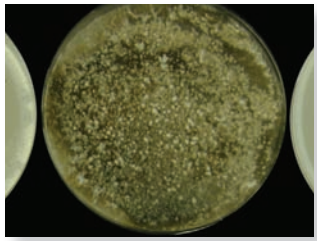
New challenge to an old foe, dollar spot fungicide resistance

Understanding the mechanisms of fungicide resistance helps superintendents combat disease.



Fungicide resistance in dollar spot caused by *Sclerotinia homoeocarpa* is a common problem on golf courses in North America (Figure 1) (2), and managing fungicide resistance is a complex and controversial topic (1,4). With dollar spot, the question of whether to use the same fungicide until resistance develops or to rotate different chemistries and tank mixtures has not been definitively answered. Will the addition of contact fungicides in rotations and in tank mixtures with systemic fungicides prevent fungicide resistance? How many times can a single fungicide be used before efficacy is reduced or complete failure occurs? Do application intervals, application timings or fungicide rates affect the development of fungicide resistance?

Fungicide resistance in dollar spot is poorly understood at the population level. In sociology and biology, a *population* is a collection of individuals of a particular species. Individual isolates in the population may vary in genetic makeup, fungicide sensitivity, pathogenicity and vegetative compatibility. (Vegetative compatibility in fungi occurs when hyphae of two individual isolates fuse.)

Our lab has attempted to carefully tease out the answers to these questions about dollar spot fungicide resistance at the population level by using DNA fingerprinting techniques and in-vitro assays for both fungicide sensitivity and vegetative compatibility. Our

Figure 1. Dollar spot on a creeping bentgrass fairway. **Photo courtesy of S. Abler**

Geunhwa Jung, Ph.D.
Young-Ki Jo, Ph.D.

recent findings revealed that a population of dollar spot isolates collected from the same location could have differences in genetic makeup, which result in a differential response to fungicides (2). A single dollar spot patch can be caused by more than one genetic isolate. The development of fungicide resistance is caused by selecting a population of resistant isolates, which do not have to be genetically identical.

Fungicide resistance is defined as the total failure or the reduced efficacy of a particular fungicide in the field against a specific pathogen. Fungicide resistance develops from two important biological steps: the existence of a small population of resistant isolates before the first application of a fungicide, and selection of the small resistant population by use of fungicides. The initial occurrence of fungicide-resistant isolates results from spontaneous mutations, which are biologically natural and possibly inevitable events. However, the selection process, which is more critical for resistance development, is mainly driven by management practices like repeated fungicide applications and the timing of applications. Fungicides suppress the population of sensitive isolates and selectively allow a subpopulation of resistant isolates to increase and dominate the population.

Development of fungicide resistance in turfgrass diseases depends heavily on the chemical mode of action. There are two types of fungicide resistance to site-specific fungicides: single-step or single-gene mutation (as seen with strobilurins such as Heritage [azoxystrobin; Syngenta], phenylamides such as Subdue MAXX [mefenoxam; Syngenta], and benzimidazoles such as Cleary's 3336 [thiophanate-methyl; Cleary Chemical Co.] and Tersan 1991 [benomyl; DuPont], for example), and multi-step or multiple-gene mutation (as seen with demethylation inhibitors such as Banner MAXX [propiconazole; Syngenta], Rubigan [fenarimol; Gowan Co.], Bayleton [triadimefon; Bayer], Eagle [myclobutanil; Dow AgroSciences], and Trinity [triticonazole; BASF], for example). The development of resistance against single-step fungicides can be rapid (Figure 2) and results in complete loss of efficacy.

Unlike resistance to single-step fungicides, resistance to the multi-step fungicides develops gradually (Figure 3) and reduces efficacy rather than causing complete failure. Efficacy is reduced because a number of mutations are required to alter the level of sensitivity to the fungicide.

Therefore, the main goal of strategies for resistance management is to minimize the chance of selection for spontaneous mutations in the population. This can be accomplished by rotating fungicides with different modes of action and practicing integrated disease management to delay the selection of resistant populations, while monitoring current dollar spot populations for resistant strains.

Mycelial growth

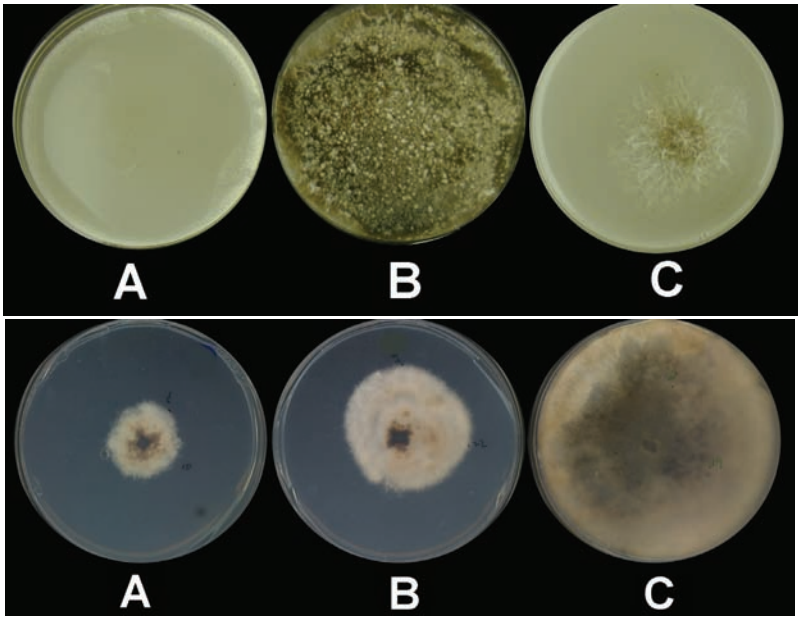


Figure 2 (top). Mycelial growth of dollar spot fungus on growth medium amended with Cleary's 3336 (1,000 micrograms a.i./milliliter). **A:** A sensitive isolate does not grow. **B:** A resistant isolate grows normally. **C:** The sensitive mycelium is chopped and spread on the medium. Spontaneous mutation conferring resistance to Cleary's 3336 occurs at a very low rate, and resistant hyphae grow. **Photos by Y. Jo**

Figure 3 (bottom). Mycelial growth of dollar spot fungus on growth medium amended with Banner MAXX (0.1 microgram a.i./milliliter). Because resistance is associated with mutation at multiple genes, it develops gradually. Sensitive **(A)**, intermediate **(B)** and highly resistant **(C)** isolates grow at different rates.

Study 1. Origins of fungicide resistance

Fungicide resistance in dollar spot originates from naturally occurring spontaneous gene mutations. How often do spontaneous mutations occur?

Methods

To test this question we carried out in-vitro petri dish assays in the lab. Several dollar spot isolates that are sensitive to Cleary's 3336 were selected from a collection of field isolates and grown on a growth medium, potato dextrose agar. Mycelium of each fully grown culture was harvested and chopped into small fragments of hyphae (100-500 micrometers). The fragmented hyphae were plated on potato dextrose agar amended with 1,000 ppm of Cleary's 3336.

Results

We found that some hyphae were capable of growth on potato dextrose agar amended with Cleary's 3336 (Figure 2). This indicates that fungicide-resistant mutants (particularly mutants resistant to Cleary's 3336) of the dollar spot fungus occur naturally and spontaneously at extremely low frequency and are inevitable. We also found that mutation rates vary significantly among dollar spot isolates tested under the in-vitro assays. Certain isolates generated mutants in a higher frequency than others.

Practical applications

Resistance is likely to occur rapidly with fungicides that have a single-step resistance response because mutation of a single gene on the specific target is the only step required for the development of complete resistance to the fungicide. However, resistance will take longer to develop with fungicides that have a multi-step resistance response because multiple gene mutations are required.

Our in-vitro petri dish assay supports this notion, and field experiments confirmed the hypothesis that the more isolates that are exposed to fungicides, the greater probability for the selection of fungicide-resistant mutants. Therefore, the first and foremost step for the management of fungicide resistance is to maintain a low amount of inoculum and keep disease pressure as low as possible by using all available cultural and chemical options:

- Plant dollar spot-tolerant cultivars if they are available.
- Follow cultural practices such as dew removal and proper fertility to minimize dollar spot pressure and maximize healthy turfgrass growth.
- Follow the recommended application intervals and rates on the fungicide labels.

Study 2. Population structure of dollar spot

Turfgrass pathologists believe that the dollar spot fungus uses only mycelium as the primary form of its life cycle in the U.S. The dollar spot fungus has yet to be observed containing sexual/asexual fruiting bodies or spores in the field or laboratory. Previous studies have provided limited information about how this life cycle influences dollar spot populations.

Study results

Our recent research shed light on the genetic structure and spatial distribution of the dollar spot field populations on a fairway and a putting green established at the University of Wisconsin–Madison and on a fairway at the University of Massachusetts–Amherst (2). The dollar spot populations were very different among the three locations in terms of genetic variation, fungicide sensitivity and vegetative compatibility. Isolates in the population at the University of Massachusetts were genetically similar and were highly sensitive to two systemic fungicides, Cleary's 3336 and Banner MAXX. In contrast, isolates collected from the fairway and the green at the University of Wisconsin were separated into two genetically distinct subpopulations (called "A" and "B"). Interestingly, these two subpopulations showed different sensitivities to the two systemic fungicides. The isolates from the two subpopulations were also vegetatively incompatible, meaning that they did not grow together, but instead competed with each other.

It was also interesting to find that, at the University of Wisconsin, the ratio of the two subpopulations, A and B, was consistent within the same fairway or within the same green, regardless of the sample size. However, even though the two subpopulations were uniformly distributed, subpopulation A had significantly greater numbers (80%) on the putting green, whereas subpopulation B had slightly greater numbers (54%) on the fairway. This preference of different subpopulations for different conditions suggests that differences in management practices such as mowing height, mowing frequency and fungicide applications can influence the population levels of subpopulations.

Furthermore, isolates on the putting green, which is under intensive fungicide management, were less sensitive to Banner MAXX than isolates on the fairway. (On potato dextrose medium amended with Banner MAXX, the mean percent of relative mycelial growth was 38% for isolates

Dollar spot

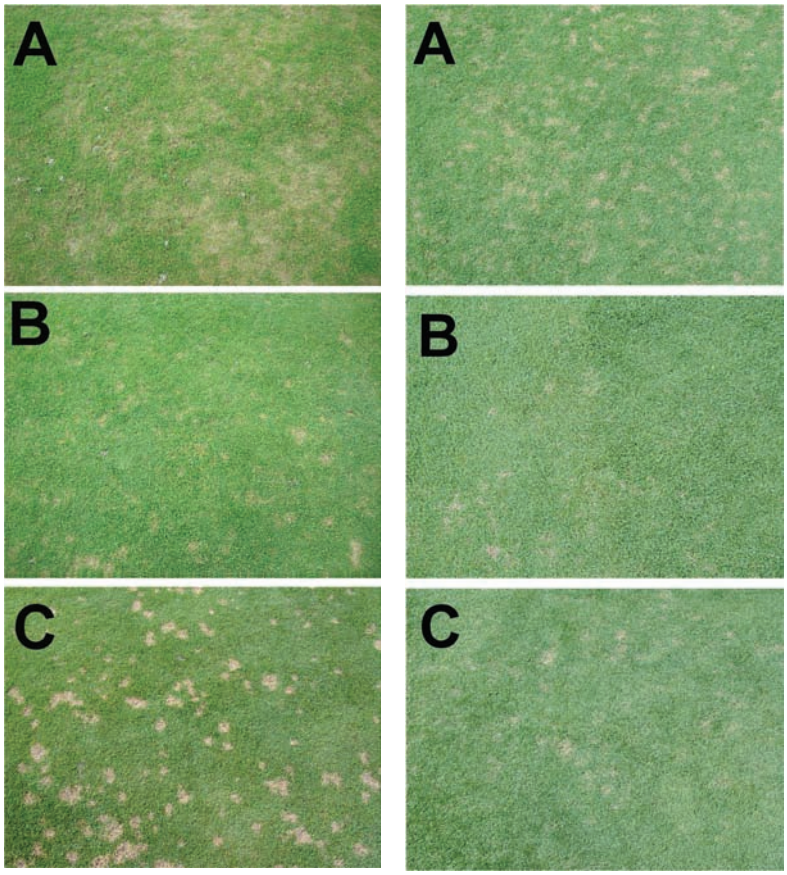


Figure 4 (left). Dollar spot on a creeping bentgrass fairway established at the University of Massachusetts, where Cleary's 3336 was applied on Aug. 4 and Sept. 8, 2006. **A:** Dollar spot before the first application. **B:** After the first application, dollar spot was significantly reduced. **C:** After the second application, dollar spot was not reduced because a population of resistant isolates was selected.

Figure 5 (right). Dollar spot on a creeping bentgrass fairway established at the University of Wisconsin, where Banner MAXX was applied once a month between June and August 2006. **A:** Dollar spot before the first application. **B:** Dollar spot was significantly reduced after the first application. **C:** In August, dollar spot broke out earlier as a population of highly resistant isolates was selected and fungicide efficacy was gradually reduced.

from the green and only 21% for isolates from the fairway.) It is speculated that continual applications of Banner MAXX resulted in greater selection for the insensitive isolates on the green. In contrast, compared to the putting green, the fairway had a higher frequency of isolates resistant to Cleary's 3336. The fairway might be under more disease pressure, which might be enhanced by fewer applications of fungicides other than Cleary's 3336 and by mowing heights and frequency.

In addition, a recent survey conducted at seven golf courses located in Wisconsin and Massachusetts (3) suggested that dollar spot populations significantly differ among golf courses and between a putting green and a fairway on the same golf course. Dollar spot resistance to Cleary's 3336 was generally higher on fairways than on greens regardless of course age or fungicide history. For Banner MAXX, higher resistance was generally observed on putting greens than on fairways.

Practical applications

Knowing the fungicide sensitivities of the dollar spot population in different areas of the golf course is key to designing a successful plan for managing fungicide resistance.

Study 3. Shifting response of dollar spot populations to repeated fungicide applications

Based on the previous population research, we have developed an idea of the composition of the dollar spot field population within the fairways and putting greens of our university research field plots.

Our next question is, how will these populations change in response to fungicide applications? To tackle this question, field experiments were conducted at the University of Wisconsin and the University of Massachusetts in 2006 and 2007. Dollar spot populations were monitored as fungicides were applied repeatedly. We speculated that consecutive applications of one fungicide under high dollar spot pressure would select a subpopulation of resistant isolates.

Two consecutive applications of Cleary's 3336 WP (4 ounces/1,000 square feet [12.2 kilograms/hectare]) with a long interval (four weeks) between applications in both 2006 and 2007 did, in fact, select a subpopulation of resistant isolates on the University of Massachusetts fairway plot (Figure 4). Figure 4 shows dollar spot severity before the

first Cleary's 3336 application (A), four weeks after the first application (B), and four weeks after the second application (C), when a population of resistant isolates had damaged the turf.

The field experiment conducted on the fairway at the University of Wisconsin in 2006 showed that three consecutive applications of Cleary's 3336 rapidly selected a resistant population. The University of Wisconsin fairway plot results were consistent with the results from the University of Massachusetts fairway plot.

To test the persistence of resistant isolates, the plots treated with Cleary's 3336 in 2006 were not treated with additional fungicides in 2007. Resistant isolates of the type that had developed in 2006 had decreased significantly in the absence of Cleary's 3336 use after one growing season. This result may be explained by a lack of fitness of the resistant isolates or by an increase of sensitive isolates, which diluted the resistant populations.

Sensitivity to Banner MAXX

Six consecutive applications (three in 2006 and three in 2007) of Banner MAXX on the University of Massachusetts fairway plot did not affect the fungicide sensitivity of the population. All isolates collected at the end of the season in 2007 were still highly sensitive to Banner MAXX.

Unlike the dollar spot populations at the University of Massachusetts, those at the University of Wisconsin showed less sensitivity to Banner MAXX. The difference in Banner MAXX sensitivity can be attributed to the wide genetic variability and a broad range of fungicide sensitivities to Banner MAXX that pre-existed in the University of Wisconsin fairway plot populations before the experiments. The origin of the insensitive populations is not known. Isolates highly insensitive to Banner MAXX were gradually selected and eventually dominated the population. Consequently, the population shifted to significantly higher insensitivity to Banner MAXX at the end of the 2006 season, resulting in reduced fungicide efficacy in the field (Figure 5).

Practical information

- The long interval between subsequent applications of the same fungicides allowed the fungus to recolonize plants and to produce massive amounts of mycelia, which increased the number of mutations occurring in the population.
- Repeated application of site-specific systemic fungicides is not recommended.
- A population's response to fungicides depends on the initial population, but the population shift to resistance can be accelerated by the repeated application of site-specific fungicides.

Conclusions

Despite our attempts to understand the mechanisms of fungicide resistance in dollar spot, more questions still remain to be answered. In a subsequent article, we will present our recent research results on the effects of repeated applications, rotation or tank mixtures on the incidence of fungicide resistance development in dollar spot.

Acknowledgments

This work was partly supported by start-up funds from the department of plant, soil, and insect sciences at the University of Massachusetts-Amherst and grants-in-aid from Cleary Chemical Corp., Bayer Environmental Science and Syngenta Professional Products.

Literature cited

1. Couch, H.B. 2003. Strategies for preventing and managing fungicide resistance. *Golf Course Management* 71(5):111-115.
2. Jo, Y., M. Boehm and G. Jung. 2007. Rapid population shift of *Sclerotinia homoeocarpa* in response to fungicide applications on turfgrass. *Phytopathology* 97:S53 (Abstract).
3. Koch, P. 2007. Effect of cultural practices on the population structure of fungicide resistant isolates of *Sclerotinia homoeocarpa* of turfgrass. M.S. thesis, University of Wisconsin, Madison.
4. Vargas, J.M., Jr. 2002. To rotate or not to rotate? *Golf Course Management* 70(4):53-56.

GCM

Geunhwa Jung (jung@psis.umass.edu) is an assistant professor and Young-Ki Jo was a post-doctoral research associate in the department of plant, soil and insect sciences at the University of Massachusetts, Amherst. Young-Ki Jo is currently an assistant professor in the department of plant pathology and microbiology at Texas A&M University.

The research says

→ Dollar spot populations have differential sensitivity to Cleary's 3336 and Banner MAXX among golf courses and between putting greens and fairways within the same golf course.

→ Dollar spot populations quickly shift from sensitivity to resistance if consecutive applications of the same site-specific, single-step fungicides such as Cleary's 3336 are made at long intervals.

→ Dollar spot populations gradually shift from high sensitivity to low sensitivity if consecutive applications of the same site-specific, multi-step fungicide such as Banner MAXX are made repeatedly at long intervals.