Development of fungicide resistance or tolerance on Illinois Golf Courses

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Over the past few years, I have observed several instances of disease control failures on golf courses in Illinois that can be attributed to development of fungal resistance or tolerance to certain systemic fungicides. In most cases, this has resulted from long term, repeated use of these fungicides under conditions of high disease pressure and highly susceptible grasses. First, let me define what I mean by resistance or tolerance. In this report, ''resistance'' refers to situations where there is near total to total loss of control under field conditions, accompanied by little or no sensitivity of fungal insolates to the fungicide(s) **in vitro**; ''tolerance'' is where there is some measurable loss of control in the field (not total), accompanied by moderately reduced sensitivity **in vitro** when compared to other strains of the same fungal species.

In order to determine the relative sensitivity of suspected resistant/tolerant fungi, I conducted standard **in vitro** toxicity tests called radial growth assays. These assays are carried out by incorporating known concentrations of a fungicide in agar media, then growing the fungi on the media and measuring their growth responses over a range of concentrations. From this, one can estimate an ''EC50'' value (effective concentration to reduce the growth rate by 50%), which is very much like an LD50 value (lethal dose for 50% of the population). The higher the EC50, the more resistant the fungus is to the fungicide **in vitro**, and gives a strong indication of **in vivo** or field resistance. Following are the results of the **in vitro** tests.

Pythium/Subdue. Possible metalaxyl (Subdue) resistant Pythium blight was observed on a fairway at the Knollwood Club in August of 1988 followed Subdue applications. Samples of diseased grass were collected and yielded a fast growing **Pythium** spp., later identified as **P. aphanidermatum**. EC50 values were calculated from radial growth assays as presented in Table 1. **Pythium** isolates from the Knollwood site were capable of growing at 500 ppm. The calculated EC50 value for this isolate was approx. 350 ppm; a sensitive isolate would have an EC50 value of around 1-2 ppm. These values are similar to those reported by Pat Sanders and coworkers at Penn State (**Plant Disease** 74:690-692). I have not **documented** any other case of Subdue resistant Pythium blight in northern Illinois, and this fungicide remains an effective control at most golf courses. (I have heard several anecdotal accounts of Pythium blight control failures, but I believe that most of these were probably due to misapplication of Subdue or misdiagnosis of the disease, and not due to resistance).

Table 1. Subdue resistant Pythium blight at the Knollwood Club, 1988.

	Per cent of control growth rate					
concn(ug/ml)	10	50	100	250	500	EC50 (ppm)
resistant	100	100	92	76	28	>300
sensitive	25	0	0	0	0	<5

(cont'd. page 8)

(Fungicide Resistance cont'd.)

Anthracnose/benzimidazoles. We have come to recognize the basal stem, crown, and root rot phases of anthracnose to be of major importance on **Poa annua** and creeping bentgrass in Illinois. Only systemic fungicides such as the benzimidazole derivatives (Tersan 1991, Fungo 50, Cleary 3336) and ergosterol biosynthesis inhibitor (EBI) products (Banner, Bayleton, Rubigan) have provided control; contact products such as chlorothalonil have not been as effective. However, in 1991 two cases of failed anthracnose control by benzimidazole products were observed. Both golf courses have long term histories of benzimidazole use. Isolates of **Collectotrichum graminicola** from diseased areas were tested **in vitro** for resistance/tolerance to benomyl (Table 2). EC50 values were 100 times higher for the resistant isolates than for isolates from other area courses with no history of benzimidazole resistance. On both courses where benzimidazoles failed to control anthracnose, applications of Banner or Rubigan controlled the disease; also, **in vitro** tests of these isolates illustrates their sentivity to these EBI fungicides (Table 3).

Table 2. Resistance to benomyl among isolates of C. graminicola and "Sclerotinia" (dollar spot).

	Tersan 1991 (ppm a.i.)	
Anthr.	EC50	EC95
MMGC	>50	>100
PaH	>50	>100
RFor	<.5	>10
Thg	<.5	1
D. Spot		
Rmr B1	9	>50
Rmr B3	5	25
Srd 01	<.5	<.5

Key: MMGC=Manteno Muni. GC; PaH=Palatine Hills GC; RFor=River Forest GC; Thg=Thorngate CC; Rmr=Ridgemoor CC Srd=Sunset Ridge CC; MMGC, Rmr, and Srd are from bentgrass, all others from Poa annua.

Anthracnose/triadimefon. I have also observed quite a difference among the EBI fungicides and their ability to control anthracnose. Bayleton (triadimefon) does not control the crown or stem base infections as well as Banner or Rubigan. This has also been observed in Wisconsin for anthracnose and "Poa decline" by Prof. Gayle Worf (**The Grass Roots** Vol. XIX, no. 6, pp. 23-25). I examined several **C. graminicola** isolates from courses where Bayleton had not provided adequate control and from courses where Bayleton had not been used. The results (Table 3) indicate that, regardless of prior exposure, Bayleton is less toxic **in vitro** to **C. graminicola** than other EBI fungicides. (This includes the experimental EBI fungicide Lynx, which is a Mobay product that is a close structural analog to Bayleton.) This reduced toxicity probably accounts for the mixed results we see in the field with Bayleton on anthracnose. There is no evidence of a developing tolerance to other EBIs among anthracnose fungi.

Table 3. Toxicity of Bayleton and other EBI fungicides to Colletotrichum graminicola.

	Approximate EC50 (ppm a.i.)			
	Bayleton	Lynx	Banner	Rubigan
MMGC (2)*	5-8	.3-.5	.3-.5	.3-.5
PaH	4-5	.3-.5	.3-.5	.3-.5
RFor (2)	4-5	.3-.5	.3-.5	.3-.5
Thg	6-7	.3-.5	NT	NT
Mdl, BBr	10-15	NT	NT	NT

*number in parenthesis indicates number of isolates tested per site. NT = not tested

Key: MMGC (Manteno - 1991); PaH (Palatine Hills - 1991); RFor (River Forest - 1991); Thg (Thorngate - 1987); Mdl (Midlane -1987); BBr (Bonnie Brook - 1987); MMGC is from bentgrass, all others are from Poa annua.

(cont'd. page 9)

(Fungicide Resistance cont'd.)

Dollar spot control failures at Ridgemoor C.C. Over the past three seasons, dollar spot disease has become increasingly difficult to control with EBI fungicides on the bentgrass fairways at Ridgemoor CC. Fungicide efficacy plots were established on a fairway in 1991 by researchers from Ciba-Geigy Corp. (Dr. C. Pearson, Field Research Repr.) which demonstrated reduced control of EBI fungicides — especially in terms of length of residual control. EBI fungicides have been used to control dollar spot at this site since the registration of Bayleton in 1982, primarily in tank mixes with contact fungicides such as chlorothalonil, thiram, and anilazine. By 1988, residual control was notably diminished; by 1991 control was unacceptable for all registered EBI products. In order to test for resistance or tolerance of the dollar spot fungi to EBI fungicides, several isolates were collected from Ridgemoor and compared to isolates from two other area golf courses (Shoreacres and Sunset Ridge CC) where no control problems have been observed.

The results of **in vitro** toxicity tests appear in Table 4. Isolates Rmr B1 and Rmr B3 were taken from an affected fairway at Ridgemoor. These isolates were, in general, five to ten times less sensitive to EBI fungicides than "wild type" isolates, even though EC50 values were less than 1 ppm for all isolates. (I would call this "tolerance".) Note that isolate Rmr R3, which was from an untreated **Poa pratensis** rough at Ridgemoor, was more sensitive to EBI products than the fairway isolates. Also, I found that greater than 25 ppm active ingredient of Bayleton and Rubigan are required to totally inhibit growth of the tolerant Rmr isolates, a concentration which the fungi are unlikely to face in nature. **In vivo** studies of these dollar spot isolates may demonstrate a greater difference between tolerant and sensitive types, since the actual process of infection of the host would be taken into account. These studies are being conducted by Ciba-Geigy.

Table 4. Tolerance of dollar spot fungi to selected EBI fungicides based on EC50 and EC95 values.

	Approximate EC50 and EC95 (ppm a.i.)							
	Banner		Bayleton		Lynx		Rubigan	
	EC50	EC95	EC50	EC95	EC50	EC95	EC50	EC95
Rmr B1	.25	3-5	.4-.6	>25	.25	<5	.6-1	>25
Rmr B3	.30	10	.6- 1	>25	.25	<5	.6-1	>25
SATD	.20	3-5	.1-.2	<5	NT	NT	.2-.3	2.5
Rmr R3	.05	1-2	.1-.2	<5	.15	<1	.2-.3	2.5
Srd 01	.05	1-2	.1-.2	<5	.1	<1	<.1	10

Key to isolates: Rmr B1,B3 (Ridgemoor CC, bent fairway)
Rmr R3 (Ridgemoor CC, bluegrass rough)
SATD (Shoreacres, bent fairway)
Srd 01 (Sunset Ridge CC, bent fairway)

It is also interesting to note that benzimidazoles failed to control dollar spot at Ridgemoor in the mid to late 1970's, which was the primary motivation to switch to Bayleton and other EBI products for dollar spot control in fairways. Even though little if any benzimidazole products have been applied to Ridgemoor fairways since 1978, the EBI tolerant fairway isolates from 1991 remain resistant to fairly high concentrations of benomyl (Table 2). Apparently, benzimidazole resistance in dollar spot fungi is fairly stable and does not lead to reduced "fitness" of resistant populations.

Avoiding resistance/tolerance problems. Systemic fungicides are advantageous for us to use because of their (generally) low application rates and long-term control. Unfor-

(cont'd. page 10)

(Fungicide Resistance cont'd.)

tunately, some of the systemic fungicides we use are single-site inhibitors, i.e. there is only one very specific site of action that confers toxicity to fungi. Therefore, only a single gene (point) mutation could alter the sensitivity to a single-site fungicide and allow a fungal population to develop resistance.

Repeated, exclusive use of a single-site fungicide places a large selection pressure on fungal populations for development of resistant or tolerant mutants. To avoid high selection pressure, the best practice (in my opinion) would be to alternate single-site fungicides with unrelated multi-site fungicides. Also, I would not use the single-site product more than once or twice per year. Examples of single-site fungicides are Subdue and the benzimidazoles (Tersan 1991, Fungo 50, Cleary 3336). Tolerance of fungi to ergosterol inhibitors, Dyrene, and Chipco 26019 has been reported for turf or other crops; these products may also be single-site inhibitors. Please note that not all systemic fungicides are single-site inhibitors, and not all contact fungicides are multi-site inhibitors.

Avoiding resistance is an often complex and controversial topic, and would probably be better addressed in more detail in another article.

Underground Storage Tank Regulations

by Steve Berning

Underground storage tanks (USTs) are regulated under subtitle C of the Hazardous and Solid Waste Amendments (HSWA) to the Resource Conservation and Recovery Act (RCRA) of 1984. The act imposes standards on all owners and operators of new and existing USTs containing petroleum and other regulated substances. The standards address tank design, construction, upgrading of existing tanks, operation and maintenance, spill and leak cleanup and closure.

What You Have to Do

It is not difficult to comply with the UST regulations. USTs of unknown age and those installed in 1979 and older, must be tested by December, 1992. The 1988 and prior USTs must be tested by December, 1993. These USTs must be tested **annually** thereafter to assure that they are not leaking. This is all you have to do with an outside contractor.

Tank levels should be measured with a stick and reconciled every month against usage records for each tank; look for discrepancies which may indicate a leak. In addition, you should supervise fuel delivery so as to assure that the quantity will fit in the UST space available.

What Kind of Contractor Do You Look For

As with any vendor, you should consider such things as experience, promptness, professionalism, and reputation. Don't hesitate to ask for and call references. You may also want to ask potential contractors about their operating philosophy and determine whether they support your industry association.

With regard specifically to USTs, contractors are required to register with the state, use certified equipment for tank testing,

(cont'd. page 12)