nutrient-poor medium is favorable for Actinobacterias, with minimal growth of fungi or other bacteria. *Pseudomonas spp.*, which are common plant-associated bacteria and frequently have biocontrol ability, were enumerated on a selective medium that we have used earlier.[4] Finally, we enumerated Oomycetes in the genus *Pythium* using a *Pythium*-selective medium.[4] These organisms may be plant pathogens or biocontrol organisms, depending on the particular species and strain that may be present.

In addition, 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. Finally, phospholipid fatty acid profiles were used to assess taxonomic diversity of microbial communities.

WHAT WE FOUND

In 1996, we sampled roots from the plots every month and evaluated changes in the microbial profiles using the various media. We detected no significant differences and the results were similar to those in 1997, so we will present only the 1997 data. Similarly, we found no significant differences in BIOLOG microbial metabolic profiling, based on principal component analyses.



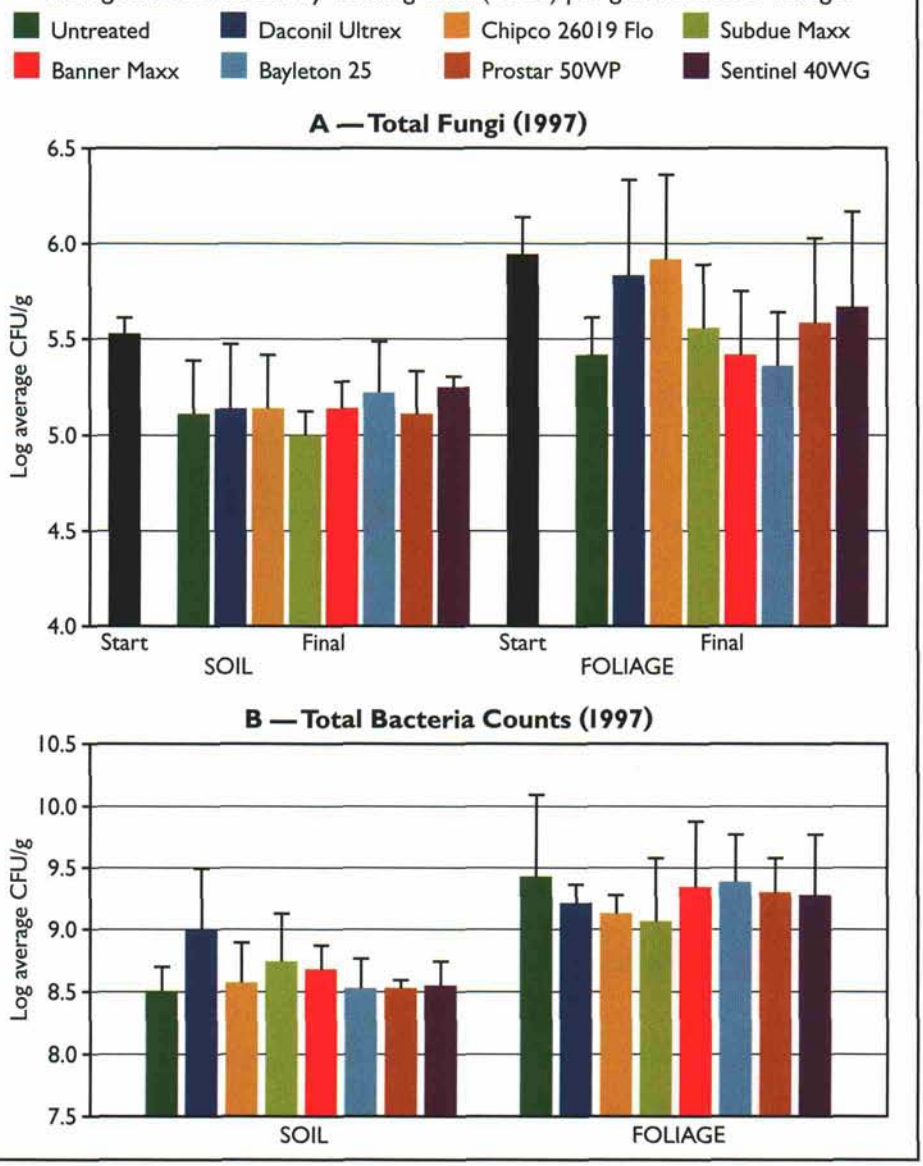
DACONIL UNTREATED

USGA 1998

These dilution plates represent the appearance of cultures from untreated plants and Daconil Ultrex-treated leaves. The dark colonies are filamentous fungi and the white to tan mucoid cultures are yeasts. Fungicides have been found to have little effect on long-term populations of putting green microbes.

Figure 1

Enumeration of total fungi (A) at the start of the 1997 season (May) and after a full season of fungicide applications (September). Total bacteria (B) are represented only at the end of the season. Populations are represented by the log of the average number of colony-forming units (CFUs) per gram of soil or foliage.



We also found no differences in general microbial activity or following phospholipid activity tests.

In 1997, we sampled both roots and leaves. The total number of fungal propagules detected was greater in soil at the start of the season than later, but there were no significant effects even after the season-long application of fungicides, regardless of the fungicide applied (Figure 1). On leaves, there were no significant effects of fungicide applications on total numbers of fungi, regardless of time or fungicide application. Most of the fungi detected were in the genus *Trichoderma*. We were able to distinguish between species similar to *T. virens* and those similar to *T. harzianum*, since the latter has a tan pigmentation on the reverse side of the acidified potato dextrose agar plates while those of *T. virens* are white.

There was no significant effect of time or treatment on either *Trichoderma spp.* in soil, but on foliage, there were initially higher levels of *T. harzianum* at the start of the season. By the end of the season, there were no differences between the two, and fungicide applications made no difference. Likewise, the fungicide applications had no effect on total numbers of *Pythium spp.*, total bacterial, Pseudomonad or Actinobacteria numbers.

In contrast, nearly all of the fungi on leaves were similar to *T. harzianum*, but by the end of the season, other fungi had largely displaced *T. harzianum*, and were predominately yeasts, *Penicillia*, and others. This was particularly true with plants that had been treated with Daconil Ultrex. On plants treated with Bayleton 25, *T. harzianum* remained the predominant culturable fungus (Figure 2).

In 1998, we performed a mini-experiment on a soil green at the Cornell University Turf Research Farm. In September and again in October, 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 sampling 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.

The relative numbers of filamentous fungi versus yeasts changed substantially on turf leaves as evidenced by both the numbers and plate appearances (Figure 3). However, there was no significant difference in total microbial metabolic activity among fungicide treatments as measured with the FDA test. Most of the fungi isolated from leaves of untreated plants were filamentous fungi, while after the season-long application of Daconil, most of the fungi isolated were yeasts. With Chipco or Banner, the change in populations of filamentous fungi versus yeasts was more transitory, dropping immediately after application and then increasing within a week.

Figure 2

Changes in leaf fungal compositions over time (start = May 1997) and at the end of the season as affected by different fungicide applications.

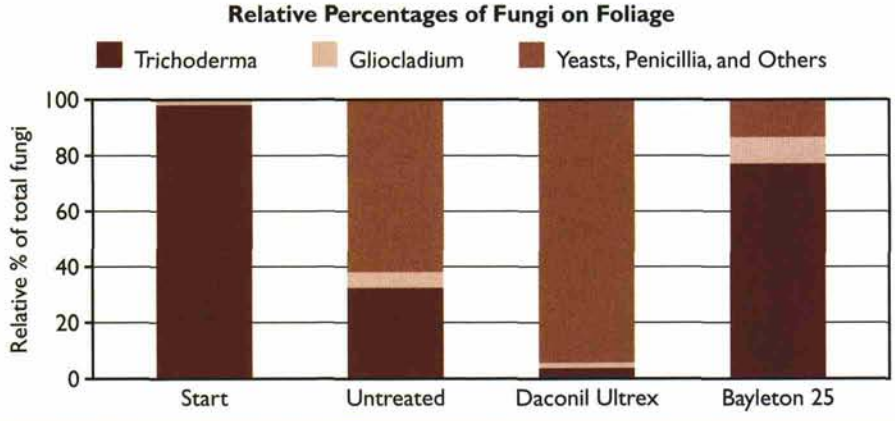
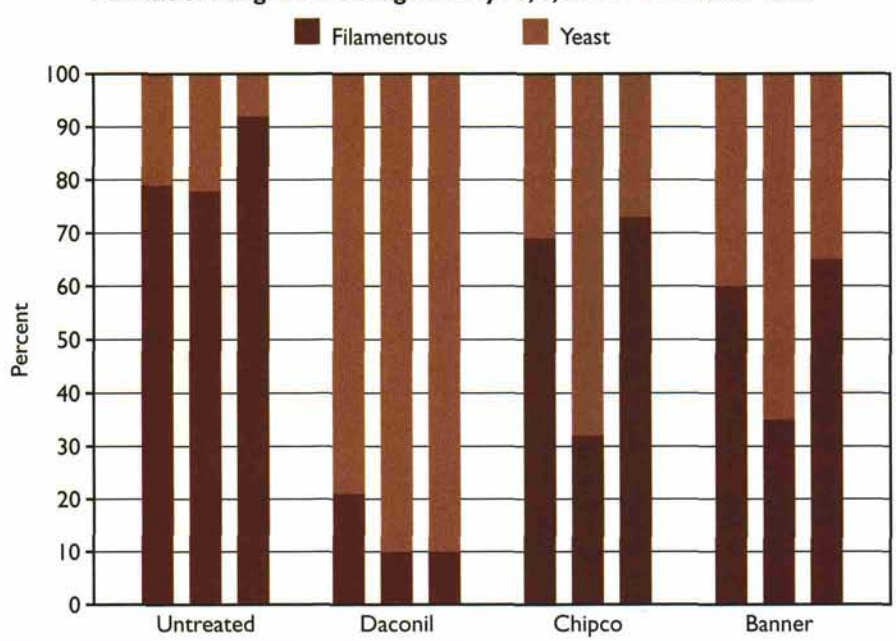


Figure 3

Changes in populations of filamentous fungi versus yeasts on turf foliage just before and shortly after fungicide applications as expressed by percentages of filamentous fungi or yeasts isolated from turf foliage.



ORIGINAL HYPOTHESIS WAS WRONG

Our hypothesis at the start of the work was that repeated applications of fungicides would dramatically change the microbial composition around roots and on leaf blades. This clearly was not the case with any of the fungicides tested. On roots, we could see no changes whatsoever with plating tests, BIOLOG tests for metabolic profiles, fatty acid microbial profiles, or tests for total microbial metabolic activity. Thus, while different results might be obtained with other assays, such as ribosomal DNA assays, it does not appear that repeated applications of fungicides have major impacts on soil microbial communities.

This may be because (a) the fungicides are mostly water insoluble and therefore do not penetrate deeply into the soil, or (b) the soil microbial community is highly competitive and resilient and able to rebound very quickly after fungicidal applications. The fact that *Trichoderma spp.* are so prevalent in the fungal community may also be significant since many members of this genus are highly resistant to a variety of fungicides[2] and their populations could be selectively favored over the years that greens are established.

We were particularly surprised at the leaf plating data, which at first glance gave little indication of change based on numbers counted on the various media. However, it now is clear that while total numbers of fungi on leaf blades do not change, the application of fungicides changes the composition in favor of yeasts relative to filamentous fungi. This effect may be transitory, as in the case of Chipco, or longer lasting, as was the case with Daconil. The fungal community on leaf blades appears highly dynamic and changing in response to fungicide applications. It is important to note that the natural dollar-spot epiphytotic that occurred each year was controlled by fungicides as expected.

LITERATURE CITED

1. Anonymous. 2005. Fungicides hit growth spurt in turf and ornamental markets. *Grounds Maintenance* 40(8):6.

2. Harman, G. E., C. R. Howell, A. Viterbo, I. Chet, and M. Lorito. 2004. *Trichoderma* species — opportunistic, avirulent plant symbionts. *Nature Rev. Microbiol.* 2:43-56.

3. Köller, W. 1992. Target sites of fungicide action. CRC Press, Boca Raton, Fla.

4. Norton, J. M., and G. E. Harman. 1985. Responses of soil microbes to volatile exudates from germinating pea seeds. *Can. J. Bot.* 65:1040-1045.

5. Smiley, R. W., P. H. Dernoeden, and B. B. Clarke. 1992. Compendium of turfgrass diseases. 2nd edition. APS Press, St. Paul, Minn.

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