USGA funded this research.

Effects of fungicide application on nontarget microbial populations of putting greens

Repeated fungicide applications did not have the anticipated effect on microbial populations in greens.

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A number of nontarget effects of fungicides have been found in turfgrass management systems. These include fungicide-resistant biotypes of pathogens, promotion of nontarget diseases, enhanced thatch buildup, decreased root or stem biomass and rapid disease resurgence following fungicide applications (4). Given the high levels of fungicides applied to turfgrass, we considered it likely that frequently used fungicides would alter or disturb soil and foliar microbial communities and thereby destroy the natural enemies of turf pathogens. This in turn could promote nontarget diseases and rapid disease resurgence. This paper summarizes three years of extensive sampling of turf microbial communities in the presence and absence of fungicide applications.

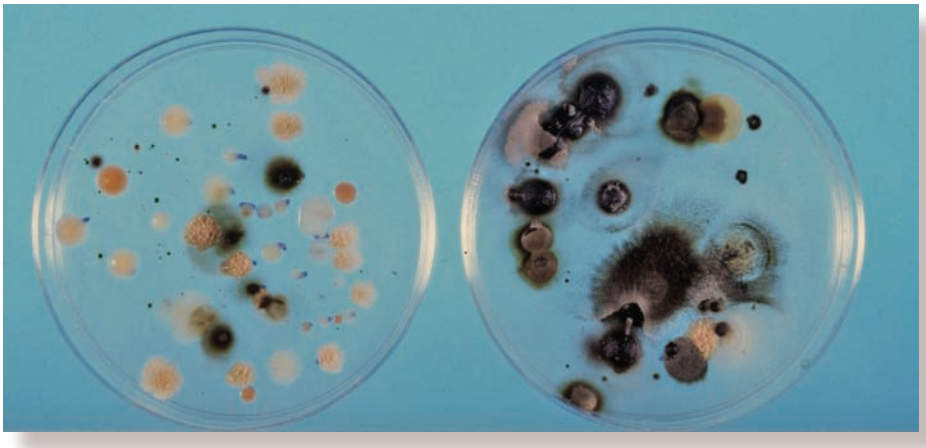

Photo by Joe Ogrodnick

Figure 1. Cultures from dilution plates from leaves treated with Daconil Ultrex (left) and from untreated plants (right). The dark colonies are filamentous fungi, and the white-to-tan mucoid cultures are yeasts.

Materials and methods

In 1996, five 8-foot (2.4-meter) diameter "swimming pool" greens constructed in 1995 at the Cornell University Turf Research Farm in Ithaca, N.Y., were used as experimental plots. The greens contained the standard USGA sand-peat root-zone profile.

Subplots were an untreated plot and seven fungicide treatments. Each subplot was 3 square feet (0.28 square meter), and each treatment was represented on each green.

The fungicides selected represent different classes with different modes of action. For example, Daconil Ultrex (chlorothalonil) is a contact fungicide with a relatively nonspecific mode of action against most classes of fungi (Figure 1). 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 (2). In all cases, if alternative rates were registered, we always used the maximum legal rate of the fungicide. The treatments, active ingredients, rates and application schedules are shown in Table 1.

Each subplot received 6.76 fluid ounces (200 milliliters) of fungicide at the appropriate rate applied with a hydraulic CO_2 sprayer. From each subplot, sample cores, 1 centimeter in diameter and 3 centimeters in depth, were

taken monthly from May (before any fungicide applications were made) through September. Nine to 12 samples were taken from each subplot over the five-month period.

Microbial assays of the samples were made in the lab. We estimated total culturable populations of fungi. Some of the most common fungi encountered were *Trichoderma* and *Pencillium* species and yeasts. These fungi are very common in soil and on roots and usually have few effects on plant growth. They also may be beneficial, acting as biological control agents against harmful organisms.

We estimated total culturable bacterial population numbers in addition to specific bacterial groups. *Pseudomonas* species, which are common plant-associated bacteria and which frequently act as biological controls, were counted (3). We also counted Oomycetes in the genus *Pythium* using a *Pythium*-selective medium (3). These organisms may be plant pathogens or biological control organisms, depending on the particular species and strain that may be present.

Results

1997 results

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.

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 2). 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 classify *Trichoderma* colonies based on their morphological similarities to two species, *T. virens* and *T. harzianum*. There was no significant effect of time or of treatment on either *Trichoderma* species in soil; but on foliage, there were higher levels of *T. harzianum* at the start of the season. By the end of the season, there were no differences between the two species, and fungicide applications made no difference. Likewise, the fungicide applications had no effect on total numbers of *Pythium*

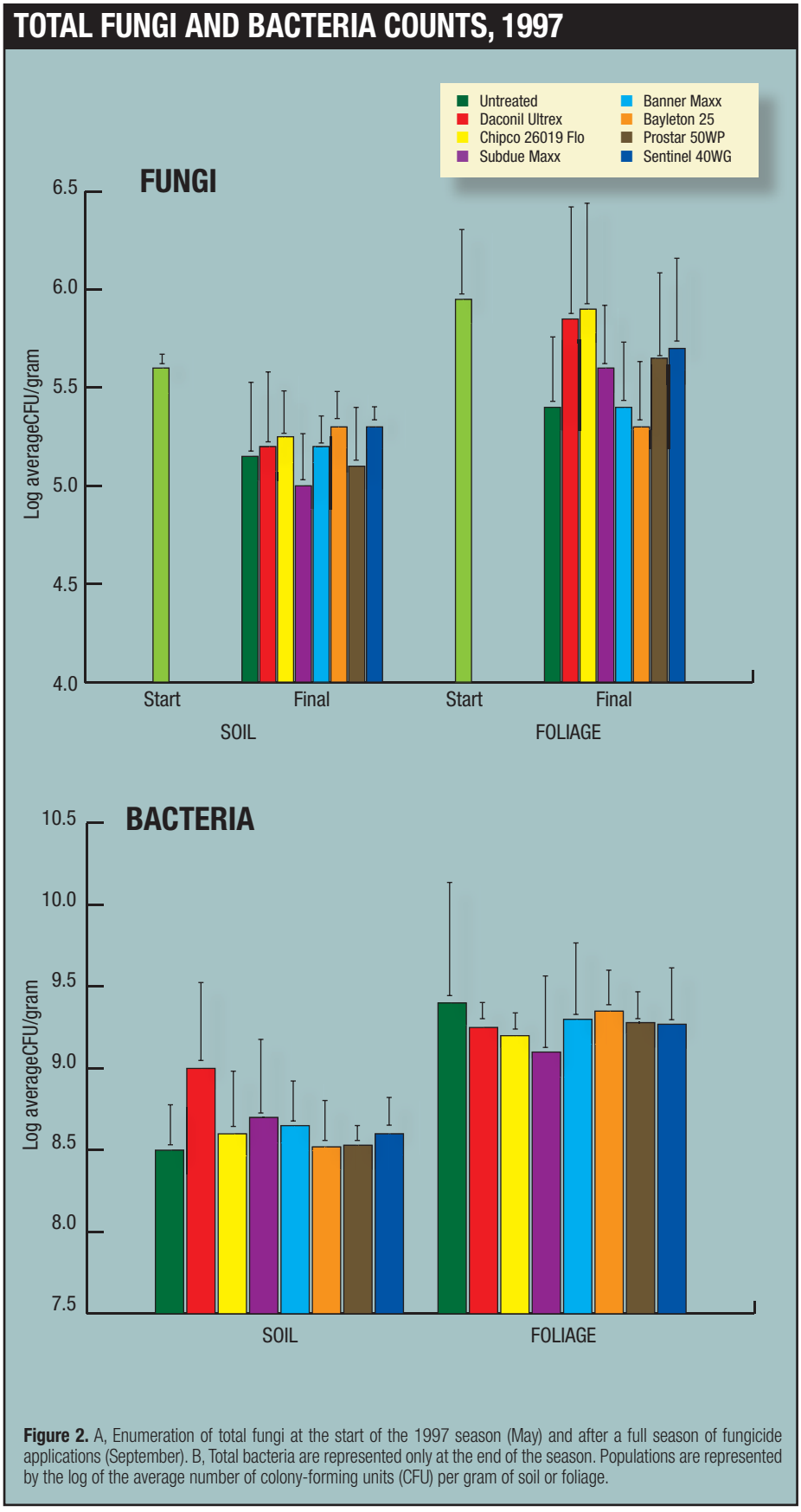

Figure 2. A, Enumeration of total fungi at the start of the 1997 season (May) and after a full season of fungicide applications (September). B, Total bacteria are represented only at the end of the season. Populations are represented by the log of the average number of colony-forming units (CFU) per gram of soil or foliage.

TURFGRASS FUNGICIDES

Treatment	Active ingredient	Rate (ounces/1,000 square feet)	Application interval (days)
Untreated	---	---	---
Daconil Ultrex	chlorothalonil	3.6	14
Chipco 26019 Flo	iprodione	8	21
Subdue Maxx	mefenoxam	1	21
Banner Maxx	propiconazole	4	21
Bayleton 25W	triadimefon	4	21
Prostar 50WP	flutolanil	3	14
Sentinel	cyproconazole	0.167	21

Table 1. Turfgrass fungicides tested to determine whether their repeated use would have significant effects on either foliar or soil-borne microbial populations of putting greens.

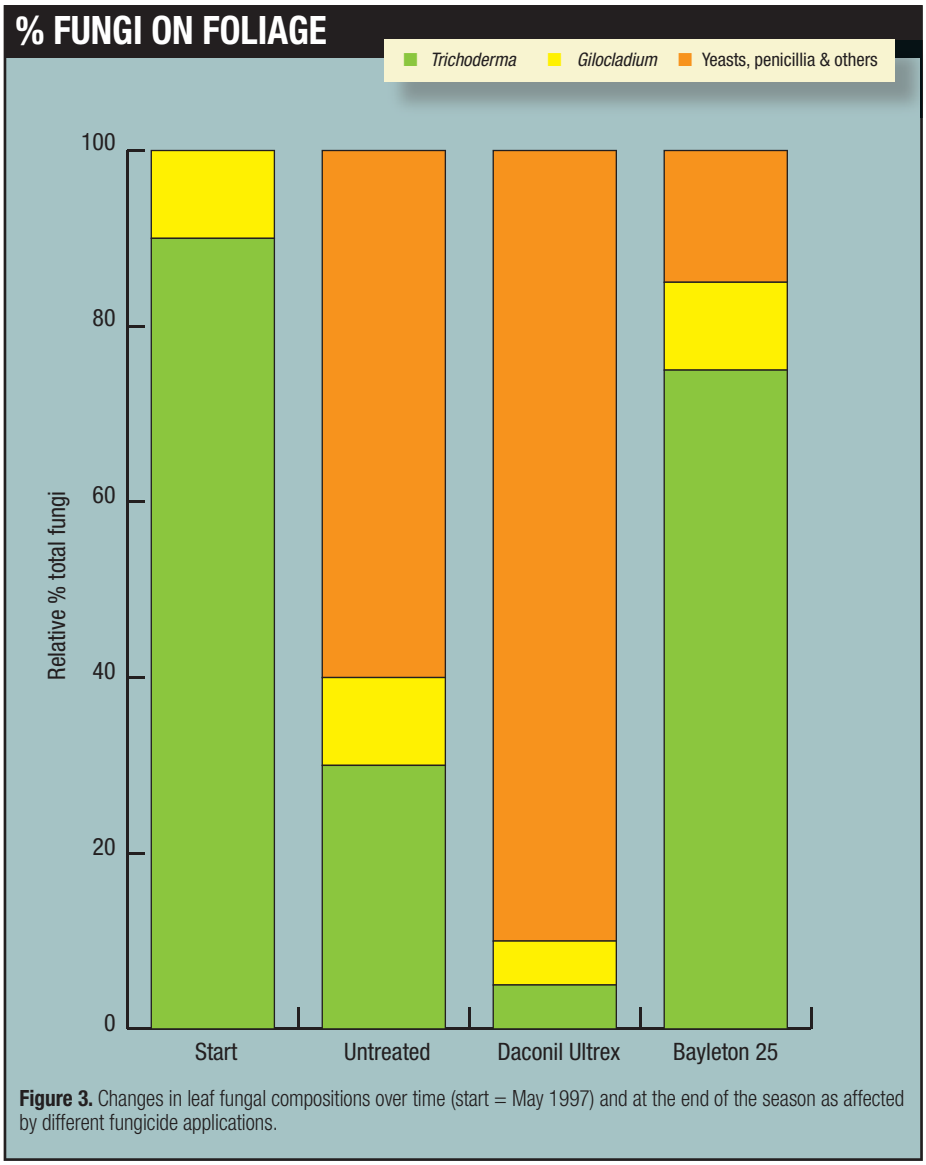

Figure 3. Changes in leaf fungal compositions over time (start = May 1997) and at the end of the season as affected by different fungicide applications.

species, total bacteria, 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, predominately yeasts, *Penicillia* and others, had largely displaced *T. harzianum*. This was particularly true with plants that had been treated with Daconil Ultrex (Figure 1). On plants treated with Bayleton 25, *T. harzianum* remained the predominate culturable fungus.

1998 mini-experiment

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). Fungal enumerations were performed at each sampling time (that is, 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 shown by both the numbers and plate appearances (Figure 3). Most of the fungi isolated from leaves of untreated plants were filamentous fungi, but 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.

Discussion

Our hypothesis 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, tests for metabolic profiles, fatty acid microbial profiles or tests for total microbial metabolic activity. Thus, although different results might be obtained with other assays, it does not appear that repeated applications of fungicides have major impacts on soil microbial communities.

This may be because the fungicides are mostly water insoluble and therefore do not penetrate deeply into the soil, or the soil microbial community is highly competitive and resilient and able to rebound very quickly after fungicidal applications. The prevalence of *Trichoderma* species in the fungal community may also be significant because many members of this genus are highly resistant to a variety of fungicides (1) and their populations could be selectively enhanced 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 is now clear that although 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 epidemic that occurs each year was controlled by fungicides as expected.

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Literature cited

1. Harman, G.E., C.R. Howell, A. Viterbo, et al. 2004. Trichoderma species — opportunistic, avirulent plant symbionts. Nature Reviews Microbiology 2:43-56.
2. Köller, W. 1992. Target sites of fungicide action. CRC Press, Boca Raton, Fla.
3. Norton, J.M., and G.E. Harman. 1985. Responses of soil microbes to volatile exudates from germinating pea seeds. Canadian Journal of Botany 65:1040-1045.
4. 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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THE RESEARCH says . . .

- **Researchers at Cornell** University tested the hypothesis that repeated applications of fungicides to putting greens would have major impacts on microbial populations of both foliar and soil-borne microbes.
- **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.
- **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.* The relative numbers of filamentous fungi versus yeasts changed substantially on turf leaves as evidenced by both the numbers and plate appearances.
- **It does not** appear that repeated applications of fungicides have major impacts on soil microbial communities.

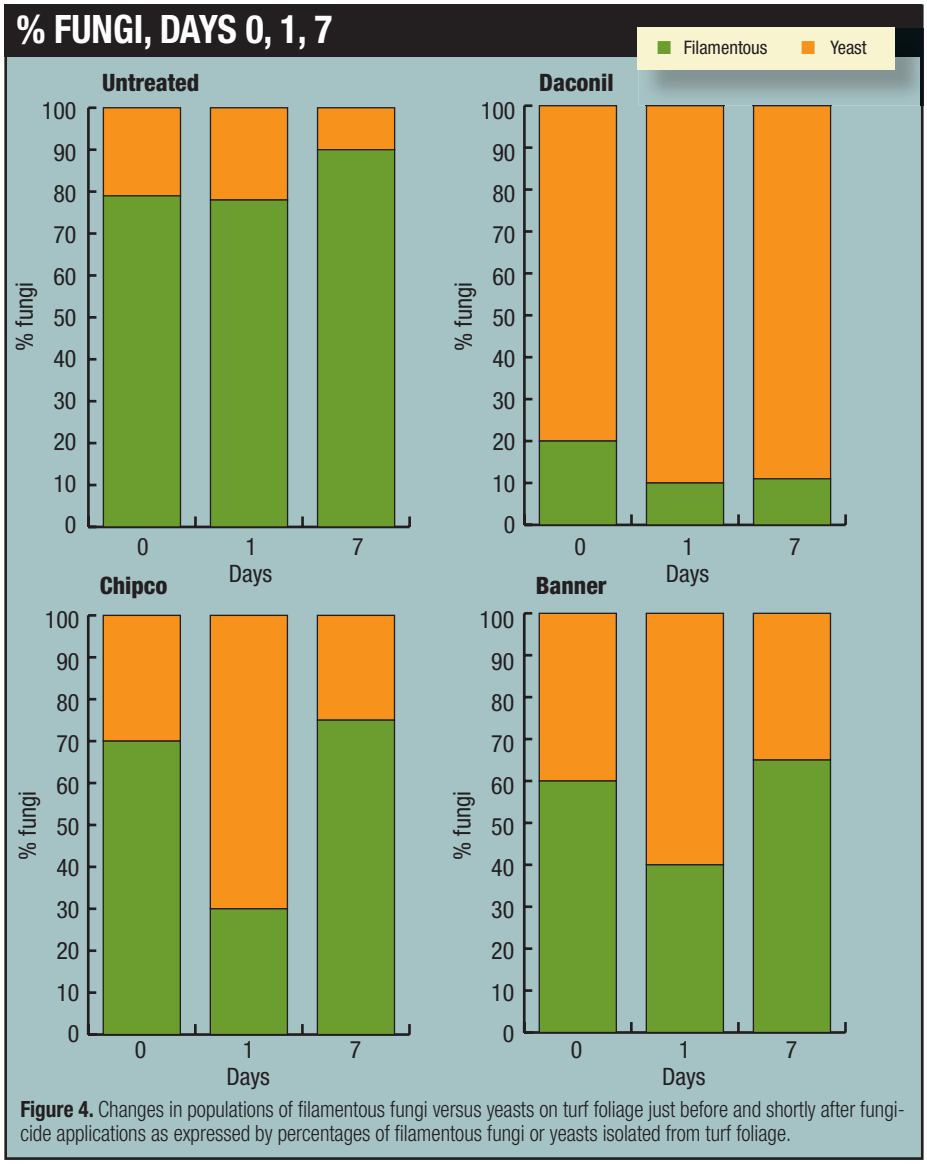

Figure 4. Changes in populations of filamentous fungi versus yeasts on turf foliage just before and shortly after fungicide applications as expressed by percentages of filamentous fungi or yeasts isolated from turf foliage.