

the Wisconsin and Northern Michigan superintendents will remember him from the Northern Great Lakes Golf Course Superintendents conference several years ago when he spoke about internet resources for turf management. For the past several years Doug has been interested in trying to quantify turf characteristics such as color and groundcover using digital imaging as a replacement for our conventional methods of simply "rating" the turf. Turf ratings require a trained investigator to achieve consistent evaluations, and many people simply are not suited to conducting consistent evaluations. Even trained evaluators can rate the same turf differently depending on the day, amount of cloud cover, personal fatigue, etc.

Doug has now developed a system using easily obtained components that allows rapid and repeatable (thus reliable) collection of turf color and cover data. The system uses a digital camera (a medium-level camera is sufficient), either a desktop or laptop computer, and SigmaScan software. A suitable camera will cost from \$500 to \$1000 and the SigmaScan software about \$1000. With this system, the user takes a digital photograph of the turf then downloads the image into the SigmaScan software. The software is used to compute the angle, hue, and saturation (brightness) of the turf as a quantitative unit of color, similar to how paint

manufacturers and photographers specify their colors. Alternatively, the user can assign one color (e.g., red) to the turf in the photo and a contrasting color (e.g., blue) to the ground or soil in the photograph, and SigmaScan will compute the percent ground actually covered by turf. Historically this has been a laborious, time-consuming process with the user having to complete several functions for each analysis. However, Doug has developed a "macro" command which users can get from him free of charge that frees the user from having to complete multiple functions for each analysis, allowing hundreds of pictures to be quickly analyzed. This work is important before it's practical to use digital imagery on golf courses to measure disease, nutrient response, drought stress, and other aspects of turf for decision-making purposes. We are starting to use the technology in some of our work to quantify the establishment rate of different grass varieties. At some point I expect the system will be sensitive enough to distinguish weeds from desirable grasses, giving superintendents accurate data to justify use of an herbicide.

Dr. Scott McElroy is a weed scientist from the University of Tennessee. While his presentation was especially geared to researchers, I thought it contained some information of interest to superintendents. He has been interested in why some turfgrasses tolerate herbicides better than others: mesotrione herbicide, for example, bleaches the color from creeping bentgrass but doesn't harm Kentucky bluegrass. In sensitive plants, mesotrione causes the loss of chlorophyll and subsequent use of the sun's energy in photosynthesis: the excessive solar radiation results in the formation of free radicals which disrupt cell membranes and kill the plant. Carotenoids are orange pigments in plants which help funnel the energy from sunlight into the photosynthetic process and also "quench" free radicals such as singlet oxygen, preventing them from damaging cells. Compounds which can prevent damage from free radicals are known as antioxidants. People are often encouraged to eat more fruits and vegetables such as spinach because of their antioxidant content. Scott has used high pressure liquid chromatography to identify and quantify carotenoids in turfgrasses. Some warm-season grasses will be bleached by an initial application of mesotrione, but are not killed. When new tissue is treated with mesotrione, it does not seem to be harmed. Scott's research shows that this new tissue contains higher levels of carotenoids which appear to prevent mesotrione from damaging the grass. This type of information is useful to breeders who can then identify genes responsible for the response and breed them into desirable grasses. While I doubt people will start eating creeping bentgrass anytime soon, Scott

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showed that turfgrasses can have a carotenoid content as high as spinach!

My own presentation was on ways to measure low temperature tolerance and injury to turfgrasses. With the routine loss of turf, particularly *Poa annua*, each winter we are fortunate enough to have newer technologies such as video thermography and chlorophyll fluorometers to measure turf response to low temperatures. Video thermography is a system we've used in the past to show how turfgrass plants freeze. An infrared camera is connected to a video recorder, and used to "watch" grass plants freeze when they are placed in a freezing chamber. Our work has shown that small roots freeze first, after which freezing progresses rapidly through the root system then into the lower crown which is the site of new root formation. The upper crown where shoots form, and the shoots and leaves themselves, are the last tissues to freeze. This work has shown why we occasionally will have green grass emerging from winter, only to see it die several weeks into spring as late winter freezes have killed the root systems while leaving the shoots and upper crowns undamaged (Stier and Filault, 2000; Stier et al., 2003).

Determining the actual cold tolerance of a grass is time-consuming as the grasses have to be properly cold-acclimated, frozen, then regrown. A single experiment can take 4-12 months. Fluorometers are sophisticated machines that allow us to measure the efficiency of photosynthesis, and identify potentially harmful agents such as low and high temperatures. A burst of light is used to saturate a leaf or leaves. Since not all of the light energy can be used in photosynthesis, some of the energy is "fluoresced" just as fluorescent paint works on a watch in the dark. Fluorescence levels rise as stress increases and photosynthetic efficiency decreases. We are finding that cold tolerant grasses such as Kentucky bluegrass have photosystems that are more responsive to low temperatures than grasses with poor cold tolerance such as perennial ryegrass. Fluorometry may allow us to rapidly identify annual bluegrass ecotypes with superior cold tolerance which can then be used in breeding programs, providing a grass which is just as competitive with the weedy type of annual bluegrass but with much better cold tolerance.

I don't expect that all of these techniques will necessarily become common tools for golf course superintendents. Devices like the fluorometer, which currently cost \$15,000-\$20,000 depending on options, are unlikely to have a routine use for golf course maintenance. However, technology such as ground-penetrating radar will likely become a contracted service by which superintendents may have their fairways "mapped" to reveal problem areas which can then be

properly handled to improve course conditions. Devices such as the soil moisture probes and digital analysis are available now. At least one superintendent in the Madison area has started using the soil moisture probe on a daily basis to monitor moisture levels in each putting green. Stay tuned for future techno-advances!

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- Stier, J., and D. Filault. 2000. Mechanisms of tolerance of freezing in turfgrass. *Golf Course Mgt.* 68(10):76-79.
- Stier, J.C., D.L. Filault, and J.P. Palta. 2003. Visualization of freezing progression in turfgrasses using infrared video thermography. *Crop Sci.* 43:415-420. ♣

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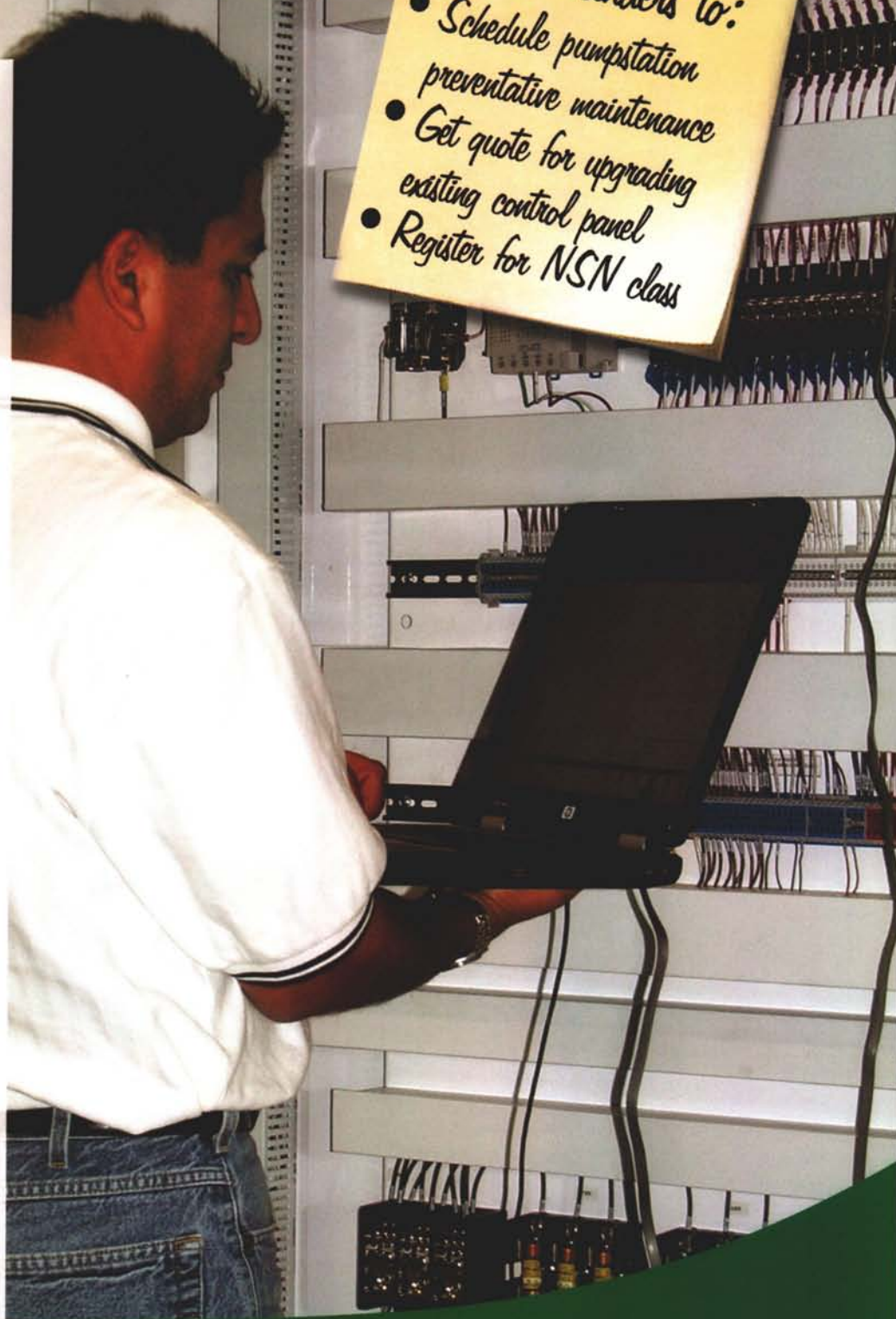
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Fungicide Resistance in Turfgrass - Current Ideas and Research



By Paul Koch, Turfgrass Diagnostic Lab, University of Wisconsin - Madison

Author's note: This is the second in a three-part series looking at fungicide resistance in turfgrass.

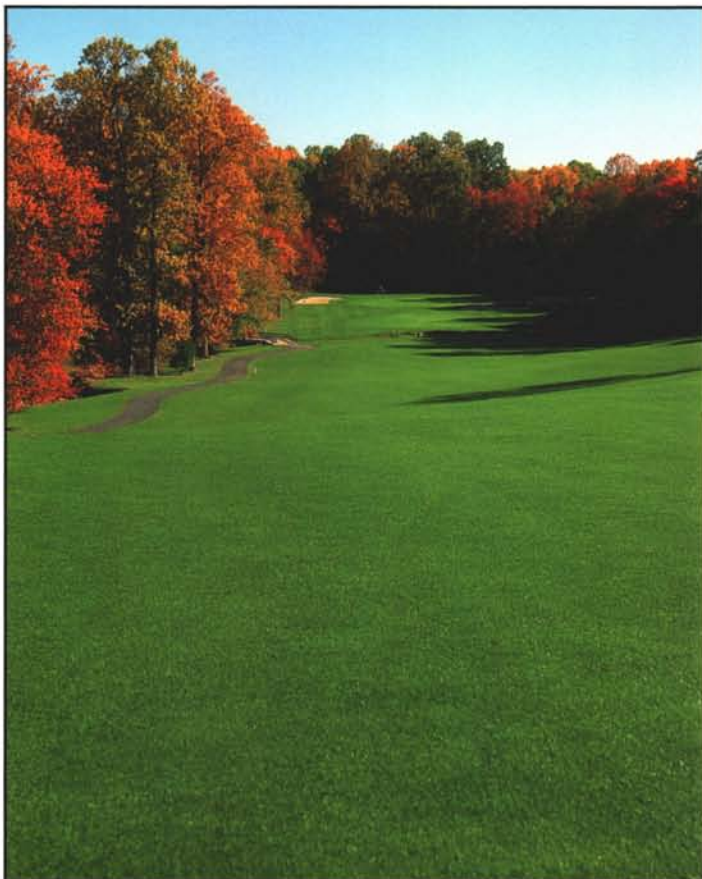
In the May/June 2007 issue of *The Grass Roots*, I briefly reviewed the past history of fungicide resistance as it pertained to turfgrass, as well as the different selection pressures employed on fungal organisms by the benzimidazole, DMI, and strobilurin classes of fungicides (Koch, 2007). But in recent years, as researchers have continued to study fungicide resistance, conflicting results and ideas have failed to greatly further our understanding of fungicide resistance.

A golf course environment is a particularly confusing site to try to manage fungicide resistance. In traditional agriculture, great pains are taken to make the field as uniform an environment as possible. In turfgrass, and especially golf course maintenance, just the opposite is true. In accordance with how the game of golf is played the course is divided up into several

distinct areas, each with a significantly different maintenance regime.

Putting greens are mowed daily during the growing season at heights around an eighth of an inch (3.2 mm). Daily mowing removes dew from the turfgrass leaf blade every morning, significantly reducing the duration of leaf wetness and reducing disease pressure (Williams *et al.*, 1996). Due to the extreme value most golf courses put on their putting greens, fungicides are applied routinely to prevent any disease from blemishing their investment. These factors suggest a relatively low amount of disease pressure when compared to other areas of the golf course.

Fairways are mowed approximately three times per week at heights of approximately half an inch (13 mm). Mowing three times per week removes the dew off those leaf blades in the morning only on those days, significantly increasing the duration of leaf wetness and making the leaf surface more conducive to



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disease infection. The increased height of the fairway turf results in a denser canopy, trapping moisture and exudates from the turf at the ground level and increasing ground-level humidity (Giesler *et al.*, 2000). Fungicides are expensive to purchase, and with the large acreages that golf course fairways can occupy, many clubs may opt to spray fairways less often with fungicides in an attempt to reduce costs. This suggests an increased level of disease pressure when compared to putting greens. Golf course tee boxes are often maintained in a similar fashion to golf course fairways.

The rough areas of a golf course are managed quite differently from either a putting green or a fairway. Golf courses in the temperate regions of the world usually have roughs that consist of Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), and/or fine fescues (*Festuca* spp.). They are mowed two or three times per week at heights of approximately 2 inches (5 cm). Many golf courses do not irrigate their rough areas, and those which irrigate do not do so every day. Fungicide applications are seldom applied to roughs due to the extreme cost, even though dollar spot and other diseases can damage these areas. Disease pressure on roughs may be lower than a fairway or green due to its species composition and lack of irrigation, but significant disease infections can occur in optimum environmental conditions.

With such different environments it is likely that a significant variation in disease pressure would also be observed, and we do see this most noticeably with *Sclerotinia homoeocarpa*, the causal agent of dollar spot. With differences in disease pressure amongst sites within a single golf course, would it also be fair to assume that there is variation amongst the rate of fungicide resis-

tance development at these same sites? This was the major objective of a study done by myself and Dr. Geunhwa Jung, former turfgrass pathologist at the University of Wisconsin - Madison who is now at the University of Massachusetts - Amherst.

Materials and Methods

The study was done during the summer of 2006. Six golf courses in Wisconsin and one golf course in Massachusetts were selected for *S. homoeocarpa* sampling to represent a range of course ages, fungicide history, and current fungicide application frequency (Table 1). Indicator areas or areas of high dollar spot disease pressure on one putting green, one fairway, and one rough area were chosen for isolate collection at each golf course. A 10 X 10 m grid was kept free of pesticide applications until our isolate collection was complete. Only courses A, B, and C did not apply fungicides to the putting green sampling grid throughout the season, allowing *S. homoeocarpa* isolates to be collected from

all three course areas only from those golf courses. Isolates were collected from the fairway and rough sampling grids at the remaining four golf courses. Isolates were sampled throughout the summer of 2006 as symptoms became present at each site.

A total of 900 leaf blades showing lesions symptomatic of dollar spot were collected from courses A, B, and C with 100 collected from one putting green, one fairway, and one rough area at each course. A total of 800 symptomatic leaf blades were collected from courses D, E, F, and G with 100 samples collected from one fairway and one rough area at each course. Each sample collected was at least one meter apart. Samples were processed for pathogen isolation within 24 hr of collection.

These isolates were then subjected to an *in vitro* fungicide resistance assay, which in this particular case is a laboratory measurement of how well each isolate grows on fungicide-amended potato dextrose agar (PDA) media

Table 1. Golf course age, location, and fungicide history for each course used for *Sclerotinia homoeocarpa* isolate collection.

Golf Course	Location	Year Opened	Five year benzimidazole history ^x	Five year DMI history ^x
A	WI	2003	None	Moderate on greens Moderate on fairways
B	WI	1958	None	Intensive on greens Sporadic on fairways
C	WI	1967	Sporadic on greens None on fairways	Moderate on greens Sporadic on fairways
D	WI	1921	Moderate on greens Sporadic on fairways	Intensive on greens Intensive on fairways
E	WI	1991	None	Intensive on greens Moderate on fairways
F	WI	2001	Sporadic on greens None on fairways	Intensive on greens Moderate on fairways
G	MA	1916	None on greens Sporadic on fairways	Moderate on greens Moderate on fairways

^xIntensive = >15 applications over the past 5 years, moderate = 5-15 applications over the past 5 years, and sporadic = < 5 applications over the past 5 years

compared to un-amended PDA media. Resistance assays were done using two different active ingredients from two separate fungicide classes. Cleary's 3336 (thiophanate-methyl) was selected to represent the benzimidazole class of fungicides, while Banner MAXX (propiconazole) was selected to represent the demethylation inhibitor (DMI) class. Because of the directional selection employed by propiconazole, growth of the fungus on the fungicide-amended media was measured with a ruler and taken as a percentage of the growth of the fungus on un-amended media (Figure 1). The disruptive selection of the thiophanate-methyl allowed a simpler rating system; if the fungus was growing at all on the media amended with thiophanate-methyl than it was rated with the number one, where if there was no growth than it was rated with a zero. Statistical analyses were performed on the data to determine significant relationships.

Results and Discussion

With over 1,400 isolates of *S. homoeocarpa* subjected to *in vitro* fungicide resistance assays, the data set was large and very complex. But there are three important observations that were made from this study that can be used as superintendents develop their fungicide program for 2008.

The first and most telling observation was that there were significant differences in the level of fungicide resistance of both DMI and benzimidazole fungi-



Figure 1. This is an example of the process used for rating the level of resistance to DMI fungicides. The plate in the middle is growing on media not amended with fungicide, while the outside four plates are growing on media amended with propiconazole. The lack of growth of the two on the right suggest it is sensitive to the fungicide, while the significant growth of the two on the left suggest a certain level of resistance to propiconazole.

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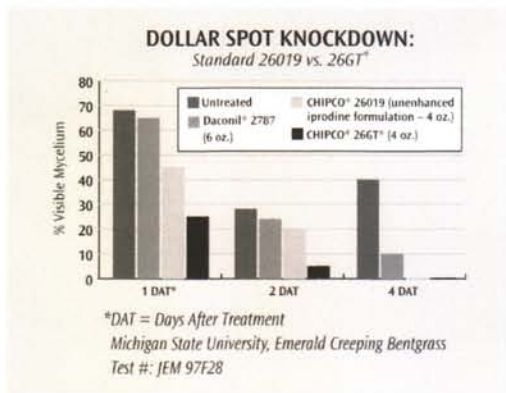
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cides based on if the isolates were collected from a putting green, fairway, or rough area. When dealing with resistance to propiconazole this roughly correlated with fungicide history, as those sites that tended to have more intensive fungicide application histories had higher levels of resistance. For example, the majority of the *S. homoeocarpa* population collected from the rough area of course "A" had very low levels of resistance to propiconazole. On the fairways of course "A", there was a shift in the *S. homoeocarpa* population to where the majority of the population now had higher levels of resistance to propiconazole, and on the greens the population shifted further towards increased resistance (Figure 2). This is not surprising in the sense that it agrees with the traditional model of fungicide resistance where the more fungicide applications made at a site equals a higher level of resistance. But with thiophanate-methyl the data seemed to suggest that higher proportions of the *S. homoeocarpa* population were resistant in the fairways regardless of the fungicide history, though the data was much more inconclusive on this point.

The second important observation from this study was that proportions of *S. homoeocarpa* populations resistant to both fungicides could generally be linked to their five year fungicide history. Those populations that received what I termed an intensive level of fungicide applications over the last five years generally had a higher proportion of resistant isolates than those populations with a moderate fungicide history, and always had a higher proportion of resistant isolates than those sites that received a sporadic level of fungicide applications. There was at least one major exception to this rule though. Isolates collected from the fairway at Course D received twice as many fungicide applications over the last five years as those collected from the fairway at course G, but the level of resistance in the population was significantly lower at course D than course G. After inspecting the fungicide records at these two courses, the only notable difference in the two spray schedules was the spray interval. Course D kept a fairly tight spray interval on their fairway of 14 days, while course G oftentimes let its interval go to 21 or even 28 days. This observation supports a traditional recommendation of fungicide resistance management that suggests that spray interval can affect the buildup of isolates with reduced sensitivities to fungicides.

The third important observation from this study was that to keep the proportion of the *S. homoeocarpa* population resistant to fungicides at or near baseline levels, an average of one fungicide application a year or less was required. The rough areas at five of the seven golf courses in this study were used as a baseline due to the very low and statistically similar resis-

tance levels found at those courses. And the only two populations that had resistant proportions statistically similar to the baseline populations were the fairway populations from course B and C, where a total of five fungicide applications were made in the past five

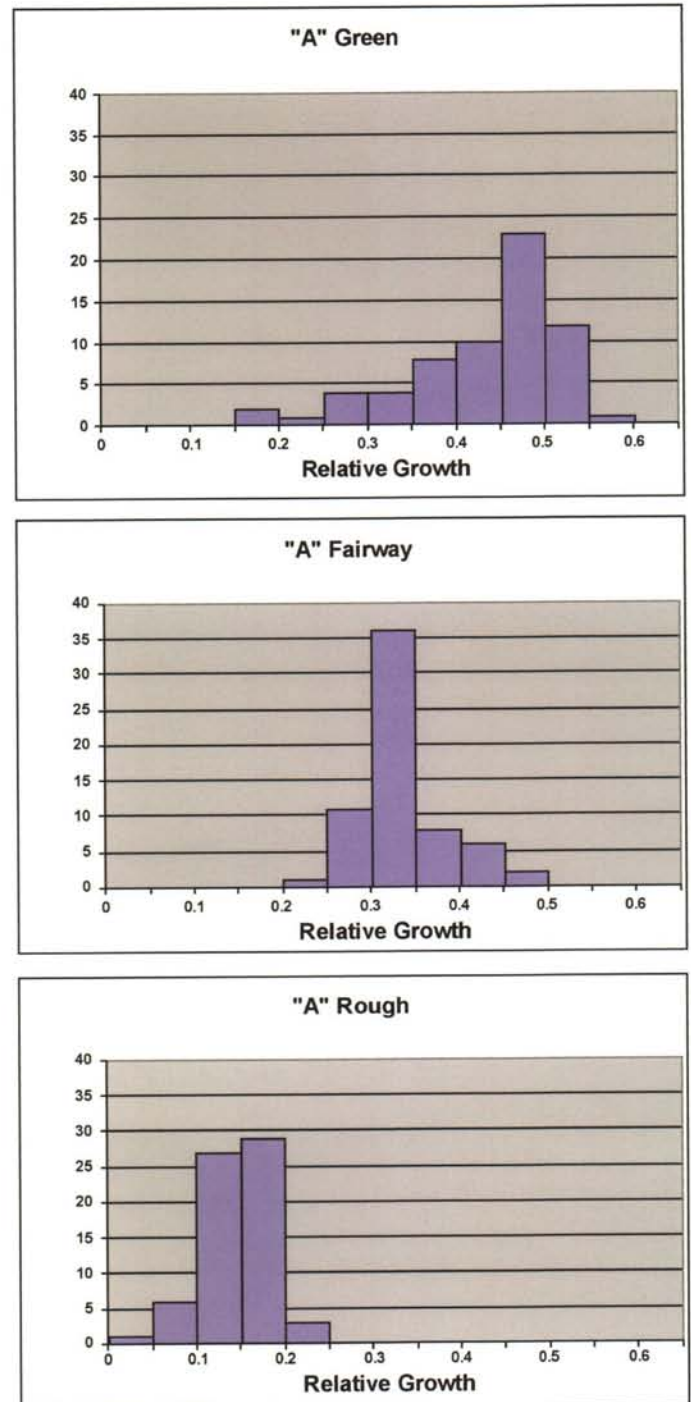


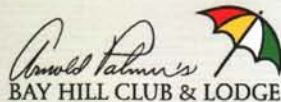
Figure 2. Histograms of *Sclerotinia homoeocarpa* populations obtained from a putting green, fairway, and rough area at course "A". Where applications of DMI fungicides were more frequent, the frequency of isolates with high mean relative mycelial growth values on propiconazole-amended media increased.

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