Understanding Fungicide Resistance

The fundamental elements and practical consequences of turf disease control.

BY RICHARD LATIN, PH.D.

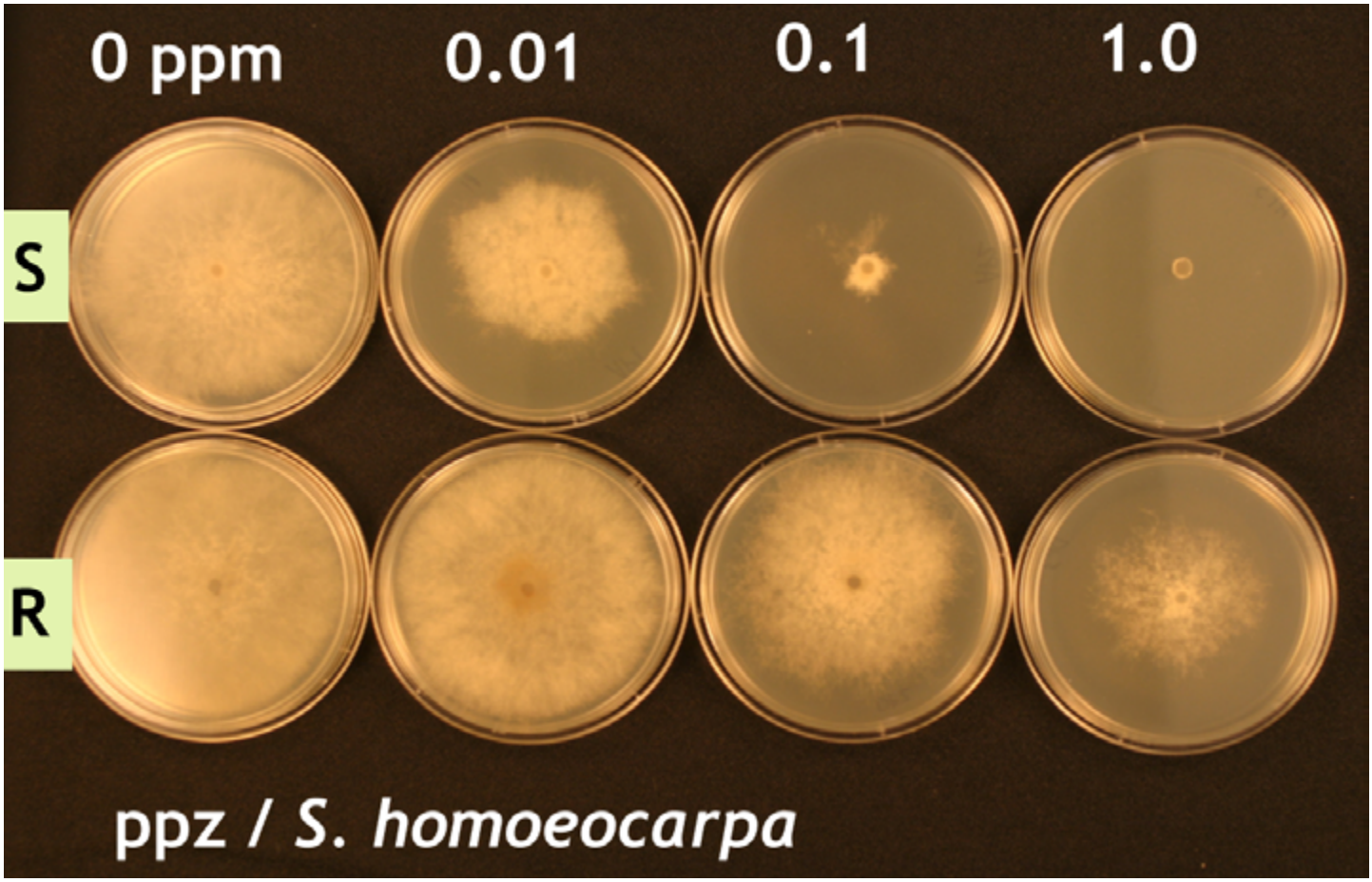

Figure 1 – As increasing amounts of fungicide are applied, the sensitive population (top row) decreases while the resistant population persists. Fungicide-resistant isolates of S. homoeocarpa *are identified by comparing colony growth on culture media amended with fungicide in several concentrations (0, 0.01, 0.1, and 1.0 ppm). For the sensitive isolate (S) growth is inhibited at 0.01 ppm (top), while growth of the resistant isolate (R) continues through 1.0 ppm (bottom).*

Everyone engaged in establishing and maintaining healthy turf should be familiar with the term fungicide resistance. In many cases, resistance is a contributing factor in unsatisfactory fungicide performance. Fungicide resistance occurs when a once-effective active ingredient is no longer able to stop pathogen growth and control disease (Figure 1). The phrase "once-effective" is essential to understanding fungicide resistance. It implies that something has changed in the pathogen to reduce the efficacy of a fungicide. The change is genetic, meaning the resistance trait can be passed on to future pathogen generations. Pathogen strains that are not resistant are said to be sensitive — i.e., sensitive to a fungicide's effect. Although the term "insensitive" is sometimes used to describe fungicide resistance, the term "resistant" is used here with the understanding that there are different degrees of resistance depending on the pathogen and fungicide. This narrative provides an overview of fungicide resistance, addressing the fundamental elements and practical consequences as it relates to turf disease control.

RESISTANCE HAS CHEMICAL AND BIOLOGICAL COMPONENTS

Fungicide resistance has chemical and biological components. The chemical component is defined by the nature of the active ingredient inhibitor. Fungicides are classified as having multisite inhibitors or single-site — i.e., site-specific — inhibitors (Table 1). Fungicides with multisite inhibitors tend to be among the older compounds. Chlorothalonil is our most valuable multisite fungicide. Once inside a fungal cell, chlorothalonil targets groups of atoms called thiol functional

groups that are common components of many essential proteins. Such proteins regulate thousands of metabolic functions required for fungal growth. In order to overcome the effects of chlorothalonil, thousands of simultaneous changes in fungal protein chemistry would need to occur without interrupting other life functions of the pathogen. The likelihood of that scenario is zero, or nearly as close to zero as biologically possible. Therefore, it can be said with confidence that populations of fungal pathogens are not likely to evolve to the point where they are resistant to chlorothalonil or other multisite compounds.

Almost all modern fungicides are single-site inhibitors — i.e., they disturb only a single metabolic function in the target pathogen. For example, DMI fungicides disrupt the biosynthesis of a single compound called ergosterol. Ergosterol is an essential component of cell membranes in pathogenic fungi. Without ample supplies of ergosterol, fungal growth will stop because of cell membrane failure and turf will recover. Changes in a pathogen's genetic makeup to overcome a fungicide's inhibitory effect on ergosterol biosynthesis will allow fungal growth to continue, rendering DMI active ingredients less effective or ineffective. The likelihood that a single metabolic change will naturally appear in a population is reasonably high, especially if a pathogen produces large populations.

Table 1
Active ingredients categorized as multi-site and single-site inhibitors. Common product names are given in parentheses.

Fungicides with multi-site inhibitors	**Fungicides with single-site inhibitors**
chlorothalonil (Daconil)	azoxystrobin (Heritage)
mancozeb (Fore)	fluoxastrobin (Disarm)
thiram (Spotrete)	trifloxystrobin (Compass)
fluazinam (Secure)	pyraclostrobin (Insignia)
	mandestrobin (Pinpoint)
	metconazole (Tourney)
	myclobutanil (Eagle)
	propiconazole (Banner)
	tebuconazole (Torque)
	triadimenol (Bayleton)
	triticonazole (Triton, Trinity)
	triticonazole (Trinity)
	difenoconazole (B-way)
	fluxapyroxad (Xzempler)
	propamocarb (Banol)
	boscalid (Emerald)
	iprodione (26GT)
	vinclozolin (Curalan)
	fludioxonil (Medallion)
	mefenoxam (Subdue)
	T-methyl (3336, et al.)
	polyoxin (Endorse/Affirn)
	cyazofamid (Segway)
	fluopicolide (Stellar)
	ethazole (Terrazole)
	flutolanil (Prostar)
	chloroneb (Terrachlor)
	penthiopyrad (Velista)
	isofetamid (Kabuto)

The biological component of resistance is a characteristic of the pathogen. Resistance issues are most likely to occur where pathogens produce vast populations. To date, fungicide resistance has been identified in turf pathogens responsible for five diseases: dollar spot, anthracnose, gray leaf spot, *Microdochium* patch and *Pythium* blight. In the northeastern quadrant of the U.S., the dollar spot pathogen, *Sclerotinia homoeocarpa*, is active during the entire season. *Colletotrichum cereale*, the anthracnose pathogen, also will infect over a broad temperature range, and a single infected plant can produce hundreds of thousands of infectious spores. Likewise, vast numbers of aerially disseminated *Pyricularia grisea* spores are produced within individual gray leaf spot lesions. The *Microdochium* patch pathogen, *Microdochium nivale*, is another spore producer that can be active in the Pacific Northwest of the U.S. for nine or more months every year. Finally, under ideal environmental conditions, the *Pythium* spp. infect quickly causing blight, have a short generation time, and can create an abundance of spores during a 12-hour period. The only characteristic common to all of these pathogens is that they produce massive populations.

Several turf pathogens are capable of producing large populations, but not all are equally likely to develop fungicide resistance. Table 2 shows the biological and chemical combinations with confirmed cases of fungicide resistance. All of the pathogens listed in Table 2 generate massive populations of infectious cells, and all of the active ingredients are single-site inhibitors. As new active ingredients are introduced into the turf market, we

Table 2
Disease and fungicide class combinations where resistance has been documented.

Disease name	Fungicides
anthracnose	benzimidazole
anthracnose	DMI
anthracnose	QoI
dollar spot	benzimidazole
dollar spot	DMI
dollar spot	dicarboximide
gray leaf spot	QoI
Microdochium patch	dicarboximide
Pythium blight	QoI
Pythium blight	phenylamide

must remain cognizant that all single-site fungicides are vulnerable to the evolution of resistance in pathogen populations, especially in the five previously discussed pathogens. This is important because SDHI compounds, the newest single-site inhibiting class of fungicide chemistry in the turf market, target two of the most notorious pathogens with known resistance issues: *S. homoeocarpa* and *C. cereale*.

Depending on the nature of the biological and chemical components, resistance expression can be classified as qualitative or quantitative. Qualitative expression means that a pathogen population is comprised of two types, totally resistant and totally sensitive. Quantitative expression indicates that a pathogen population is comprised of numerous types with various levels of fungicide sensitivity. Interpreting qualitative and quantitative expression is important from a practical sense. In the field, quantitative expression — e.g., DMI fungicides against dollar spot — is characterized by a gradual erosion of sensitivity. A fungicide does not completely lose its ability to control a pathogen and, in some cases, satisfactory levels of disease control can be achieved with higher application rates or shorter application intervals. Qualitative expression is characteristic of benzimidazole — e.g., thiophanate-methyl — resistance in *S. homoeocarpa* populations. Where pathogen strains are benzimidazole-resistant, increasing the product application rate or shortening the application interval of benzimidazole will not result in disease control.

CROSS RESISTANCE AND MULTIPLE RESISTANCE

Cross resistance: The term "mode of action" is often used when discussing fungicides. Mode of action refers to the interaction between a fungicide and a pathogen. Fungicides with the same mode of action disturb the same metabolic function in pathogens. For example, all DMI fungicides have the same mode of action — i.e., they all interfere with ergosterol biosynthesis in the same manner. The DMI class of fungicides includes metconazole, myclobutanil, propiconazole, tebuconazole, triadimefon, and triticonazole. Mode of action is an important consideration because when a pathogen develops resistance to one active ingredient in a class of fungicides, it should be expected that it will also develop resistance to the other active ingredients in that class. Resistance within a fungicide class is referred to as cross resistance (Figure 2). Table 3 includes a list of active ingredients grouped in important fungicide classes. It cannot be emphasized strongly enough — pathogen populations that develop resistance to one fungicide active ingredient have the potential to develop resistance to all of the other active ingredients within that class of fungicides.

Multiple resistance: When active ingredients from two or more fungicide classes with single-site inhibitors are used to control a disease, resistance to more than one class of fungicide — i.e., multiple resistance — can occur. A population's natural tendency is to change or adapt to survive in the presence of existential threats. Where single-site fungicides from different classes have been used against crop pathogens, resistance to multiple fungicide classes has developed. As presented in Table 4, observations of multiple resistance have been reported for several turf pathogens. The issue with multiple resistance is not if it will occur, rather how quickly it will occur and what can be done to delay its development.

EVOLUTION OF RESISTANCE IS A TWO-STEP PROCESS

A mutation — i.e., some alteration in the DNA of a pathogen that allows it to grow and infect in the presence of a fungicide — is the first step in developing fungicide resistance. Current scientific thinking holds that the appearance of a fungicide-resistant individual in a population is a natural but rare phenomenon. Since mutation is rare, the likelihood that a resistant individual will appear is a function of population size. As implied earlier, larger populations have a greater chance of harboring an individual with a fungicide-resistant mutation. The Powerball lottery provides a good analogy. If you only purchase a few lottery tickets — representing a small population size — the chance of winning the Powerball is extremely low. If you purchase hundreds of thousands of lottery tickets — representing a much larger population— the odds of winning are considerably improved. With regard to turf disease control, implementing a preventive management strategy to keep pathogen populations low reduces the chance that a resistant strain will materialize. Conversely, the odds of developing a fungicide-resistant strain will increase if large

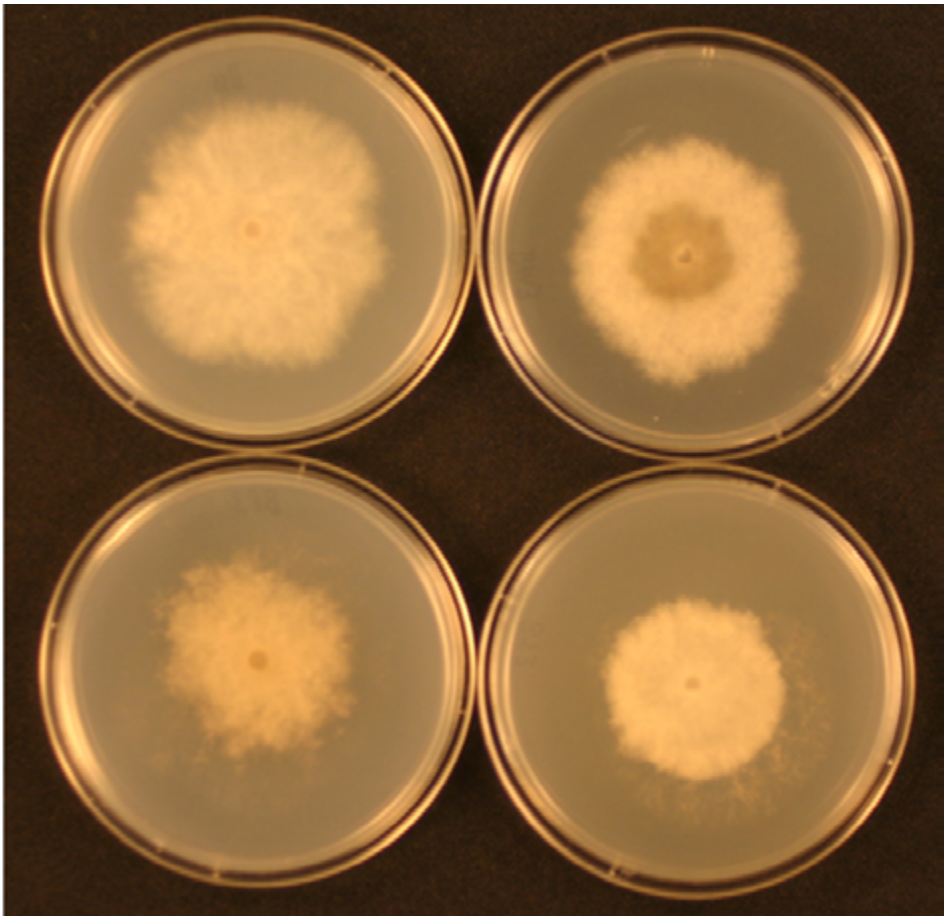

Figure 2 – Applying different fungicides from the same class will not control a cross-resistant pathogen population. In this illustration of cross resistance, colony growth of a resistant isolate of S. homoeocarpa *on culture media amended with 1 ppm of three DMI fungicides (myclobutanil, propiconazole, and triadimefon) is similar to colony growth where no fungicide was included.*

pathogen populations are allowed to develop. For instance, only applying fungicides post-outbreak or curatively without any preventive disease management may allow large populations to develop, increasing the risk of resistance.

The first step towards resistance — i.e., a mutation — is likely a natural occurrence. The second step toward resistance, however, is a human action — applying selection pressure. One fungicide-resistant pathogen cell will not cause a problem on its own; in fact, its existence may go unnoticed. The situation becomes problematic only when a resistant individual reproduces, causing a pathogen population to shift toward fungicide resistance as resistant offspring proliferate. Ultimately, the evolution of fungicide-resistant populations is a function of selection pressure. Selection pressure increases when fungicides from the same class are repeatedly applied. When a fungicide is applied to a pathogen population with resistant individuals, the sensitive strains are neutralized but the resistant strains are still able to grow (Figure 3). Therefore, applying the fungicide provides a competitive advantage to the resistant strain, eventually allowing it to become predominant in the population. As a result, the efficacy of that fungicide or fungicide class will decrease. Selection pressure influences the rate at which a pathogen population may evolve into one that is predominantly resistant. Greater selection pressure hastens the evolution toward resistance. There should be no question that the quickest way to develop a fungicide-resistant population is to exclusively apply fungicides within the same class. During 1980s and 1990s this mistake was often made using DMI fungicides to control dollar spot. Hopefully the mistakes of the past will not be repeated with the newer SDHI compounds.

PERSISTENCE OF FUNGICIDE-RESISTANT STRAINS

The two-step process of resistance evolution establishes that fungicide-resistant populations evolve in response to repeated application of single-site compounds from the same fungicide class. Given that a resistant strain has a competitive advantage in the presence of a fungicide, is there also a competitive disadvantage — i.e., a fitness penalty — in the absence of that fungicide? In other words, if we remove the resistant strain's competitive advantage by discontinuing use of a fungicide, will the population revert back to the original, sensitive type? It is an important question in a very practical sense because if a fitness penalty is attached to fungicide-resistant strains, once-defeated fungicides may again be useful in the future. The answer depends on the fungicide, the pathogen, and the mechanism of resistance at the molecular level. Understanding the fundamental nature of fungicide resistance provides valuable insight into the persistence of resistant strains over time.

MOLECULAR MECHANISMS OF RESISTANCE

For a single-site fungicide to be effective, the fungicide molecule must bind to a fungal protein molecule like a three-dimensional puzzle piece. When binding occurs, enzyme function is disrupted, fungal growth stops, turf recovers, and the disease is controlled. In one resistance mechanism, a fungicide-resistant strain undergoes a change or genetic alteration at the fungicide binding site. The change prevents the fungicide active ingredient from binding to the target enzyme, allowing enzyme function and pathogen growth to continue as normal. When such a change occurs, increasing fungicide rates will have no effect because the target enzyme structure is fundamentally different. This resistance mechanism is referred to as target-site mutation and, in probably

all cases, it is not associated with a fitness penalty. Because these fungicide-resistant mutants have no competitive disadvantage in the absence of fungicide, they are likely to persist over time.

Other resistance mechanisms include overexpression and active efflux. In the case of overexpression, target enzymes in a pathogen are not changed at all. Rather, the pathogen produces the target enzyme in such high quantities that fungicide molecules cannot bind to enough target sites to have a significant impact. Active efflux, on the other hand, is a condition where pathogen cells essentially pump out fungicide molecules faster than they can accumulate to harmful levels. Although the target site remains unchanged, active efflux prevents the fungicide from accumulating to concentrations sufficient enough to cease cell function and pathogen growth.

Where resistance is a function of overexpression or active efflux and the fungicide label allows, at least partial disease control can be achieved by increasing fungicide rates or shortening application intervals (Figure 4). Laboratory research on the mechanisms of resistance supported by monitoring the persistence of resistant and sensitive strains in the field is scarce. However, evidence generated from research on fungicides and crop pathogens suggests that there is a fitness penalty associated with non-target site resistance mechanisms. Although almost all of the work has been conducted on crop pathogens, some insight into the behavior of turf pathogens can be gained because many fungicide active ingredients are used in both systems.

CASE STUDIES: FUNGICIDES FOR TURF DISEASE CONTROL

Benzimidazoles (thiophanate-methyl): There seems to be ample empirical and experimental evidence that benzimidazole resistance in populations of *S. homoeocarpa* and *C. cereale* is the result of target-site mutations and does not carry a fitness penalty. This is supported by numerous studies on crop pathogens, indicating a very high likelihood that benzimidazole-resistant pathogen types will persist over time.

Table 3
Active ingredients and brand-name products in five major fungicide classes, benzimidazole, DMI, QoI, dicarboximides, and SDHI.

Fungicide class	Active Ingredient	Product
Benzimidazole	thiophanate-methyl	Cleary 3336
DMI	metconazole	Tourney
DeMethylation Inhibitors	myclobutanil	Eagle
	propiconazole	Banner Maxx
	tebuconazole	Torque/Mirage
	triadimefon	Bayleton
	triticonazole	Triton
		Trinity
QoI	azoxystrobin	Heritage
Quinone outside Inhibitors	fluoxastrobin	Fame (Disarm)
	pyraclostrobin	Insignia
	trifloxystrobin	Compass
	mandestrobin	Pinpoint
Dicarboximides	Iprodione	26GT
	vinclozolin	Curalan
SDHI	flutolanil	Prostar
Succinate Dehydrogenase Inhibitors	boscalid	Emerald
	penthiopyrad	Velista
	fluxayroxad	Xzempler
	fluopyram	Exteris
	isofetamid	Kabuto

QoI fungicides (strobilurins): Target-site mutations confer resistance to QoI fungicides in the gray leaf spot pathogen *P. grisea*. Research shows that resistance is maintained over several pathogen generations, confirming the absence of any fitness penalty attached to QoI-resistant strains. Cross resistance also has been demonstrated, so resistance to one of the strobilurin fungicides often means resistance to all other fungicides in the strobilurin family. QoI resistance in the anthracnose pathogen *C. cereale* is believed to be the result of a target-site mutation, as suggested by its expression in the field and by evidence generated for some crop pathogens. Although some variation exists in QoI-resistant strains of crop pathogens, there appears to be strong evidence for the absence of a fitness penalty.

Dicarboximides (iprodione): Evidence of iprodione-resistant strains of *S. homoeocarpa* that grow more slowly than sensitive types is thought to indicate a fitness penalty. Similar evidence has been reported for dicarboximide-resistant crop pathogens. However, there are few studies to confirm this in the field, none of which involve turf pathogens. In a field study of *Botrytis* pathogen in European vineyards, dicarboximide use was discontinued after resistance developed. After a few years, dicarboximide was returned to the fungicide rotation and used sparingly — i.e., applied once per season — with some satisfactory results. Molecular mechanisms of resistance to dicarboximides remain unclear; some research suggests

target-site mutations are responsible for the loss of fungicide efficacy while others suggest active efflux is involved.

DMI fungicides: At least three molecular mechanisms of DMI resistance have been identified in a variety of pathogens. Recent research with *S. homoeocarpa* suggests that active efflux and target-site mutation are involved. The active efflux mechanism explains why high rates of DMI fungicides applied at short intervals can result in satisfactory control, especially under conditions of low disease pressure. Experiences with DMI-resistant strains of *S. homoeocarpa* in the field support the notion that resistant types remain high in the population despite the possibility of a fitness penalty. Given that cross resistance has been demonstrated and that two plant growth regulator compounds (flurprimidol and paclobutrazol) have fungistatic activity similar to DMIs, it is most likely that the usefulness of DMI compounds is severely limited once resistant strains become predominant.

SDHI fungicides: Resistance to SDHI fungicides has been studied in numerous crop diseases. In those cases, the resistance mechanism is associated with several target-site mutations. Resistant pathogen populations quickly evolved with extensive use of SDHIs such as boscalid, fluopyram, fluxapyroxad, and penthiopyrad. Cross resistance occurs but is influenced by the type of mutation and the active ingredient's binding strength. In almost all cases there was no fitness penalty associated with SDHI resistance, suggesting that resistant strains will indefinitely remain in the population. To date, SDHI resistance among turf pathogens has not been reported. However, the development of resistance should be expected if selection pressure is increased by exclusively using SDHI compounds. Lessons learned from other crops suggest long-term survival of SDHI-resistant pathogen strains.

Multiple Resistance: The long-term survival of multi-resistant strains of *S. homoeocarpa* and *C. cereale* is influenced by the resistance mechanisms involved and the continued use of the ineffective fungicides. Ultimately, the survival ability of multi-resistant strains remains uncertain. Results of crop disease research that monitored the frequency of fungicide-resistant and fungicide-sensitive strains over time suggest a measure of fitness penalty associated with multiple resistance — i.e., pathogen types with multiple resistance declined in the absence of fungicides during winter but did not disappear from the popu-

Table 4
Documented cases of cross resistance and multiple resistance for turf fungicides

Cross Resistance

Disease Name	Fungicides
anthracnose	QoI class, azoxystrobin and trifloxystrobin
dollar spot	DMI class, myclobutanil, propiconazole, triadimefon
gray leaf spot	QoI class, azoxystrobin, trifloxystrobin
Microdochium patch	Dicarboximides, vinclozolin, iprodione

Multiple Resistance

Disease Name	Fungicide Classes
anthracnose	QoI class and benzimidazoles
dollar spot	DMI class and benzimidazoles
dollar spot	Dicarboximides and benzimidazoles

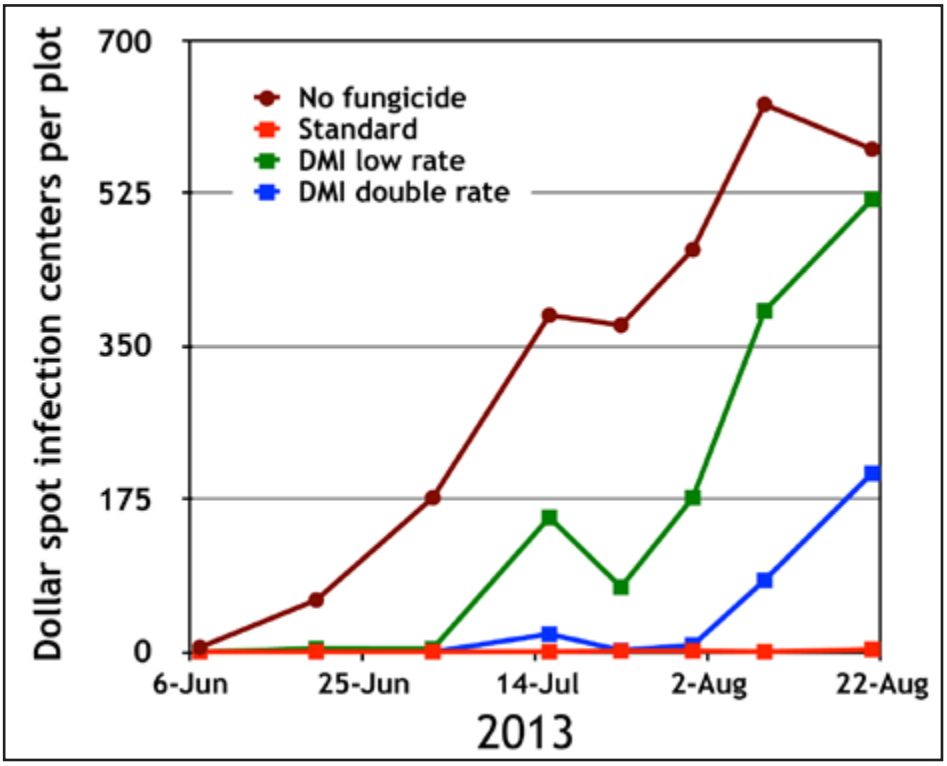

Figure 3 – Applying higher rates of a fungicide to a moderately resistant population can provide some control, but using a different fungicide class yields better results. A DMI fungicide was applied at low and high rates at two-week intervals on a site inhabited by a moderately DMI-resistant strain of S. homoeocarpa *beginning on June 1. In the DMI-treated plots, dollar spot severity was reduced compared to the check plots, but it was greater than a standard treatment that included chlorothalonil and a non-DMI fungicide.*

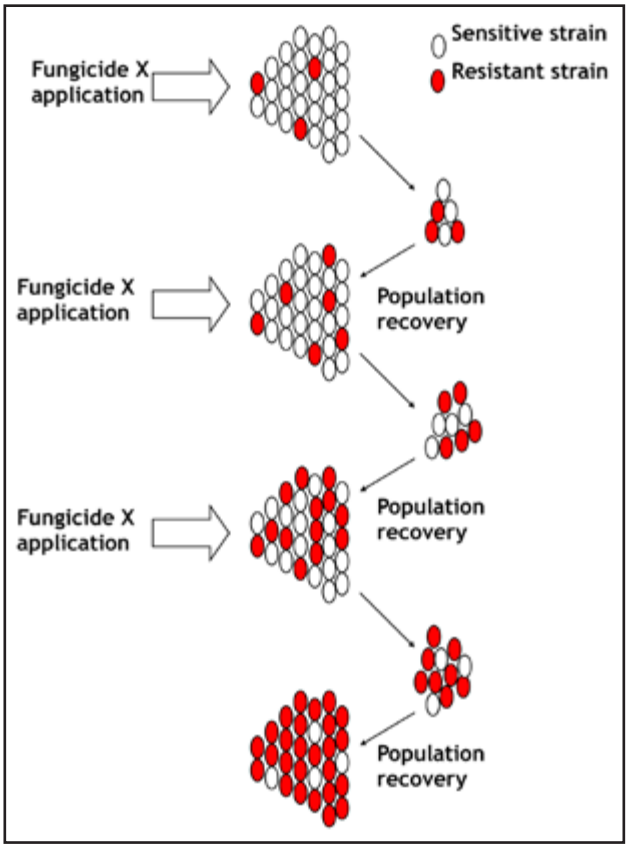

Figure 4 – Making repeated applications of the same fungicide gives a competitive advantage to resistant strains, allowing them to become more prevalent. In this simple representation, repeated application of the same fungicide (Fungicide X) provides a competitive advantage to the fungicide-resistant strain over time. Growth of sensitive strains is limited, but resistant strains continue to multiply to the point where they make up the majority of the population.

lation. The fitness penalty allows the possibility for once-ineffective fungicides to be returned to a fungicide rotation, provided they are used sparingly — i.e., applied once per season — after being discontinued for a year or longer.

A PRACTICAL APPROACH TO FUNGICIDE RESISTANCE

Eventually, a comprehensive understanding of resistance mechanisms will guide the use of fungicides for turf disease control. Until then, disease control should be based on a simple strategy to manage the two-step process that drives the evolution of resistance. Since mutations toward fungicide resistance are more likely to occur in large populations, tactics designed to keep pathogen populations low can help avoid resistance. Exercise all non-chemical options to control fungal populations. Preventive disease control is encouraged, especially programs that do not involve fungicides with single-site inhibitors. Fungicides with multisite inhibitors are also important tools because they decrease the likelihood of resistant pathogen populations. Examples of fungicides with multisite activity for dollar spot control include chlorothalonil and fluazinam. Limiting the use of single-site compounds, or tank mixing them with multisite products, will greatly reduce the rate at which resistance develops. Finally, and most importantly, avoid the exclusive use of one fungicide class to control a disease. Until experimental evidence convinces us to do otherwise, rotating among different fungicide classes is in our best interest.

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RICHARD LATIN, PH.D., *is a professor of plant pathology at Purdue University.*