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EFFECT OF AZOXYSTROBIN ON DOLLAR SPOT DISEASE DEVELOPMENT IN CREEPING BENTGRASS (AGROSTIS STOLONIFERA) AND KENTUCKY BLUEGRASS (POA PRATENSIS)

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ABSTRACT

Dollar spot disease, caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett is common on intensively managed creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.), and other turfgrasses. Frequent chemical applications during the growing season are made to control this disease. Previous observations state that application of fungicides such as azoxystrobin can increase the level of disease within the same growing season. The purpose of this study was to quantify the extent of disease increase caused by an application of azoxystrobin the previous year. In three field tests on creeping bentgrass between 2005 and 2007, a single application of azoxystrobin in the late fall was found to increase the incidence of dollar plot several fold on treated plots in the following summer. In addition, this study also found a slight enhancement of dollar spot incidence on Kentucky bluegrass (*Poa pratensis* L.), which has not been previously reported. Although the cause of this disease enhancement is not known, the physical and chemical characteristics of azoxystrobin, along with turfgrass cultural practices and the finding of the longer term effect from this study, implies that the phenomenon stems from non-target effects on microbial populations of leaves or soil rather than a direct long term effect on the plants. Studies on phyllosphere communities may shed light on this phenomenon and point out directions for future research.

Keywords: enhancement, fungicide, resurgence, non-target, pathogen

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INTRODUCTION

The turfgrass disease dollar spot is caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennett. It is widespread on cool season grasses worldwide (Smith et al., 1989). The small, bleached and sunken patches characteristic of this disease pose an aesthetic and playability problem on golf courses since ball speed and direction are affected. Golf course superintendents and bowling green managers may spend a great deal of their pest protection budget controlling and preventing this disease (Vargas, 2005). These control efforts may lead to other problems such as fungicide resistance development. *Sclerotinia homoeocarpa* has developed resistance toward demethylation-inhibiting (DMI) fungicides (Golembiewski et al., 1995; Vargas et al., 1992; Hsiang et al., 1997), dicarboximide fungicides (Detweiler et al., 1983), and benzimidazoles (Warren et al., 1974; Burpee, 1997).

In addition to fungicide resistance issues, interactions with fungicides targeting other diseases may also increase disease incidence. Disease increase may be due to resurgence or enhancement, which are closely related and often referred to as the same effect. The first refers to the chemical effect in which the target disease is successfully controlled, but once the chemical effect fades, the disease surges with more intensity than on non-treated areas. Disease enhancement occurs when the target disease is controlled, but the incidence of a non-target disease is higher than in areas where the chemical was not applied (Vincelli, 2002). Disease enhancement or resurgence is a phenomenon that is not well understood. Examples of this can be found in the turfgrass environment such as the increase incidence of summer patch (caused by *Magnaporthe poae* Landsch. & Jacks.) in Kentucky bluegrass *(Poa pratensis* L.) after applications of chlorothalonil (Vincelli, 2007), or the increased severity of Pythium blight (*Pythium* spp.) after benomyl applications (Warren et al., 1976). Dollar spot in particular has been reported to be enhanced by the application of azoxystrobin (Cook and Hsiang, 2002; Cook and Hsiang 2004; Dernoeden, 2000; Hsiang and Cook, 2006; Schumann, 2003; Vincelli, 2007). These reports give observational and anecdotal details on disease resurgence or enhancement soon after application, but they have not examined the mechanisms behind this effect, nor have they probed the longer-term effects of fungicide applications on non-target disease incidence.

The mechanisms proposed to produce resurgence and disease enhancement can be divided in two main factors: direct effects on the plants, or effects on phyllosphere microbial communities (Vincelli, 2007). A direct pesticide effect on the plants includes changes to their physiology, such as hormonal changes, carbohydrate content changes and increased plant stress (Couch, 1995). Any of these factors could alter the susceptibility of the plant to particular diseases. The second common explanation for the occurrence of disease enhancement or resurgence after fungicide application is the suppression of phyllosphere antagonist microorganisms (Smith et al., 1989). Most of the turfgrass pathogens that have been found to increase in incidence after pesticide application are foliar pathogens attacking and infecting plants through their leaves, such as in the following cases: red thread (Dernoeden et al., 1985), melting out (Dernoeden and McIntosh, 1991), Rhizoctonia yellow patch (Smiley, 1981) and Helminthosporium leaf spot (Jackson, 1970). The pathogens causing these diseases are part of the phyllosphere, and this environment plays a key role in their infection processes. The inhabitants of this microenvironment are affected by conditions such as temperature and nutrient availability (Lindow and Brandl, 2003). Additionally, interactions among the members of the microbial community itself have been shown to affect the survival of certain species (Bélanger and Avis, 2002). The biological control of leaf pathogens relies on the antagonism that certain phyllosphere microorganisms exert on leaf pathogens (Bélanger and Avis, 2002).

Dollar spot disease has been reported to be enhanced in creeping bentgrass (*Agrostis stolonifera* L.) treated with commercial fungicides. This includes casual observations in the golf industry (Dernoeden, 2000; Schumann, 2003) as well as specific experimentally supported results (Hsiang and Cook, 2006; Vincelli, 2002). Carboxin-derived fungicides such as flutolanil (Vincelli, 2007), strobilurins such as azoxystrobin (Dernoeden, 2000; Hsiang and Cook, 2006; Schumann, 2003), pyrrol fungicides such as fludioxonil, chitin synthetase inhibitors such as polyoxin D (Vincelli, 2007) and organochlorine fungicides such as pentachloronitrobenzene (Smiley, 1981) are some of the fungicides reportedly related to dollar spot disease resurgence or enhancement.

In order to investigate the enhancement phenomenon, the effects of azoxystrobin on dollar spot incidence were assessed. Multi-season trials on two turfgrass species were conducted over two years with treatment of azoxystrobin at different rates. The objective of this study was to obtain data to examine the link between azoxystrobin application in the winter and dollar spot enhancement in the following summer.

MATERIALS AND METHODS

Study site

Plots were established at the Guelph Turfgrass Institute (GTI) at the University of Guelph (Guelph, Ontario, Canada). The first experiments ran from Nov 2005 to Oct 2006 (11 months), and the second set of experiments ran from Nov 2006 to Aug 2007 (9 months). At the GTI, two areas of *A. stolonifera* and one area of *P. pratensis* were chosen. These areas were maintained using cultural regimes commonly practiced at golf courses in Ontario for putting greens, fairways or fringes, and experience levels of disease similar to what is found in commercial turfgrass areas nearby in the absence of fungicide use.

One set of plots was placed on a sward of *A. stolonifera* referred to as the pathology green. This area had been constructed in 1994 on a 30 cm soil base of 80:20 sand:peat (v/v) mixture on top of a 10 cm drainage layer of gravel following specifications recommended by the United States Golf Association (USGA). The pathology green was fully surrounded by large trees and shrubbery. It was initially seeded and has been frequently overseeded with Penncross creeping bentgrass. The plot area at the time of the experiments, however, contained approximately 30% annual bluegrass (*Poa annua* L.) which is commonly found as an invader of closely mown *A. stolonifera* swards. The area was irrigated as needed and mowed daily during the growing season at 5.5 mm height with clippings removed.

On the pathology green in 2006, homogeneous methylene urea complex Country Club (N-P-K: 18-3-18) (Lebanon Seaboard Corp., Lebanon, Pennsylvania, USA), was applied monthly as a fertilizer from May to Aug at 35 kg N/ha, and in Sep and Nov, it was applied at 50 kg N/ha. Additionally, in Jul 2006, the area was topdressed with 1 cm of calcareous sand (west half) or non-calcareous sand (east half) and aerated twice with 0.5 inch (1.25 cm) diameter hollow tines on a 2 inch (5.8 cm) center. Similarly, in Sep 2006 the area was topdressed twice. In 2007, the fertilizer Country Club (N-P-K: 18-3-18) was applied at 50 kg N/ha each month from May until Aug, and in Sep, 2007, Urea Micro-Prill (N-P-K: 46-0-0) (Dyno Nobel, Salt Lake City, Utah, USA) was applied twice at 50 kg N/ha. Except for the experimental treatments, no other fungicides were applied to the plot areas during this experiment, and fungicide use is generally very limited in research plot areas.

The other *A. stolonifera* plots were in an area called the native sand fairway. This was built as a push-up or natural soil green in 1994 with local Fox sandy loam, and seeded with Penncross creeping bentgrass, but it contained approximately 20% *P. annua* at the time of these trials. During the growing season, it was mowed every other day at 10 mm height and was topdressed using the methods and amounts previously stated for the pathology green. The fertilizers Scotts Pro Turf (N-P-K: 21-3-21) (Scotts Miracle-Gro, Marysville, Ohio, USA) at 25 kg N/ha or Country Club (N-P-K: 18-3-18) at 25 kg N/ha were applied in May 2006, followed by monthly fertilizations with Country Club (N-P-K: 18-3-18) at 35 kg N/ha from Jun to Aug 2006. In Sep and Nov 2006, the native sand fairway was fertilized with Country Club at 50 kg N/ha. In 2007, the same fertilizer was used at 50 kg N/ha once a month from May to Aug 2007. Except for the experimental treatments, no other fungicides were applied to the plots areas during this experiment, and fungicide use is generally very limited in research plot areas.

Another set of plots was placed on a sward of *P. pratensis*. This area was located on a fringe of a putting green and had been sodded in 1994 on native soil. It was irrigated as needed and cut at 3.8 cm height every other day during the growing season, and the clippings left in place. The fertilizer Agromart® 25-4-10 (Agromart Group, London, Ontario, Canada) was applied at 40 kg N/ha in May, Aug, Sep and Nov 2006. No pesticide was applied to this area, except for designated treatments as part of these experiments.

Field Experiments

Four sets of plots were established in this study at the Guelph Turfgrass Institute in 2005 and 2006. The plots were established on different areas of the pathology green, native sand fairway and the *P. pratensis* fringe. At the time of plot establishment, no dollar spot disease was evident. Except for *P. pratensis*, these plot areas generally experience moderate levels of dollar spot disease from natural inoculum. All plots consisted of areas of 16 m^2 (eight plots of 1 m x 2 m). Plots with odd numbers (1, 3, 5 and 7) were not treated, while even numbered plots (2, 4, 6 and 8) were treated with fungicide. No artificial inoculum was used.

Plots were visually evaluated weekly throughout the course of the trials. Evaluations consisted of assessing plant injury and incidence of dollar spot. Symptoms of other diseases, abiotic injury and weed presence were also recorded. Plant injury was assessed as the percentage of plot area with yellow, straw-like or dead leaves caused by all biotic and abiotic agents, such as temperature, drought, insects, pathogens or unknown reasons. Dollar spot incidence was estimated by counting all characteristic dollar spot patches. On the *A. stolonifera* plots, the patches counted were generally less than 4 cm across, round, yellowish and sunken spots, while *P. pratensis* patches were roughly round, approximately 10 cm in diameter with hourglass symptoms on leaves.

The four multi-season trials were established and treated in the fall (two in 2005 and two in 2006), and evaluations done monthly or weekly until the next fall. On 28 Nov 2005, the first trial was established and treated on the southwest portion of the *A. stolonifera* native sand fairway. A second trial was established and treated at the same time near the southeast fringe of the upper green in a *P. pratensis* area. On 25 Nov 2006 a third multi-season trial (2006-2007) was established and treated on the southwest portion of the native sand fairway. The final multi-season trial was established and treated at the west end of the pathology green on 25 Nov 2006.

The two 2005-2006 multi-season trials were treated with Heritage® 50WDG applied at the maximum rate used for anthracnose blight of 12 g/100 m^2 (Anonymous, 2005), giving an active ingredient (azoxystrobin) rate of 6 g/100 m^2. The two subsequent multi-season trials of 2006-2007 were treated with Heritage® MAXX® at 126 ml/100 m^2 (12 g/100m^2 of active ingredient), which is the amount recommended for gray snow mold (Anonymous, 2005). The fungicide applications were made with a wheel-mounted compressed air boom sprayer at 140 kPa in water at 10 l/100 m^2 using Lurmark 03-F110 nozzles (Hydro EU, Cambridge, UK). The same amount of water was applied to the non-treated control plots.

Weather Data

Weather data were obtained for the duration of the trial, specifically snow cover depth, temperature and precipitation. Snow cover depth of winter 2005-2006 in Guelph was visually estimated on a daily basis. For winter 2006-2007, snow cover records were obtained from the University of Waterloo weather station web site (www.uwaterloo.ca), which uses a sonic range sensor to measure snow depth. Daily maximum and minimum temperatures (ambient air) and precipitation (tipping bucket) data were also obtained from the same weather station from Oct 2005 to Oct 2007.

Statistical Analyses

Data were analyzed with ANOVA using SAS (SAS Institute, Cary, North Carolina, USA) PROC GLM. When a significant treatment effect was observed in the ANOVA ($p = 0.05$), the means were compared using Fisher's LSD (Least Significant Difference) at $p = 0.05$. To obtain standard error values, SAS PROC MEANS was used.

RESULTS and DISCUSSION

In spring, temperatures were similar in both years (Figures 1 and 2), while in summer, the gap between highest and lowest levels was greater in 2007 than 2006. The warmer weather in 2007 was more conducive to summer disease development. Precipitation levels were also different between the years. In 2005-2006, precipitation occurred more frequently and at higher levels than in 2006-2007.

Native sand fairway multi-season trial of 2005-2006

Dollar spot patches began to appear on the *A. stolonifera* plots in 1 Jun 2006, and immediately a difference between treated and non-treated plots was apparent and significant (Figure 3). From the first appearance through most of summer, dollar spot counts on treated plots remained approximately 50% greater than non-treated plots. Only on 13 Jul 2006 were the counts not significantly different, although the mean count for the treated plots was higher than that of the non-treated plots. Treated and non-treated plots had the same dollar spot incidence by the end of Aug 2006 (Figure 3).

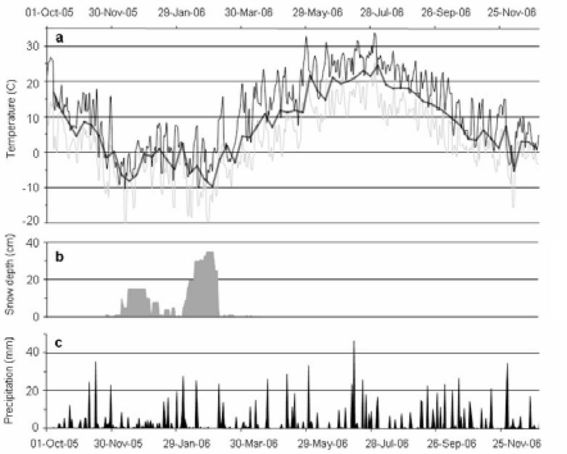

Figure 1. Temperature and precipitation from 1 Oct 2005 to 1 Dec 2006: (a) maximum, minimum and weekly average temperatures recorded at the Waterloo Wellington weather station (Climate ID 6149388), (b) visual estimation snow depth (cm) at the Guelph Turfgrass Institute, Ontario, and (c) total precipitation (mm) recorded at the Waterloo University weather station, Ontario.

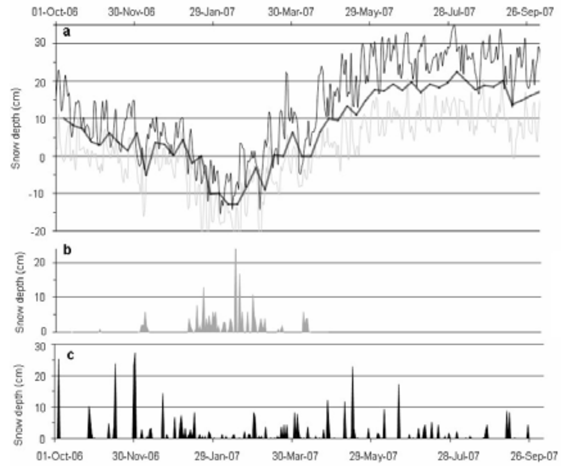

Figure 2. Temperature and precipitation from 1 Oct 2006 to 1 Oct 2007: (a) Maximum, minimum and weekly average temperatures recorded at the Waterloo Wellington weather station (Climate ID 6149388), (b) snow depth (cm) measured with a sonic range sensor and (c) total precipitation (mm) recorded at the Waterloo University weather station, Ontario.

Poa pratensis multi-season trial of 2005-2006

Dollar spot disease on the *P. pratensis* plots was first observed on 20 Jul 2006 at low levels (Figure 4). The disease continued to spread reaching 6 spots per square meter, and remained at that level through July until mid August. On 25 Aug 2006, the disease level had doubled (from 6 to 12 spots/m^2) in non-treated plots, and tripled in treated plots (from 6 to 18 spots/m^2). On 20 Jul and 25 Aug 2006, dollar spot incidence was significantly greater in azoxystrobin-treated plots than in non-treated plots.

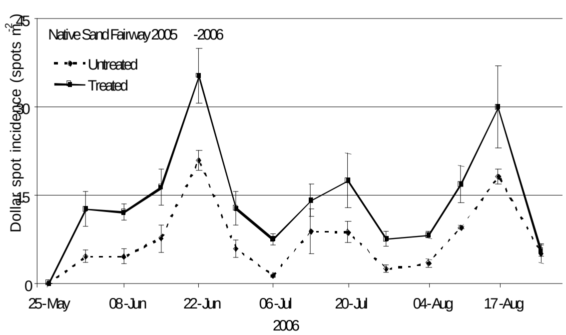

Figure 3. Dollar spot incidence on the *Agrostis stolonifera* native sand fairway multi-season trial of 2005-2006. Dollar spot incidence was evaluated weekly to monthly from 13 Mar 2006 until 20 Oct 2006, and was assessed as number of patches per m^2, based on four replicate 1 m x 2 m plots per treatment. Heritage® 50WDG was applied on 28 Nov 2005 at 6 g azoxystrobin per 100 m^2. The bars represent standard error.

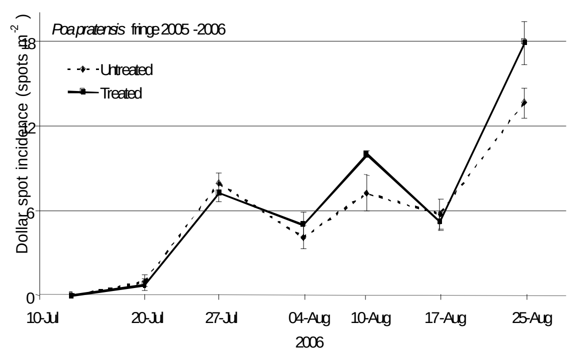

Figure 4. Dollar spot incidence on the *Poa pratensis* multi-season trial of 2005-2006. Dollar spot incidence was evaluated weekly to monthly from 13 Mar 2006 until 20 Oct 2006, and was assessed as number of patches per m^2, based on four replicate 1 m x 2 m plots per treatment. Heritage® 50WDG was applied on 28 Nov 2005 at 6 g azoxystrobin per 100 m^2. The bars represent standard error.

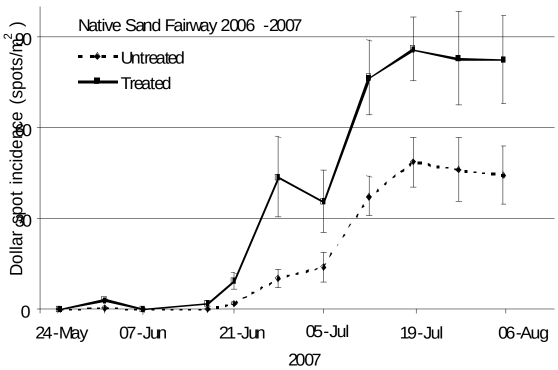

Figure 5. Dollar spot incidence on the *Agrostis stolonifera* native sand fairway multi-season trial of 2006-2007. Dollar spot incidence was evaluated weekly to monthly from 23 Nov 2006 until 8 Aug 2007, and was assessed as number of patches per m^2, based on four replicate 1 m x 2 m plots per treatment. Heritage® 50WDG was applied on 25 Nov 2006 at 12 g azoxystrobin per 100 m^2. The bars represent standard error.

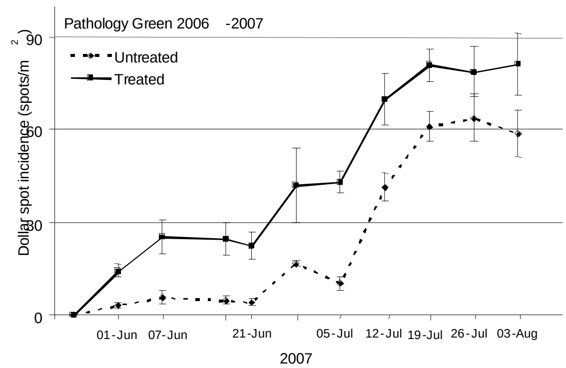

Figure 6. Dollar spot incidence on the *Agrostis stolonifera* pathology green multi-season trial of 2006-2007. Dollar spot incidence was evaluated weekly to monthly from 23 Nov 2006 until 8 Aug 2007, and was assessed as number of patches per m^2, based on four replicate 1 m x 2 m plots per treatment. Heritage® MAXX was applied on 25 Nov 2006 at 12 g azoxystrobin per 100 m^2. The bars represent standard error.

Native sand fairway multi-season trial of 2006-2007

Dollar spot disease levels on the *A. stolonifera* native sand fairway plots of 2006-2007 had almost the same pattern in treated and non-treated plots (Figure 5). Dollar spot disease appeared on 21 Jun 2007, reaching the highest incidence one month later. Dollar spot incidence remained steady until the last evaluation when it decreased. Although disease on non-treated and treated plots followed the same up and down patterns, they were significantly different in levels of dollar spot incidence. From the beginning of the experiment, treated plots showed more spots. The ratio varied through the growing season from 2 to 3 times more spots on the treated plots than non-treated plots. Increasing incidence was observed until the levels plateaued on 19 Jul 2007 at 80 spots/m^2 in the treated plots and 40 spots/m^2 in the non-treated plots until the end of observation of 6 Aug 2007 (Figure 5).

Pathology green multi-season trial of 2006-2007

Dollar spot disease was first observed on 1 Jun 2007 on the *A. stolonifera* pathology green multi-season trial of 2006-2007. This was earlier than was observed for the *A. stolonifera* native sand fairway trial of 2006-2007. Three months later, dollar spot incidence reached a maximum of 80 spots/m^2 (Figure 6). As on the native sand fairway plots, the disease levels increased until late Aug, after which they remained constant. Dollar spot incidence was observed to increase in treated and non-treated plots, but there were higher numbers in the treated plots (Figure 6). The first symptoms of dollar spot on 1 Jun 2007 showed five times more spots on treated plots than non-treated plots, but the difference was reduced by the end of Jul 2007, although incidence dollar spot continued to be greater in treated plots non-treated plots.

Dollar spot incidence was found to be affected by multiple factors such as plant height, (pathology green vs. native sand fairway trial multi-season trials of 2006-2007), environmental conditions (2005-2006 vs. 2006-2007 multi-season trials) and plant species (*A. stolonifera / P. annua* vs. *P. pratensis*). Among these, the largest differences were seen between plant species. The timing of overall plant injury (which includes dollar spot injury) was similar in both species differing only in the levels reached. After winter, *P. pratensis* plots were almost completely yellow (90%) from abiotic sources, while *A. stolonifera* plots reached 60%. Snow mold was scarce and did not make a large contribution to the plant injury recorded. Plots of both species promptly recovered in spring, with less than 10% injury (7-8 of weeks after snow melted).

As different species, *A. stolonifera* and *P. pratensis* have different characteristics that make them naturally more resistant to some diseases than others and tolerant of different environmental and cultural conditions. It is not surprising then, that dollar spot disease development was different on *P. pratensis* plots than on *A. stolonifera* plots at the same time of the year. In stands of *P. pratensis*, dollar spot patches are 6 to 12 cm in diameter (Vargas, 2005), while on bentgrass putting greens, mature patches average 3 cm in diameter (Charbonneau and Hsiang, 2003), with single spots covering average areas of ~100 cm^2 vs. ~7 cm^2, respectively. Consequently, the maximum number of dollar spots per square meter will be less in lawns (*P. pratensis*) than on golf greens (*A. stolonifera*) by virtue of size patch alone.

For *A. stolonifera*, patch numbers of 126 spots/m^2 up to 380 spots/m^2 have been reported in different areas of the United States (Lee et al., 2003; Tredway and Butler, 2004) while in previous studies conducted at GTI, the maximum dollar spot incidence was over 170 spots/m^2 on non-inoculated plots (Cook and Hsiang, 2002; Liu et al., 1995). Studies with dollar spot on *P. pratensis* are scarce, and the disease incidence is not usually recorded as number of spots per area, but rather as a quality rating. This may be because *S. homoeocarpa* is not considered an agronomic problem on *P. pratensis* fairways (Toshikazu 1997). In recent years however, greater incidence of dollar spot on *P. pratensis* has been reported, such as the first evidence of dollar spot in *P. pratensis* in Saskatchewan (Smith et al., 2001).

Dollar spot disease is not controlled or has been enhanced in *A. stolonifera* treated with commercial fungicides containing azoxystrobin. This is based on casual observation in the golf industry as well as specific experiments carried out to test the effect (Cook and Hsiang, 2002 and 2003). Application of azoxystrobin did not reduce the number of dollar spot patches in the summer when compared with non-treated areas (Dernoeden et al., 2000). In other trials, the number of dollar spot patches was twice as high as the non-treated areas (Cook and Hsiang, 2003; Dernoeden, 2000; Gleason et al., 1996; Grogan and Scott, 1997; Hsiang and Cook, 2006). Schumann (1996) reported dollar spot enhancement on *A. stolonifera* 10 times higher after a single application of azoxystrobin two weeks before. In this study, all the *A. stolonifera* plots treated with azoxystrobin had significantly higher number of dollar spots than the non-treated plots many months after a single azoxystrobin application. The current study is the first one in which dollar spot enhancement caused by azoxystrobin was reported after more than one season. Furthermore, although previous studies have reported the enhancement effect for putting greens containing *Agrostis* spp. with some *P. annua* (Dernoeden, 2000; Hsiang and Cook, 2006), this is the first study to document or even mention the effect of azoxystrobin on the resurgence or enhancement of dollar spot incidence on *P. pratensis.*

The enhancement of dollar spot caused by azoxystrobin has not been commonly observed by turfgrass managers on golf courses or sport fields. The reason for this may be because other fungicides are commonly used to control dollar spot, and in intensively managed turf areas that suffer from dollar spot, it would be rare to have no treatments for dollar spot control during the growing season as was done in this study. The cause of the dollar spot enhancement is not well understood, but there are two major hypotheses for enhancement and resurgence in general. First, there may be a direct longer lasting effect of the fungicides on the plants that makes them weaker or stressed, facilitating infection. Secondly, the microbial population of soil or leaves may mediate the enhancement effect, since longer lasting alteration of microbial communities or changes in biodiversity may create different conditions for pathogen growth and infection (Dernoeden, 2000).

In support of the physiological explanation for enhanced disease, azoxystrobin is known to alter plant growth and delay senescence periods of leaves (Grossmann and Retzaff, 1997). These effects have not been reported on turfgrass, but they have been found for other graminaceous plants such as wheat (Wu and Tiedemann, 2001) and barley (Kleven et al., 2003). Alteration of plant hormonal and water-conserving metabolism is considered possible causes of the growth effects of some strobilurins (Vincelli, 2002). These could also alter conditions in which pathogens attack the leaves. The changes produced by azoxystrobin on the plant metabolism could also indirectly modify microbial populations that lead to the disease enhancement, although no study reporting this was found.

Although cited as a possible source of disease resurgence and enhancement, some experimental conditions indicate that the plant effect may not be the main cause in this study. First, it seems unlikely that plants treated more than six months previously with azoxystrobin still contain sufficient chemical to cause some effect. A study of azoxystrobin movement suggests that it is localized in intercellular spaces of the leaves and does not enter the cytoplasm of grape plants (Wong and Wilcox, 2001). Thus, the direct effects on plant cellular metabolism may be limited. Since the photodegradative half-life of azoxystrobin in the soil was found to be 11 days (Anonymous, 1997), there should be no remaining pool to be drawn from the soil over time. Biotransformation also increases the rate of azoxystrobin dissipation over time. Persistence studies of azoxystrobin in the Canadian central provinces indicate a wide range of persistence with a 50% dissipation time (DT_{50}) of 14 to 62 days (Anonymous, 2000). This implies that the total dissipation (biotransformation) of azoxystrobin can take more than nine months, however the application of 50 g a.i. 100 m^{-2} used in the cited report was four to eight times greater than that used in the current study.

Among other factors that argue against a direct plant effect as the cause of resurgence, is the speed at which turfgrass plants grow. At the time when enhancement was observed in summer in this study, the plant leaves that were treated had already been removed months earlier by continuous growth and mowing. The *A. stolonifera* shoot growth rate reported by Xu and Huang (2001) ranges from 0.5 to 3 mm/day depending on the temperature. Thus, when mowing at 5.5 mm daily, all treated plant foliage would have been replaced in less than a week (5.5 days assuming a growing rate of 1 mm/day). Consequently, the long lasting effect observed might be an indirect effect of azoxystrobin such as the alteration of microbial communities rather than a direct effect on plants.

Azoxystrobin is known to have strong inhibitory effects on a variety of organisms such as many of the true fungi (Ascomycota, Deuteromycota, and Basidiomycota) and even the stramenopile Oomycota. A single application of this broad spectrum fungicide may have wide reaching and long term effects. Microbial communities including bacteria and fungi as major groups, are delicate ecosystems that once altered may take months to recover the original biodiversity, richness and distribution, and might never return to their pre-treatment composition. The dollar spot disease enhancement effect produced by azoxystrobin on *A. stolonifera*, although clearly observed in both years, differed depending on year and plot location. In the 2005-2006 trial (native sand fairway), the levels of dollar spot were not as high as in the two experiments set up in 2006-2007 (native sand fairway and pathology green). However the ratio of dollar spot incidence between treated and non-treated plots differed from 2:1 on the native sand fairway plots (2006-2007), 3:2 native sand fairway plots (2005-2006) to 5:1 on the pathology green plots (2006-2007). The most probable causes of these differences were the rate of azoxystrobin applied, and variation in weather and cultural practices. In 2005-2006, the fungicide rate was half (6 g a.i./100 m^2) of the rate used in 2006-2007 plots. The doubled azoxystrobin rate might have allowed for the increased disease enhancement observed in 2007, but since the weather conditions were also very different between 2006 and 2007, a definitive conclusion cannot be drawn from the data. Experimental work with different rates within the same period is required to resolve this.

CONCLUSION

In summary, dollar spot disease incidence can be affected by many factors such as turfgrass species, climatic conditions and cultural practices. Previous studies and anecdotal reports have found an enhancement or resurgence of dollar spot within the same growing season of fungicide application. This is the first study to show a longer term effect where, a single azoxystrobin application on mixed *A. stolonifera* and *P. annua* in the fall produced a several fold enhancement of dollar spot many months later in summer. In addition, this study also found a slight enhancement of dollar spot incidence on *P. pratensis* in multi-season trials, which has not been previously reported. Although the cause of this disease enhancement phenomenon is not known, the physical and chemical characteristics of azoxystrobin along with turfgrass cultural practices and the finding of the longer term effect from this study, implies that the phenomenon stems from non-target effects on microbial populations of leaves or soil rather than a direct long term effect on the plants. Studies on phyllosphere communities may shed light on this phenomenon and point out directions for future research.

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