Strategies for preventing and managing fungicide resistance

Rotating fungicides with different biochemical modes of action and using fungicide mixtures are the two primary strategies for managing fungicide resistance.

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The turfgrass disease research program at Virginia Tech has identified combinations of fungicides that are synergistic in the control of Sclerotinia dollar spot and Pythium blight. In addition to providing increased levels of disease control, these mixtures are also effective in fungicide resistance management. The results of the disease control aspect of these studies have been reported in earlier articles (4,7). This paper covers the nature of resistance to fungicides by turfgrass pathogens and describes specific risk-management strategies, including the use of synergistic mixtures to prevent the development of resistance or manage existing resistance in the Sclerotinia dollar spot and Pythium blight pathogens.

EDITOR'S note:

This is the second of two articles by Houston Couch about using synergistic mixtures of fungicides. The first article appeared in the November 2002 *GCM*.

Photo courtesy of Houston Couch

To assess the toxicity of fungicides to pathogenic turfgrass fungi in the lab, a fungus is placed in a number of petri dishes containing agar and various levels of the fungicide being tested. Growth rates of the fungus under the different test conditions are measured.

The workings of fungicide resistance

Resistance risk

The degree of vulnerability of turfgrass fungicides to resistance by the target organisms is linked to the fungicides' biochemical mode of action. *Biochemical mode of action* refers to the effects of the fungicide on the metabolic processes of the fungal cell. Action of individual fungicides against metabolic pathways will be either multisite (affecting several sites simultaneously) or site-specific (affecting only one site). The majority of fungicides developed since the 1960s are site-specific.

Resistance develops more frequently in site-specific situations than in cases of multiple metabolic target sites. A single target site can be overcome by one mutation, but several changes occurring simultaneously are needed to overcome multiple-site fungicide sensitivity. For example, if the probability of occurrence of a single mutation that affects one target site is 10^{-8}, then the chance of two such mutations affecting two target sites and occurring simultaneously is 10^{-16} (2).

KEY points

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- **Continuous applications** of the same single-site fungicide at close intervals accelerate the development of resistance.
- **Development of a resistant strain of the target pathogen** is determined by the frequency of application of the fungicide.
- **Rotating fungicides** with different biochemical modes of action will significantly reduce the risk of developing resistance.
- **Synergistic fungicide combinations** are effective in preventing and managing fungicide resistance.

Genetic changes in the target pathogen

Fungicide-resistant populations begin with genetically controlled modification(s) of the primary metabolic site(s) of action of the fungicide in question. The lone mutant survives and increases in numbers during continued exposure to applications of a specific fungicide.

Instances of fungicide resistance may be regulated by one gene (monogenic) or by multiple genes (polygenic). In cases of monogenic fungicide resistance, full impact of the mutation on reduced effectiveness of the fungicide develops immediately. Monogenic resistance also tends to be stable. Resistance of the Sclerotinia dollar spot pathogen to benzimidazoles (Cleary 3336, Fungo 85) is monogenic.

In contrast to monogenic resistance,

increased disease severity caused by polygenic resistance develops incrementally. This means that in instances of polygenic resistance, monitoring for progressing reduction in fungicide effectiveness can detect the early stages of its development before failure to control the disease becomes severe enough to cause significant damage. Resistance of the Sclerotinia dollar spot pathogen to the demethylation inhibitors (Banner, Bayleton, Eagle, Rubigan) is polygenic (1).

Survival fitness of resistant strains

To sustain themselves indefinitely at high population levels, fungicide-resistant forms must compare favorably with fungicide-sensitive strains as primary pathogens and as thatch- and soil-inhabiting saprophytes. The ability of resistant strains to compete with fungicide-sensitive strains for survival is referred to as "fitness" (2).

A pathogenic fungus that has become resistant to different turfgrass fungicides may have a high level of fitness in relationship to one fungicide and poor fitness in relationship to another. For example, at Virginia Tech, we have observed in the field that the strain of *Sclerotinia homoeocarpa* resistant to the demethylation inhibitors has a low level of fitness. It usually persists no more than two to three years after the application of demethylation inhibitors has ceased. We have also observed that, in contrast, the form of the Sclerotinia dollar spot pathogen resistant to benzimidazole fungicides (Cleary 3336, Fungo 85) shows a very high level of fitness.

Fungicide Resistance Action Committee

Assessing risk of resistance to fungicides and establishing appropriate use strategies for risk reduction is now a major factor in the development of new fungicides. Internationally, government regulations for registration are beginning to require that each main use of a new fungicide be given a separate risk assessment. In order to provide a common source of information on use strategies that minimize risk of pathogen resistance to specific fungicides, pesticide manufacturers have formed a standing intercompany organization known as the Fungicide Resistance Action Committee (FRAC). FRAC has working groups that formulate strategies for each class of fungicides. Publications on the nature of fungicide resistance and current recommendations on resistance management for specific fungicides can be accessed on FRAC's Web page, http://www.frac.info.

VULNERABLE FUNGICIDES

Fungicide group	Common names	Representative trade names
Benzimidazoles	benomyl thiophanate methyl	Benomyl Fungo 85 Cleary 3336
Demethylation inhibitors	triadimefon propiconazole fenarimol myclobutanil	Bayleton Banner Rubigan Eagle
Strobilurins	azoxystrobin trifloxystrobin pyraclostrobin	Heritage Compass Insignia
Dicarboximides	iprodione vinclozolin	Chipco 26019 Vorlan Curalan

The turfgrass fungicides listed are vulnerable to cross-resistance within the group.

Resistance extending to other fungicides

Pathogens that develop resistance to one fungicide may become simultaneously resistant to other fungicides. Generally, these fungicides have either a close chemical relationship or a similar mechanism of fungitoxicity. This phenomenon is known as *cross-resistance* (10).

Cross-resistance is usually confined to members of the same fungicide group. For example, certain target pathogens are cross-resistant to individual fungicides within the strobilurins, benzimidazoles, demethylation inhibitors and dicarboximides. However, cross-resistance can also extend into fungicide groups with different chemical structures. Resistance to the dicarboximides (iprodione and vinclozolin) is known to occur simultaneously with resistance to the aromatic hydrocarbon fungicides (quintozene [PCNB] and chloroneb) (10).

Resistance to fungicides from different chemical groups and with different biochemical modes of action can develop in the same isolate of a pathogenic fungus. This is known as *multiple resistance*. A strain of the Sclerotinia dollar spot pathogen has been identified that is resistant to both benzimidazole fungicides and demethylation inhibitors (3).

Increasing the risk of resistance

The basic level of risk of resistance of a pathogen to a specific fungicide is determined by the factors listed above. The magnitude of risk in the field, however, can be modified by disease-management strategies. Some strategies will increase the risk of fungicide resistance; others will either prevent or delay its development; and others can deal effectively with existing cases of resistance.

Strategies that have a direct impact on the risk of development of fungicide resistance include continual applications of the same fungicide at close intervals, rotation of fungicides with different biochemical modes of action, and use of mixtures of fungicides.

Continual applications of the same fungicide at close time intervals

Continual use of the same single-site fungicide without intervening applications of materials with different biochemical modes of action hastens the development of resistance on the part of the pathogen. Consecutive applications of a fungicide at close intervals until resistance occurs may also generate certain disease-management problems.

- *Extensive disease damage to the turf.* With single-site monogenic resistance, an unexpected and severe outbreak of the disease can develop before an alternative fungicide can be brought into use.
- *Fungicide-enhanced resurgence of the target disease.* After the disease has gone into remission and fungicide applications have ceased, severe outbreaks of the target disease may occur. For example, in field studies at Virginia Tech, 21 days after Sclerotinia dollar spot had gone into remission, plots that had been treated continuously throughout the season with either chlorothalonil (Daconil 2787) or iprodione (Chipco 26019) had a disease level 500 percent greater than that of the untreated controls (5,6).
- *Resistance to an entire group of fungicides.* If the fungicide belongs to a group with closely related biochemical modes of action, cross-resistance to the entire group will develop simultaneously.
- *Resistance to a fungicide with different chemistry.* The target pathogen may automatically develop cross-resistance to a chemically unrelated fungicide.
- *Development of multiple resistance.* Switching applications of an alternative single-site fungicide for control of the same target disease in the same location without intervening applications of materials with different biochemical modes of action will eventually result in the development of multiple fungicide resistance in the pathogen.

Rotating fungicides

Development of resistance to a single-site fungicide is not necessarily inevitable. The suggestion that each fungicide chemistry has a fixed number of applications that can be made before resistance develops has been hypothesized (15). The degree of selection pressure for the development of a resistant strain of the target pathogen is determined primarily by the frequency of application of the fungicide in question in rotation with other fungicides during the season, not the grand total of times it has been used (1).

Increasing the time between applications of a specific single-site fungicide through rotation with other fungicides decreases selection pressure for resistant strains. However, rotating among fungicides can reduce the risk of resistance only when certain guidelines are followed. Reduction of selection pressure can be achieved only if the fungicides used in the rotation have different biochemical modes of action. For the rotation to be successful, it must be tailored to the fitness level of the fungicide-resistant strain.

- *Low-fitness-level program.* Strains of *Sclerotinia homoeocarpa* resistant to either demethylation inhibitors (Banner, Bayleton, Eagle, Rubigan) or iprodione (Chipco 26019) and *Pythium aphanidermatum* resistant to mefenoxam (Subdue Maxx) have low levels of fitness. In these instances, if the interval between applications of the fungicide at risk is long enough to cause the resistant strain to remain at a nonpathogenic population level, it is possible to avoid resistance problems indefinitely. Specifically, during the growing season, individual rotations should progress through a schedule of one single-site fungicide application followed by two applications of multisite fungicides, or one multisite fungicide application followed by two single-site fungicides with different biochemical modes of action (9).
- *High-fitness-level program.* When the fungicide-resistant strain of the pathogen has a high level of fitness (for example, *Sclerotinia homoeocarpa* resistance to benzimidazoles), the risk of resistance can be reduced significantly if time between applications of the benzimidazole is increased by using three intervening fungicides, each with different biochemical modes of action. During the growing season, the individual rotations should progress through one benzimidazole application followed by three applications of multisite fungicides, or one benzimidazole application followed by three applications of single-site fungicides with different biochemical modes of action (9).

Use of fungicide mixtures

Full-label-rate mixtures of fungicides with different biochemical modes of action for control of the same target pathogen are effective in both the prevention and management of fungicide resistance (9). The downside of this strategy, however, is that the additional fungicide increases both financial and environmental costs. These shortcomings can be avoided by using synergistic fungicide mixtures.

Synergistic mixtures increase the effectiveness of disease control significantly while using less fungicide during the season (4,5,12).

FUNGICIDES VS. DOLLAR SPOT

Fungicide & EC level*	Fungicide concentration (µg/ml) Sensitive strain	Resistant strain
Banner (propiconazole)		
EC_{50}	0.197	2.911
EC_{95}	2.458	23.684
Bayleton (triadimefon)		
EC_{50}	0.002	1.780
EC_{95}	0.003	15.282
Eagle (myclobutanil)		
EC_{50}	2.900	24.710
EC_{95}	21.057	741.770
Lynx (tebuconazole)		
EC_{50}	0.078	6.963
EC_{95}	1.943	60.390
Rubigan (fenarimol)		
EC_{50}	0.003	7.582
EC_{95}	0.796	5,478.390
Sentinel (cyproconazole)		
EC_{50}	0.357	3.316
EC_{95}	0.494	18.209

*EC levels refer to amount of fungicide required to reduce fungus growth rates in culture by 50 and 95 percent, respectively.

Comparative toxicity of six demethylation inhibitors to sensitive and resistant strains of *Sclerotinia homoeocarpa.*

In addition, they prevent the initiation and buildup of fungicide-resistant populations by the target pathogens (8,14). Synergistic combinations are also very effective in disease control situations where resistance to one of the fungicides in the mixture already exists (13).

Managing resistance by the dollar spot pathogen

Tests were conducted by our research group for toxicity of the synergistic fungicide mixtures to a strain of *Sclerotinia homoeocarpa* cross-resistant to six demethylation inhibitors (Banner, Bayleton, Eagle, Lynx, Rubigan and Sentinel). (Note that Lynx and Sentinel are not available in the United States at this time.) Petri dishes were used containing agar amended with known amounts of fungicide at varying dosage levels. A 3/16-inch agar plug of *S. homoeocarpa* was placed at the center of each plate. Radial growth for each fungicide entry was measured when fungus growth in the plates not amended with fungicide covered approximately 95 percent of the agar surface. The EC_{50} and EC_{95} values (fungicide concentrations required to reduce radial growth rate by 50 and 95 percent, respectively) were calculated for each fungicide-amended treatment by using a computer software program for probit analysis and probit plot. These tests showed that the strain of *S. homoeocarpa* used in our experiments was highly resistant to all six demethylation inhibitors.

Management of fungicide resistance by the Pythium blight pathogen

Our Pythium blight control experiments were carried out with container-grown perennial ryegrass (*Lolium perenne*) plants. These studies included tests for the toxicity of the mancozeb (Fore) + metalaxyl (Subdue) mixture to the strain of *Pythium aphanidermatum* that is resistant to metalaxyl/mefanoxam. Fungicide applications were made and inoculations performed when the plants reached the four-leaf stage of growth. Individual treatments were replicated four times, and each experiment was performed twice. Disease ratings were based on visual estimate of the percentage of foliage blighted per container, and the data were subjected to analysis of variance and compared by means of protected LSD values (7).

Four fungicide mixtures were identified that are synergistic in the control of Pythium blight: mancozeb: (Fore) + metalaxyl (Subdue), (Fore) + propamocarb (Banol), fosetyl-Al (Signature) + propamocarb (Banol), and fosetyl-Al (Signature) + metalaxyl (Subdue). Each of these mixtures provided a significant increase in disease control, and, in addition, the mancozeb (Fore) + metalaxyl (Subdue) combination was found to be highly toxic to the Subdue-resistant strain of the pathogen (7).

Summary and conclusions

Various disease management strategies can significantly modify the inherent risk of fungicide resistance in the target pathogen. The risk can be lowered by rotation among fungicides with different biochemical modes of action or by using synergistic fungicide combinations. The synergistic combinations listed in this paper are very effective in the control of Sclerotinia dollar spot and Pythium blight and highly toxic to the fungicide-resistant strain of each pathogen.

No research has been conducted to show that each fungicide chemistry is limited to a fixed number of applications before pathogen resistance develops. The degree of selection pressure for the development of a resistant strain of the pathogen is determined primarily by the frequency of applications of the fungicide in question during the season, not the grand total of times it has been used (1).

Continuous applications of the same single-site fungicide at close intervals accelerate the development of resistance on the part of the pathogen. Several disease management problems may be generated by consecutive applications of the same fungicide until resistance occurs. These can include extensive disease damage to the turf from an unforeseen outbreak of the disease, fungicide-enhanced resurgence of

DMIs VS. A RESISTANT FUNGUS

Fungicide	Concentration (µg/ml)	% growth rate reduction Expected*	Actual†
Banner (propiconazole) and Curalan (vinclozolin)			
Control	—	—	0.0 a
Banner	1.0		35.9 b
Curalan	1.0		43.1 c
Banner + Curalan	1.0 + 1.0	63.5	100 d syn
$LSD_{0.50}$			3.6
Banner (propiconazole) and Bayleton (triadimefon)			
Control	—	—	0.0 a
Bayleton	0.1		8.9 b
Banner	0.001		11.1 b
Banner + Bayleton	0.001 + 0.1	19.0	46.6 c syn
$LSD_{0.50}$			7.5
Banner (propiconazole) and Chipco 26019 (iprodione)			
Control	—	—	0.0 a
Banner	0.0001		2.5 a
Chipco 26019	0.1		3.6 a
Banner + Chipco 26019	0.0001 + 0.1	6.0	14.6 b syn
$LSD_{0.50}$			3.9
Banner (propiconazole) and Daconil 2787 (chlorothalonil)			
Control	—	—	0.0 a
Banner	1.0		22.6 b
Daconil 2787	1.0		37.3 c
Banner + Daconil 2787	1.0 + 1.0	51.5	76.9 d syn
$LSD_{0.50}$			4.5

*Synergy computed according to the method of Gowing (11).
†In each group, means followed by different letters are significantly different from each other. syn = synergistic.

The values show the percentage of growth rate reduction caused by four different combinations of fungicides to a strain of *Sclerotinia homoeocarpa* that is resistant to demethylation inhibitors (DMIs). All experiments were conducted in petri dishes in the laboratory.

the target disease, simultaneous cross-resistance to an entire group of closely related fungicides or to fungicides with different chemistry, and the eventual development of multiple resistance.

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Literature cited

1. Brent, K.J. 1995. Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph No. 1. Global Crop Production Federation, Brussels.
2. Brent, K.J., and D.W. Hollomon. 1998. Fungicide resistance: The assessment of risk. FRAC Monograph No. 2. Global Crop Production Federation, Brussels.
3. Burpee, L.L. 1997. Control of dollar spot of creeping bentgrass caused by an isolate of *Sclerotinia homoeocarpa* resistant to benzimidazole and demethylation-inhibitor fungicides. *Plant Disease* 81:1259-1263.
4. Couch, H.B. 2002. Better dollar spot control with less fungicide. *Golf Course Management* 70(11):89-93.
5. Couch, H.B. 1995. Diseases of turfgrasses, 3rd ed. Krieger, Melbourne, Fla.
6. Couch, H.B., P. Keating, S. Abler and D. McCall. 1998. Results of 1998 Virginia Tech Turfgass Disease Control Trials. Annual Report of the Turfgrass Pathology Laboratory, Blacksburg, Va.
7. Couch, H.B., and B.D. Smith. 1991. Synergistic and antagonistic interactions of fungicides against *Pythium aphanidermatum* on perennial ryegrass. *Crop Protection* 10:386-390.
8. Dekker, J. 1982. Countermeasures for avoiding fungicide-resistance. p. 177-186. *In:* J. Dekker and S.G. Georgeopoulos (eds.), Fungicide resistance in crop protection. Netherlands Center of Agricultural Publishing and Documentation, Wageningen.
9. Dekker, J. 1986. Preventing and managing fungicide resistance. p. 347-354. *In:* U.S. National Research Council Committee on Strategies for the Management of Pesticide-Resistant Pest Populations, Pesticide resistance: Strategies and tactics for management. National Academy Press, Washington, D.C.
10. Georgopolus, S.G. 1982. Cross-resistance. p. 53-59. *In:* J. Dekker and S.G. Georgeopoulos (eds.), Fungicide resistance in crop protection, Netherlands Center of Agricultural Publishing and Documentation, Wageningen.
11. Gowing, D.P. 1960. Comments on tests of herbicide mixtures. *Weeds* 8:379-391.
12. Samoucha, Y., and U. Gisi. 1987. Use of two- and three-way mixtures to prevent buildup of resistance to phynylamide fungicides in *Phytophthora* and *Plasmopara. Phytopathology* 77:1405-1409.
13. Sanders, P.L., W.J. Houser, P.J. Parish and H. Cole Jr. 1985. Reduced-rate fungicide mixtures to delay fungicide resistance and to control selected turfgrass diseases. *Plant Disease* 69:939-943.
14. Scardavi, A. 1966. Synergism among fungicides. *Annual Review of Phytopathology* 4:335-341.
15. Vargas, J.M., Jr. 2002. To rotate or not to rotate? *Golf Course Management* 70(4):53-56.

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SYNERGISM VS. DOLLAR SPOT

Preventive disease control rates*

Banner MAXX (14.3 FL) 0.25 oz fp + Bayleton (50 WP) 0.25 oz fp
Banner MAXX (14.3 FL) 0.25 oz fp + Chipco 26 GT (23.3 FL) 0.75 oz fp
Banner MAXX (14.3 FL) 0.25 oz fp + Curalan or Touche (50 EG) 0.25 oz fp
Banner MAXX (14.3 FL) 0.25 oz fp + Daconil Weather Stick (54 FL) 1.0 oz fp

Curative disease control rates*

Banner MAXX (14.3 FL) 0.5 oz fp + Bayleton 1.0 oz fp
Banner MAXX (14.3 FL) 0.5 oz fp + Chipco 26 GT (23.3 FL) 3.0 oz fp
Banner MAXX (14.3 FL) 0.5 oz fp + Curalan or Touche (50 EG) 1.0 oz fp
Banner MAXX (14.3 FL) 0.5 oz fp + Daconil Weather Stick (54 FL) 4.0 oz fp

*Rates = formulated product (fp)/1,000 square feet.

Synergistic fungicide combinations for resistance management and increased control of dollar spot. The rates listed for the preventive disease control mixtures will be adequate for most Sclerotinia dollar spot management programs. If the disease has become high in incidence and severity before the fungicide program can be initiated and a rapid rate of recovery is important, then one of the curative rate mixtures should be used for the first application.

FORE + SUBDUE VS. PYTHIUM BLIGHT

Fungicide	Label amount/ 1,000 square feet	% disease control Expected*	Actual†
Control	—	—	0.0 a
Subdue FL (21.3%)	0.2×		0.0 a
Subdue FL (21.3%)	1.0×		0.0 a
Fore WP (80%)	0.5×	64.0 b	
Fore WP (80%)	1.0×		77.0 c
Subdue FL (21.3%) + Fore WP (80%)	0.2× 0.5×	64.0	84.4 c syn
$LSD_{0.05}$			8.8

*Synergy computed according to the method of Gowing (11).
†Means followed by different letters are significantly different from each other; syn = synergistic.

Verification of the effectiveness of combinations of Fore (mancozeb) and Subdue (metalaxyl) in the control of Pythium blight of perennial ryegrass caused by the metalaxyl–resistant strain of *Pythium aphanidermatum* (7).

SYNERGISM VS. PYTHIUM BLIGHT

Preventive disease-control rates*

Fore WP (80%) 4.0 oz fp + Subdue MAXX FL (21.3%) 0.5 oz fp†
Fore WP (80%) 4.0 oz fp + Banol 6 F 2.0 oz fp
Signature 4.0 oz fp + Banol 6 F 2.0 oz fp
Signature 4.0 oz fp + Subdue MAXX FL (21.3%) 0.5 oz fp

Curative disease-control rates*

Fore WP (80%) 6.0 oz fp + Subdue MAXX FL (21.3%) 1.0 oz fp†
Fore WP (80%) 6.0 oz fp + Banol 6 F 4.0 oz fp
Signature 6.0 oz fp + Banol 6 F 4.0 oz fp
Signature 6.0 oz fp + Subdue MAXX 1.0 oz fp

*Rates = formulated product (fp)/1,000 square feet.
†Fore + Subdue is also highly toxic to the Subdue-resistant strain of *Pythium aphanidermatum.* (7)

Synergistic fungicide combinations for resistance management and increased control of Pythium blight. Rates listed for the preventive disease-control mixtures will be adequate for most Pythium blight management programs. If the disease has become high in incidence and severity before the fungicide program can be initiated, and if a rapid rate of recovery is important, then one of the curative rate mixtures should be used for the first application.