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Effects of Early Spring and Preventative Snow Mold Fungicide Applications on DMI Sensitive and Insensitive Populations of Sclerotinia Homoeocarpa

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EFFECT OF EARLY SPRING DOLLAR SPOT AND PREVENTATIVE SNOW MOLD FUNGICIDE APPLICATIONS ON DMI SENSITIVE AND INSENSITIVE POPULATIONS OF *SCLEROTINIA HOMOEOCARPA*

A Thesis Presented

by

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ABSTRACT

EFFECT OF EARLY SPRING DOLLAR SPOT AND PREVENTATIVE SNOW MOLD FUNGICIDE APPLICATIONS ON DMI SENSITIVE AND INSENSITIVE POPULATIONS OF *SCLEROTINIA HOMOEOCARPA*

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Dollar spot, caused by the pathogen *S. homoeocarpa* (F.T. Bennett), is a common disease that infects a wide variety of turfgrasses all over the world. Yet it is significant problem on golf course putting greens and fairways consisting of creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.). It is active in a wide variety of environmental conditions ranging from 16-30˚C but favors warm, humid days, followed by cool nights. *Sclerotinia homoeocarpa* overwinters as dormant mycelium in dead plant tissue. In the spring, germinating mycelia begin to infect leaf blades causing foliar lesions, which then spread via mycelium by means of wind, rain, animals and equipment. While there are a number of cultural practices that can reduce disease severity, frequent fungicide applications are required to maintain acceptable playing conditions on a golf course. The repeated use of fungicides with the same mode of action has led to the development of fungicide resistance of *S. homoeocarpa* to certain fungicide

classes. Most notably, demethylase inhibitor (DMI) fungicides have been found to have varying levels of inefficacy against *S. homoeocarpa* across North America. The cause for reduced efficacy is suspected to the shifted sensitivity levels of many *S. homoeocarpa* populations, which are resulted from repeated use of the DMI fungicide. Recently, "early-spring fungicide applications" targeting to reduce initial inoculum density of dollar spot have gained popularity in an attempt to reduce dollar spot severity. In addition, preventative fungicide applications (from late October through mid-November) containing DMI fungicides have been traditionally practiced to target snow molds (caused by *Microdochium nivale, Typhula* spp.) in the northeastern United States. To date, there is not a clear understanding as to what effect, if any, these applications have on *S. homoeocarpa* DMI sensitivity or residual dollar spot control the following year. Traditional preventative snow mold applications were also investigated on the effect of *S. homoeocarpa* DMI sensitivity and early-season dollar spot control. The objective of this study was to investigate the effect of early-spring dollar spot application and late-fall snow mold application on *S. homoeocarpa* population with a bimodal distribution of DMI sensitive and insensitive isolates.

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LITERATURE REVIEW

Introduction

Since the days of production based agriculture, plant pests such as weeds, insects, and diseases have been causing problems for those who oversee the crops these pests invade. Weeds create additional competition for sunlight, water, and nutrients while insects physically damage the plant or hinder it from functioning normally. Plant diseases are not unlike insect damage in that they can mar fruit, reduce yields, and hinder our ability to cloth and feed ourselves (Schumann and D'Arcy, 2006). Diseases such as late blight of potato (caused by *Phytophthora infestans*), apple scab (*Venturia inaequalis*), rice blast (*Magnaporthe grisea*), and dutch elm disease (*Ophiostoma novo-ulmi*) have forever changed the way humans view plant diseases and the effect that they can have on society. In order to combat the negative effects of these diseases, humans invented and further developed fungicides. Today, fungicides are by the far the most common and effective way of controlling plant diseases (Russell, 2005). Unfortunately, their initial success has led to an overdependence and overuse that has led to fungicide resistance (Schumann and D'Arcy, 2006). Fungicide resistance has become an increasing problem as environmental concerns coupled with government regulations have led to more site-specific fungicides and away from older multisite fungicides. These site-specific fungicides then need only a minor change in the fungal deoxyribonucleic acid (DNA) to be passed down and rendered less effective.

History of Fungicides

Simply put, fungicides are chemical substances that inhibit the growth of a fungus. Mankind's long history with fungicides dates back hundreds of years ever since humans tried to limit the destructiveness of plant diseases (Latin, 2011). The earliest fungicides were developed almost by coincidence as farmers noticed that wheat bunt was less severe if the seeds were coated with copper sulfate prior to planting (Latin, 2011). These early mixtures of cadmium, copper, mercury, and sulfur were very effective at controlling plant diseases yet due to their toxicity cadmium and mercury are no longer available (Schumann and D'Arcy, 2006). The next wave of fungicides occurred in the 1950's with the introduction of chloroneb, ethazole, and pentachloronitrobenzene (PCNB). The 1960's marked the introduction of a multisite fungicide, chlorothalonil (Syngenta Crop Protection Greensboro, NC). Chlorothalonil is still a widely used fungicide highly effective against many plant pathogens. It was also in the 1960's that the first penetrant fungicides became commercially available. Thiophanate-methyl and benomyl were the first fungicides capable of penetrating plant tissue and limiting existing infection (Latin, 2011). This led to a rapid development of fungicides that utilized lower applications rate and increased field efficacy (Russell, 2005)

The 1970's brought about an expansion and modernization of the agrichemical industry (Latin, 2011). This, coupled with the expansion of government regulation, was the reason for new site-specific chemistries such as iprodione (Bayer Greensboro, NC), vinclozolin (BASF Greensboro, NC), and triadimefon and the cancellation of mercury, cadmium and cyclohexamide. One of the negative consequences of having fungicides with specific modes of action is the ability of the pathogen to develop resistance. Practical resistance to benomyl in the benzimidazole class was first reported in powdery mildew (*Sphaerotheca xanthii*) on cucurbits in

1969 (Schroeder and Provvidenti, 1969; Smith, 1988). This marked the first documented case of resistance to a local penetrant and it came only after one year of benomyl applications (Schroeder and Provvidenti, 1969; Smith, 1988). Practical resistance of gray mold (*Botrytis cinerea*) to the dicarboximides was first documented in 1982 (Katan, 1982). And finally, the further development of the DMI fungicides in the 1980's ultimately led to resistance in barely powdery mildew *(Erysiphe graminis* f. sp. *hordei*) in 1984 (Butters *et al*., 1984).

Fungicide Resistance

In its simplest form, fungicide resistance is the loss of fungicide efficacy against a particular pathogen (Brent and Holloman, 2007). This sudden or slow development of reduced sensitivity and resistance can be characterized by the biological side (the pathogen) and the chemical side (the fungicide) (Latin, 2011). Both of these have to possess certain attributes in order for resistance to develop and it is important to note that not all pathogens and fungicides carry concerns of resistance (Latin, 2011). Resistance is a concern with site-specific fungicides within the benzimidazoles, dicarboximides and DMI's. Resistance has yet to be documented within the succinate dehydrogenase inhibitor (SDHI) fungicide class, however, extreme care should be taken since this class encompasses site-specific active ingredients. Multi-site compounds such as chlorothalonil attack numerous physiological processes within the fungal cell. In order for resistance to occur, mutations would need to overcome the active ingredient at numerous sites while still allowing for the fungi to grow (Latin, 2011). While this is possible, it has yet to be documented, and possible resistance to the multi-site fungicides is considered quite low.

The rise of resistant populations can occur in either a qualitative or quantitative pattern. The sudden shift toward a resistant population as observed in the benzimidazole, dicarboximide, and phenylamide classes exemplifies qualitative resistance (De Waard *et al*., 1993). Qualitative resistance is marked by fungal isolates that are either resistant (immune) or sensitive to the fungicide class (Latin, 2011). Qualitative resistance is more susceptible to dramatic shifts in fungal populations and is exacerbated by repeated applications of a fungicide with the same mode of action. Qualitative sensitivity distribution can easily be observed in dollar spot and anthracnose to the benzimidazole fungicide class (Latin, 2011). The shift to resistance remains stable even after discontinuing use of the particular fungicide. This differs quite dramatically from what is observed in the DMI class exhibiting quantitative resistance.

When the sensitivity distribution is considered quantitative, the population is composed of isolates showing a continuous range of variation in resistance or insensitivity. This gradual shift towards insensitivity can be attributed to multiple genes being responsible for reduced efficacy leading to a population that lies somewhere in between the two extremes (Georgopoulos, 1988). Evidence of various mechanisms of resistance has been suggested within differing crop species. For example, resistance associated with target site mutations for the pathogen powdery mildew on barley, the ability for *V. inaequalis* to overproduce resistant enzymes target by DMI fungicides, and in the case of *B. cinerea* on grapevines, the ability for the mutant strain to prevent the toxic accumulation of the fungicide within the fungal cell by transporting it though the cell wall and out of the cell are all examples of various mechanisms of resistance (Latin, 2011). This could explain why complete failure of these fungicides is rarely observed and simply increasing the application rate and/or shortening the application interval can provide and acceptable control interval (Köller and Wilcox, 1999). However, repeated

application with the same mode of action will put increasing selection pressure on the population and push it further towards insensitivity and reduced fungicide efficacy (Köller, 1987; Köller and Scheinpflug, 1987; Skylakakis, 1987). The azole portion of the DMI class is an example of quantitative resistance, and this gradual shifting of the population has been observed in pathogens *E. graminis*, *S. homoeocarpa,* and, *V. inaequalis* (Butters *et al*., 1984; Eckert, 1988; Golembiewski *et al*., 1995; Köller *et al*., 1997). The plant growth regulators (PGR's) paclobutrazol and flurprimidol that are frequently used in turfgrass management are closely related in chemistry to the DMI class. Research that shown these specific PGR's had higher EC_{50}'s (effective concentration to inhibit 50% growth) on DMI insensitive *S. homoeocarpa* isolates than sensitive isolates and suggested that both PGRs may contribute to the selection of DMI insensitive isolates (Ok *et al*., 2011).

Dollar Spot

Dollar spot, caused by the multinucleate pathogen *S. homoeocarpa* (F.T. Bennett), is a major foliar turfgrass disease affecting many varieties of turfgrasses across the world. While it can occur on a wide variety of turfgrasses it's most common on creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*). These turfgrass species make up the vast majority of golf course greens, tees, and fairways in the northern United States.

The pathogen becomes active when air temperatures are between 16-30°C and favors high humidity and periods of extended leaf wetness (Smiley *et al.*, 2005). Initial lesions will appear straw colored and as the disease progresses the leaves turn bleached white. Under severe infections sunken patches of 1 - <10cm infection centers will coalesce, negatively affecting the playability on a golf course green. *Sclerotinia homoeocarpa* is believed to overwinter as

dormant mycelium within decaying plant material and to a lesser extent the stromata on the leaf surface (Couch, 1995; Smiley *et al.*, 2005; Smith *et al*., 1989).

While there are many cultural practices turfgrass managers can do to reduce the severity of dollar spot (i.e. dew removal, adequate nitrogen fertility, proper irrigation, rolling, and thatch control), they still do not provide sufficient control, and fungicides are the predominant way dollar spot is controlled. In fact, more money is spent to control dollar spot than any other turfgrass pathogen (Goodman and Burpee, 1991). Because of the frequent fungicide applications needed to sustain high quality of turfgrass, selection of resistant/insensitive *S. homoeocarpa* isolates have been documented within the benzimidazole, DMI, and dicarboximide fungicide classes (Golembiewski *et al.,* 1995; Detweiler *et al.,* 1983; Warren *et al*., 1974). Reduced sensitivity to the DMI's is of great concern due to their broad-spectrum disease control and relatively cost effective.

Taxonomy

Originally labeled "little brown patch" by Monteith and Dahl (1932) dollar spot was once thought to belong the *Rhizoctonia* genus. However, it eventually sustained the name dollar spot while continuing to have its proper classification in doubt. In 1937 F.T. Bennett examined isolates from Great Britain, North American, and Australia and determined that there were several different types. This included isolates that produced ascospores and conidia, "ascigerous strain" that produced ascospores and microconidia, and a non-spore forming isolate in North America and Australia (Bennett, 1937). All of these isolates were believed to be the same fungus and thus in 1937 the pathogen was named *Sclerotinia homoeocarpa* (Bennett, 1937).

Even though the Latin name has not changed, there are doubts as to whether the aforementioned classification is correct for two main reasons. First, the absence of sclerotia suggests that it cannot be a *Sclerotinia,* and secondly there is an absence of fertile apothecia. This suggests that this pathogen may belong to *Lanzia, Moellerodiscus* or *Rutstroemia* genus (Carbone and Kohn, 1993; Smith *et al.*, 1989). More recently, the name *Clarireedia homoeocarpa* has been offered as a suggestion and final approval should be coming soon (Clarke and McDonald, 2013). Whatever the decision may be, it is our hope that it will provide us with a better understanding of the pathogen and the ways to control it in a more sustainable manner.

Fungicide Resistance to *Sclerotinia homoeocarpa*

DMI Fungicides

The demethylase inhibitors (DMI) are the largest group of fungicides used in turfgrass management. In fact, there are currently eight active ingredients (fenarimol, metconazole, myclobutanil, propiconazole, tebuconazole, triadimefon, triticonazole, and difenoconazole) labeled for dollar spot control within the DMI family and countless combination products under various trade names (Rimelspach *et al.,* 2011). Their ability to provide broad-spectrum disease control at a relatively low cost has made them a very popular choice among golf course superintendents. Unfortunately, this led to their overuse, causing DMI resistance in *S. homoeocarpa* to be first reported in 1995 (Golembiewski *et al.,* 1995). DMI insensitivity exhibits a quantitative resistance response and is best described by a gradual reduction in control at recommended application intervals and rates (Latin, 2011). The genetic mechanisms governing DMI insensitivity were investigated by Hulvey *et al*. (2012). Minor levels of overexpression in the *ShCYP-51B* gene and high levels of overexpression in the *ShatrD* gene

were associated with *S. homoeocarpa* isolates collected from locations that displayed practical DMI field resistance. Hulvey *et al.* also reported that the *ShatrD* gene was closely related to ABC-transporter (ATP-Binding Cassette) genes in other fungi (*B. cinerea*) that have been implicated in DMI insensitivity. *Sclerotinia homoeocarpa* isolates that displayed overexpression of the *ShatrD* gene were sampled from Hickory Ridge Golf Club in Amherst, MA and Hartford Golf Club in Hartford, CT and both locations have experienced documented reductions in DMI field efficacy (Hulvey *et al*., 2012; Popko *et al*., 2012).

The aforementioned popularity of the DMI's has led to resistance monitoring in field or greenhouse studies aimed at determining the loss of fungicide efficacy (Burpee, 1997; Gilstrap *et al*., 1997; Hsiang *et al*., 1997; Hsiang *et al*., 2007; Jo *et al*., 2006; Jo *et al*., 2008; Koch *et al*., 2009; Miller *et al*., 2002). In 1995, Golembiewski *et al.* found that three golf courses with anecdotal DMI resistance exhibited reduced relative growth (RG) as well as cross-resistance within the DMI family (Golembiewski *et al.,* 1995). Moreover, golf courses without prior DMI exposure showed five to eight times lower in RG than the exposed populations (Golembiewski *et al.,* 1995). These findings suggest that *in-vitro* sensitivity is an accurate measure in determining field efficacy of the DMIs.

In order for accurate and consistent *in-vitro* field resistance testing to occur, proper DMI discriminatory concentrations within the *in-vitro* fungicide assay were determined as well as *in-vitro* sensitivity values that correlate to decreased field efficacy. In 2006, Jo *et al.* suggested that propiconazole amended media at a discriminatory concentration of 0.1 µ. a.i. ml^{-1} was an accurate measure for screening large numbers of isolates and determining DMI sensitivity. In 2009, Koch *et al.* proposed that RMG values at 40% or higher (on propiconazole amended media at the concentration of 0.1 µg a.i. ml^{-1}) might serve as a threshold at determining decreased field

efficacy. However, most recently, Popko *et al.* results suggest that relative mycelium growth (RMG) (grown on propiconazole amended media at 0.1 μg a.i. ml^{-1}) greater than 50% may be a suitable threshold for detection *of S. homoeocarpa* that causes practical field resistance (Popko *et al*., 2012). It is this threshold that we will be using in determining what effects early spring and snow mold fungicide applications have on population dynamics of *S. homoeocarpa*.

Early Spring Dollar Spot Applications

Typically, golf course superintendents apply fungicides to control dollar spot while the pathogen is actively growing and disease symptoms are visible. In the northeastern United States, that is generally from May through October. Possibly driven by an attempt to gain better control of dollar spot and reduce fungicide applications, non-traditional fungicide applications have gained popularity in recent years. These applications are made prior to disease symptoms, but while the fungus may be actively growing in an attempt to reduce pathogen inoculum early in the season (Putman and Kaminski, 2008). Numerous reports from Illinois, Connecticut, and Maryland have shown that early spring fungicide applications reduced dollar spot severity (McDonald and Dernoeden, 2006; Putman and Kaminski, 2008; Koenig, 2009; Settle *et al.*, 2007). Research conducted by Koch 2008 showed that penetrant fungicides such as propiconazole and iprodione are more effective at delaying the onset of dollar spot than contact fungicides such as chlorothalonil (Koch, 2008). Koch (2008) examined common dollar spot rates of propiconazole at 1.0 kg a.i. ha^{-1}, iprodione at 3.1 kg a.i. ha^{-1}, and boscalid (BASF Greensboro, NC) at 0.4 kg a.i. ha^{-1} and determined that when applied in early May these treatments could delay symptom development by approximately one month when compared to the untreated.

Many golf course superintendents begin dollar spot spray programs in early June. An informal study of 8 Wisconsin golf course superintendents revealed that most facilities could tolerate up to 5% disease severity (Koch, *unpublished data*). Using a 5% disease threshold, Koch concluded that initial dollar spot applications could be delayed until mid-July when implementing an early dollar spot fungicide application (Koch, 2008). By allowing up to 5% dollar spot severity, Koch concluded that 1 application could be eliminated, thus saving a golf course with 34 acres of fairway $6,700 (as cited in Koch, 2008). Moreover, Wilson *et al.* suggested that significant economic savings could be realized by implementing an early spring fungicide application program. She concluded that by tank mixing propiconazole (0.67 kg a.i. ha^{-1}) and chlorothalonil (7.54 kg a.i. ha^{-1}) after the initial early application, golf course superintendents could achieve acceptable turfgrass quality (<5% disease severity) by making applications every 28 days. If this would end up eliminating 1 application, a savings of $1,982-$4,014 could be realized on 24 acres of fairway (Wilson *et al.,* 2011). These economic savings are substantial, especially during a time when there are declining revenues due to an oversupply golf course in many parts of the United States (Keegan, 2012).

Late Fall (Snow Mold) Preventative Applications

While the evidence is clear that early spring fungicide applications on asymptomatic turfgrass can be an effective way to reduce disease occurrence, the research has been inconclusive as to what effect late fall fungicide applications have on *S. homoeocarpa*. Koenig found that single late fall fungicide applications targeting dollar spot had no effect on disease severity the following July (Koenig, 2009). However, multiple fall applications significantly reduced dollar spot the following year July (Koenig, 2009). The success of multiple applications might suggest that at varying time periods *S. homoeocarpa* was actively growing undetected

and/or metabolically active. Coincidently, many northeastern golf course superintendents are making preventative snow mold fungicide applications close to this time frame.

A review of a snow mold fungicide trial revealed that many of the same fungicides used to treat snow mold are also used to treat dollar spot (Jung *et al.*, 2007). Active ingredients such as chlorothalonil, propiconazole, iprodione thiophanate-methyl and vinclozolin) are all very effective in tank-mix combinations for both snow mold and dollar spot control. In 2009, Koenig suggested that if air temperatures are at least 4.4°C *S. homoeocarpa* can be actively growing in the absence of symptom development and that at temperatures between 4.4°C and 15.5°C golf course superintendent are typically making fungicide applications (Koenig, 2009). Wilson *et al.* stated that disease development could occur at temperatures as low as 10°C (Wilson *et al*., 2010). Temperatures around 10°C are common during the month of November when golf course superintendents are making snow mold applications. Historical weather data (2008-2012) show that the mean high temperatures for Chicopee, MA in November were 10.4°C and 11.1°C in Hartford, CT (Weather Underground 2013). This leads us to hypothesize that traditional preventative snow mold applications may affect the DMI sensitivity *S. homoeocarpa* populations in the fall.

In order to measure and analyze population structure accurately, a specific population of *S. homoeocarpa* must be present. Popko *et al.* (2012) presented data that suggested a bimodal population of *S. homoeocarpa* at Hartford Golf Club in Hartford, CT and Hickory Ridge Golf Course in Amherst, MA. This means that two distinct sub-populations composed of both sensitive and insensitive isolates are present at the respective sites. Moreover, this allows researchers to monitor *S. homoeocarpa* population changes in response to DMI fungicide exposure. For this reason we will be using these two sites for this study.

CHAPTER 2

EFFECT OF EARLY SPRING DOLLAR SPOT AND LATE FALL PREVENTATIVE SNOW MOLD FUNGICIDE APPLICATIONS ON DMI SENSITIVE AND INSENSITIVE POPULATIONS OF *Sclerotinia homoeocarpa*

Introduction

Dollar spot is caused by the sterile, multinucleate, ascomycete fungus *S. homoeocarpa* (F.T. Bennett) and affects many species of turfgrass across in the world. Dollar spot occurs most commonly on creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*). These turfgrass species make up the vast majority of golf course greens, tees, and fairways in the northern United States.

While there are many cultural practices turfgrass managers can utilize to reduce the severity of dollar spot (i.e. dew removal, adequate nitrogen fertility, proper irrigation, thatch control and rolling), cultural practices alone still do not provide sufficient control. Therefore, fungicides are the predominant control method for dollar spot. In fact, more money is spent to control dollar spot than any other turfgrass pathogen (Goodman and Burpee, 1991). As a result frequent fungicide applications required for high quality turfgrass, selection of insensitive/resistant *S. homoeocarpa* isolates has been documented within benzimidazole, demethylation inhibitor (DMI), and dicarboximide fungicide classes (Golembiewski *et al.,* 1995; Detweiler *et al.,* 1983; Warren *et al*., 1974). Among them, reduced sensitivity to the DMI class is of great concern due to the broad-spectrum disease control and relative low cost this fungicide class provides. Fungicide resistance likely controlled by multiple genes in a quantitative fashion

can lead to unexpected, reduced efficacy of fungicide, which can result in additional applications each year.

Dollar spot is extremely difficult to control due to its persistent pressures throughout a growing season and this has led to considerable research focusing on alternative or different application timings to control dollar spot. One method that has been widely investigated on is "early spring" applications, targeting for reduction of accumulation of initial inoculum. Numerous reports from Connecticut, Illinois, Maryland, and Wisconsin have shown that early-spring fungicide applications reduced dollar spot severity (Koenig, 2009; McDonald and Dernoeden; 2006; Putman and Kaminski, 2008; Settle *et al.*, 2007; Wilson *et al.,* 2011). Research conducted by Koch (2008) showed that penetrant fungicides such as propiconazole and iprodione are more effective at delaying the onset of dollar spot than contact fungicides such as chlorothalonil. In addition to early-spring applications, late-fall applications have also been examined for the potential to suppress dollar spot in the following year. Koenig (2009) found that single late-fall fungicide application targeting dollar spot had no effect on disease severity the following year, but, multiple fall applications significantly did (Koenig, 2009). The success of multiple applications might suggest that at varying time periods *S. homoeocarpa* was actively growing undetected and/or metabolically active.

Coincidently, many northeastern golf course superintendents are making preventative snow mold fungicide applications with active ingredients such as chlorothalonil, propiconazole, iprodione, PCNB and thiophanate-methyl. The timing of preventative snow mold applications is very close to the timing of late-fall dollar spot applications. Furthermore, all of these fungicides are effective for control of both snow molds and dollar spot. Microdochium patch (*Microdochium nivale*), gray snow mold (*Typhula incarnata*), and speckled snow mold (*T.*

ishikariensis) are the three most common snow mold spp. They can infect all turfgrasses in northern and alpine climates but are most prevalent on creeping bentgrass and annual bluegrass (Hsiang *et al*., 1999; Jung *et al.*, 2007). While many other perennial crops are able to survive under winter months by translocating carbohydrate reserves to the roots, intensively managed turfgrasses are typically kept a lush green late into the fall creating a highly susceptible host before winter and necessitating fungicide applications (Hsiang *et al.,* 1999).

The aforementioned information has been substantial enough for some superintendents to adopt early-spring and late-fall fungicide applications for control of dollar spot, however, the early-spring are more widely used. Many golf course superintendents routinely implement these practices without considering the impact of these applications on selection pressure for fungicide resistance. Since DMI fungicides are prominently used at both application timings, we want to examine the possibility of selecting DMI insensitive *S. homoeocarpa* isolates by early-spring and late-fall dollar spot applications. Providing practitioners with this knowledge will allow them to make more informed decisions on fungicide selection and application timing when managing populations of *S. homoeocarpa*.

The objective of this study was to determine the effect of early-spring dollar spot and late-fall fungicide applications on *S. homoeocarpa* populations with DMI sensitive and insensitive isolates.

Materials and Methods

Study Sites

This study was conducted at Hickory Ridge Golf Club (HRGC) in Amherst, MA and at Hartford Golf Club (HGC) in Hartford, CT. The early-spring application (April 2012, 2013 and

2014) studies were completed at HRGC and the snow mold application studies at HRGC (November 2012 and 2013) and HGC (November 2012). These sites were chosen because of prior confirmation of bimodal *S. homoeocarpa* populations in regard to DMI sensitivity (Popko *et al.,* 2012). Both sites were chosen to test if fungicide treatments are selecting DMI insensitive *S. homoeocarpa* isolates. The fairway turf at HRGC was mowed at 1.6 cm, mowed three times per week, received 98.5 kg N ha^{-1} per year, irrigated as needed, and clippings were not removed. The tee box turf at HGC was mowed at 1.27 cm, mowed 3-4 times per week, received approximately 147.75 kg N ha^{-1} per year, and clippings are removed. Both sites were irrigated on an "as needed" basis.

Experimental Design

Two separate treatment lists for the early-spring and late-fall trials using commonly used fungicides are provided in Table 1. For the early-spring trial, the plant growth regulator (PGR) flurprimidol (Cutless™, SePRO Corporation, Carmel, IN) was included because it is a pyrimidine and thus related to the DMI fungicides (Ok *et al*., 2011). Flurprimidol was also tank-mixed with propiconazole since it is common spray mixture used by golf course superintendents. For the late-fall trial, a premixed product, Instrata™ (chlorothalonil, fludioxonil, and propiconazole, Syngenta Crop Protection, Greensboro, NC), was included since it has been commonly used by golf course superintendents for snow mold protection but is also effective on dollar spot control.

For each trial, treatments were applied once and plots were arranged in a complete randomized block design (CRBD) with four replications. The early-spring dollar spot applications had one untreated plot within each replication, while the late-fall applications had

two untreated plots to provide a more accurate benchmark for statistical comparisons. The plots measured 1.8 m by 1.8, m with 0.3 m buffer strip between each treatment. The late-fall application at HGC did not include buffer strips due to limited size of the tee box. All fungicide applications were made at a nozzle pressure of 275.8 kPa using a CO_2 pressurized boom sprayer equipped with two flat-fan XR TeeJet 8004VS nozzles. The sprayer was calibrated to deliver 81.5 ml m^{-2}.

Dollar Spot and Snow Mold Ratings

For the dollar spot trials, experimental plots were rated after dollar spot became active and individual infection centers were counted per plot. Snow mold severity (caused by *T. incarnata, T. ishikariensis,* and *M. nivale*) was visually assessed as percent snow mold damages per plot for the late-fall trials.

In-vitro Sensitivity Assay

In-vitro sensitivity was assayed to determine if the treatments had an effect on DMI sensitivity of the *S. homoeocarpa* population by analyzing the Relative Mycelium Growth (RMG). *Sclerotinia homoeocarpa* was isolated from turf plots followed the procedures of Jo *et al*. (2006) and Popko *et al.* (2012). Ten infected leaf blades were taken from each plot giving a total of 40 leaf blades per treatment. Leaf blades were placed in a 1.5 ml polypropylene micro centrifuge tubes and then filled with a 3% sodium hypochlorite solution. The tubes were inverted several times and left to sit for approximately 1 minute. The leaf blades were then taken out, rinsed in sterile distilled water, and put on sterile filter paper to dry before being placed onto a petri plates containing acidified potato dextrose agar (APDA). APDA was prepared by adding 1 ml of 85% lactic acid (Fisher Scientific, Fair Lawn, NJ) per 1 liter of potato dextrose agar (PDA)

(Difco Laboratories, Detroit, MI) after PDA was sterilized for 45 minutes at 121°C in an autoclave (Tuttnauer 3850 M, Hauppauge, NY). One leaf blade was placed on APDA petri plates and allowed to incubate for 2-3 days. Following incubation, *S. homoeocarpa* isolates were identified based on colony morphology and compared to known reference isolates. Next, pure cultures were obtained by subculturing 4 mm plugs of APDA media onto PDA and allowed to incubate. *In-vitro* fungicide sensitivity assays were conducted after *S. homoeocarpa* isolates had grown in culture 2-3 days. Propiconazole amended PDA was prepared by using a commercial grade propiconazole (Banner MAXX 1.3EC, Syngenta Crop Protection, Greensboro, NC) and the final concentration of the amended PDA was 0.1 μg a.i. ml^{-1} (Jo *et al*., 2006; Popko *et al.*, 2012). Agar plugs (5 mm in diameter) were transferred from actively growing pure cultures to the center of PDA Petri plates amended with propiconazole (0.1 μg a.i. ml^{-1}) and non-amended PDA Petri plates using a sterile 5-mm cork borer and spatula.

These plates were kept for approximately 48 hours before being measured with digital calipers (Mahr 16EX, Göttingen, Germany). Two measurements from each plate were taken with the second reading being taken by rotating the calipers 90 degrees. Measurements were averaged for each medium (non-amended PDA and propiconazole amended) and the average radial growth on propiconazole amended PDA was divided by the average non-amended radial growth and multiplied by 100 to give a percent value. Prior research conducted by Popko *et al*. (2012) concluded that RMG value above 50% exhibited practical field resistance, while RMG values below 50% represented sensitive isolates.

A qualitative *in-vitro* sensitivity assay was used to analyze DMI insensitivity for the late-fall trial. The protocol is outlined in Popko *et al.* (2013) and differs from the prior assay by using a higher propiconazole concentration (1.0 μg a.i. ml^{-1} compared to 0.1 μg a.i. ml^{-1}) to

qualitatively differentiate DMI sensitivity (growth or no growth). The aforementioned sampling process was used for the snow mold experiment and pure culture of *S. homoeocarpa* isolates were obtained. A single 5-mm agar plug was placed on the 1.0 μg a.i. ml^{-1} propiconazole amended petri plates and incubated for approximately 48 before qualitative assessment. The main advantage for using this qualitative assay technique is the conservation of time and significant reduction in Petri plates used.

Statistical Analysis

An analysis of variance (ANOVA) was used to test for differences among the treatments for the quantitative *in vitro* data and field efficacy data. Mean separation was conducted using Duncan's Multiple Range Test ($P < 0.05$) for all quantitative *in vitro* data and field efficacy data in which significant treatment effects existed according to the ANOVA. Chi-square analysis was used to analyze all qualitative *in-vitro* data and to test if fungicide treatments affected the frequency of resistant and sensitive isolates from the untreated plot. *Sclerotinia homoeocarpa* isolates that exhibited growth on 1.0 μg a.i. ml^{-1} PDA were considered resistant and *S. homoeocarpa* isolates that did not exhibit growth were considered sensitive to propiconazole. Isolates sampled from both untreated plots were pooled to increase the sample size of the untreated and to protect against poor isolation or low sample numbers.

Results and Discussion

Early-Spring Dollar Spot

Relative mycelial growth percentage was significantly different among treatments at Hickory Ridge Golf Club in the 2012 trial (Table 2). The untreated RMG (51.1%) was significantly lower than all other treatments. The RMG values of the propiconazole 0.44 kg a.i.

ha^{-1} (low labeled rate), propiconazole 0.87 kg a.i. ha^{-1} (high labeled rate), propiconazole and fluprimidol, and boscalid treatments were significantly higher than the untreated, thus suggesting selection of DMI insensitive isolates. However, chlorothalonil, vinclozolin, and flurprimidol treatments all had RMG% values closer to the untreated, but were still significantly higher than the untreated. This suggests that some level of selection pressure occurred, but to a lesser extent. Therefore, sites with a population of DMI insensitive isolates are more likely to be shifted to a higher level of insensitivity through one DMI application. In short, this site previously confirmed by Popko et al. (2012) is likely experiencing reduced control using DMI fungicides and thus shorter spray intervals are recommended in order to achieve the level of dollar spot control desired.

The results of these 2013 and 2014 studies showed no statistical difference between the treatments. In the 2013 trial, the 71% RMG for the untreated showed a clear shift in the population compared to the 2012 untreated (51 RMG%). This can be explained by the exposure of DMI fungicides when the plots were not being used for research purposes. It is estimated that 2-3 DMI fungicide applications were made to the experimental area. There were no ratings data taken during the spring of 2013 due to poor turfgrass quality caused by flooding. The plots partially recovered by sampling time (July), however, turf quality was extremely poor and ratings were omitted. In the 2014 trial, the 54% RMG for the untreated showed return near to the value expressed in 2012 (51 RMG%). However, few of the other treatments varied from 54% suggesting that the fungicide treatments had little effect on the population structure. No significant differences were observed in 2014 among treatments. One half of the experimental area showed consistent disease pressure while the other half exhibited very little. We

hypothesize that half of the experimental area was accidentally sprayed with a fungicide thus causing an increase of the error term and compromising our data.

Overall, all early-spring fungicide applications reduced the severity of dollar spot compared to the untreated control (Table 3). The penetrant fungicides in the study including propiconazole, boscalid, and vinclozolin can be very effective at delaying the onset of dollar spot activity between 37-41 days after treatment. For example, at 37 DAT the untreated plots had 37 infection centers while the low-labeled rate of propiconazole had 6, boscalid (0.38 kg a.i. ha^{-1}) had 2, and vinclozolin also had 2. While at 41 and 48 DAT we still observed reduced dollar spot activity amongst the propiconazole, boscalid, and vinclozolin treatments the control was not likely to the level that would be acceptable to many turfgrass managers. While this should be viewed positively and as a strategy for turfgrass managers these applications will still cause selection of DMI resistant isolates, which might influence dollar spot control later season. This is a research area that requires further study.

The variability of the *in-vitro* data from 2012-2014 demonstrates the importance of having a bimodal population in order to detect differences. When a population shifts from one extreme to the other it becomes difficult to discern differences among treatments. However, the 2012 data suggest that the application of non-DMI fungicides (SDHI or dicarboximide) can cause selection of DMI resistance isolates. Two studies conducted concurrently with my work provide some molecular explanation for DMI resistance selection with non-DMI fungicides. Sang *et al*. (2014) reported over-expression of the pleiotropic drug resistance (PDR) transporter gene *ShPDR1* after isolates were treated with boscalid, iprodione (dicarboximide class) and propiconazole. Furthermore, Hulvey *et al*. (2012) also reported over-expression of the *ShCYP51B* and *ShatrD* genes in *S. homoeocarpa* isolates treated with propiconazole. Both

studies included isolates from Hartford Golf Club and Hickory Ridge Golf and demonstrate that multiple genes likely govern *S. homoeocarpa* resistance to DMI fungicides.

Late-Fall Snow Mold

In the 2013 trial at Hickory Ridge Golf Club, plots treated with Instrata™ (chlorothalonil 5.46 kg a.i. ha^{-1}, propiconazole 0.86 kg a.i. ha^{-1}, and fludioxonil 0.23 kg a.i. ha^{-1}) and propiconazole 0.95 kg a.i. ha^{-1} had a significantly higher proportion of DMI resistant isolates than the untreated (Table 4). This demonstrates selection pressure from plots treated in November of 2012 to when they were sampled in July 2013. Despite a low number of isolates collected at Hartford Golf Club, a significant shift in the proportion of DMI resistant isolates was observed in the following three treatments: Instrata™ (chlorothalonil 5.46 kg a.i. ha^{-1}, propiconazole (0.86 kg a.i. ha^{-1}), and fludioxonil (0.23 kg a.i. ha^{-1}), iprodione (4.26 kg a.i. ha^{-1}), and fludioxonil (0.37 kg a.i. ha^{-1}) compared to the untreated (Table 5). Iprodione had more sensitive isolates than the untreated plots suggesting that there was no selection. Collectively, the number of fungicide treated isolates was 86 fewer at Hartford Golf Club versus Hickory Ridge Golf Club. However, this does provide some evidence that fungicide applications targeting snow molds may have an impact on dynamics of *S. homoeocarpa* population with DMI insensitive isolates. In the 2014 trial at Hickory Ridge Golf Club, plots treated with Instrata™ (chlorothalonil 5.46 kg a.i. ha^{-1}, propiconazole 0.86 kg a.i. ha^{-1}, and fludioxonil 0.23 kg a.i. ha^{-1}), iprodione (4.26 kg a.i. ha^{-1}), and propiconazole (0.95 kg a.i. ha^{-1}) had a significantly higher proportion of resistant isolates than the untreated (Table 6). This demonstrates selection pressure from plots treated in November of 2013 to when they were sampled in July 2014.

The snow mold data suggests that higher rates of propiconazole can have an effect on *S. homoeocarpa* population dynamics. At HRGC in both 2013 and 2014, the treatments Instrata (chlorothalonil, propiconazole, and fludioxonil) and propiconazole (0.95 kg a.i. ha^{-1}) alone showed a significant difference when compared against the untreated plots. Koenig (2009) suggested that minimum air temperature of 4.4°C is sufficient to support active growth of *S. homoeocarpa*, but not dollar spot symptom development on turfgrass. While the temperatures may be enough to suppress *S. homoeocarpa* from causing visible disease symptoms, it does appear to be metabolically active in some capacity, and thus the fungicide treatment does provide a selection event on *S. homoeocarpa* isolates.

Conclusion

The early spring ratings data supports similar studies (McDonald and Dernoeden 2006, Koch et al. 2009) and provides evidence that *S. homoeocarpa* is, at least, metabolically active and susceptible to fungicide applications. This present study found that penetrant fungicides such as boscalid and vinclozolin seem to be the most effective at delaying and/or reducing the amount of dollar spot practitioners experience during the early part of summer. As with all fungicide applications the primary means of degradations seems to be mowing and thus the positive effects of the early spring applications subside over time (Koch 2012). However, as Table 2 suggests, care should be taken as early-spring fungicide applications can still cause selection of DMI insensitive isolates.

Future recommendations for research include season long analysis of early spring applications. While the 2012 ratings data suggest that acceptable control can be obtained 41-DAT, significant differences in control were observed 48-DAT (Table 3). This suggests that

initial suppression of inoculum could provide some level of disease suppression throughout the entire growing season. However, each spring provides a different set of environmental conditions and treatments in some years would likely to be more effective than other years. The implementation of the early spring fungicide applications to reduce disease severity would most likely be practiced on golf course fairways, since the small acreage of golf course greens would provide little financial incentive for turfgrass managers to reduce fungicide applications (Koch, 2012).

Furthermore, more specifically designed experiments should be conducted with the snow mold portion of this thesis. These experiments would include varying rates of Instrata as well as incremental increases of propiconazole. The data presented in this thesis suggests that selection of insensitive isolates occur between 0.87 and 0.94 kg a.i. ha^{-1}. We suggest having additional propiconazole treatments that coincide with higher rates of Instrata. This may provide further scientific evidence of whether or not selection of DMI insensitive isolates of *S. homoeocarpa* is occurring.

Table 1. List of treatments for early-spring dollar spot and late-fall snow molds trials.

Treatment	FRAC#[Z]	Fungicide Class	Manufactures	Rate (kg a.i. ha^{-1})
		Early-spring dollar spot		
Untreated	-			-
Propiconazole	3	DMI	Syngenta Crop Protection	0.44
Propiconazole	3	DMI	Syngenta Crop Protection	0.87
Fluprimidol +	-	Pyrimidine +	SePro Corporation	0.42 +
Propiconazole	2	DMI	Syngenta Crop Protection	0.44
Fluprimidol	-	Pyrimidine	SePro Corporation	0.84
Boscalid	2	SDHI	BASF	0.38
Chlorothalonil[y]	M5	Nitriles	Syngenta Crop Protection	8.17
Vinclozolin	2	Dicarboximide	BASF	1.53
		Late-fall snow molds		
Untreated	-	-		
Untreated	-	-		
Propiconazole	3	DMI	Syngenta Crop Protection	0.44
Propiconazole	3	DMI	Syngenta Crop Protection	0.87
Chl + Ppz + Flu[x]	M5 + 3 + 12	Nitrile + DMI + Phenylpyrrole	Syngenta Crop Protection	5.47 + 0.86 + 0.22
Vinclozolin	2	Dicarboximide	BASF	1.53
Chlorothalonil[y]	M5	Nitrile	Syngenta Crop Protection	8.18
Iprodione	2	Dicarboximide	Bayer	4.26
Fludioxonil	12	Phenylpyrroles	Syngenta Crop Protection	0.37
Propiconazole	3	DMI	Syngenta Crop Protection	0.95
Chlorothalonil[w]	M5	Nitrile	Syngenta Crop Protection	6.59

[Z] Fungicide Resistance Action Committee.
[y] Daconil Ultrex.
[X] Chl=Chlorothalonil, Ppz=Propiconazole, Flu=Fludioxonil.
[W] Daconil Weatherstik.

Table 2. Summary of relative mycelium growth percentage (RMG%) from 2012-2014 at Hickory Ridge Golf Club.

Treatment[z]	Rate (kg a.i. ha^{-1})	RMG%[y]		
		2012	2013	2014
Untreated	-	51.1 c[x]	72.3	54.3
Propiconazole	0.44	69.0 ab	61.1	60.9
Propiconazole	0.87	71.5 ab	69.7	57.6
Fluprimidol + Propiconazole	0.42 + 0.44	74.1 a	69.8	51.1
Fluprimidol	0.84	61.8 b	61.7	51.7
Boscalid	0.38	71.0 ab	62.2	51.6
Chlorothalonil	8.17	63.9 ab	68.3	72.2
Vinclozolin	1.53	66.6 ab	64.7	58.2
P-value[x]		0.0011	0.9266	0.4662

[z] Treatments represent common name of product.
[y] RMG=Relative Mycelium Growth.
[x] Means followed by the same letter are not statistically different according to Duncan's New Multiple Range test.
[w] P-value from the analysis of variance of treatments.

Table 3. Influence of early-spring fungicide treatments on dollar spot infection center at Hickory Ridge Golf Club, 2012.

Treatment	Rate (kg a.i. ha^{-1})	Number of Dollar Spot Infection Center[z]						
		5/25	6/1	6/5	6/12	6/19	6/27	7/11
Untreated		26 a[y]	37 a	109 a	119 a	103 a	93 ab	102 a
Propiconazole	0.44	2 c	2 c	15 b	27 b	27 cd	23 c	43 cd
Propiconazole	0.87	2 c	6 c	15 b	34 b	22 d	22 c	26 d
Fluprimidol + Propiconazole	0.42 0.44	2 c	5 c	16 b	16 b	20 d	24 c	26 d
Fluprimidol	0.84	19 ab	29 ab	97 a	139 a	76 b	119 a	82 ab
Boscalid	0.38	1 c	2 c	7 b	28 b	19 d	16 c	22 d
Chlorothalonil	8.17	5 bc	12 bc	47 b	54 b	46 c	54 bc	61 bc
Vinclozolin	1.53	5 bc	2 c	27 b	27 b	19 d	40 c	48 dc
P-value		0.0038	0.0018	0.0005	0.0021	0.0001	0.0002	0.0001
DAT[x]		30	37	41	48	55	63	77

[z] Ratings data started once approximately 10 or more infection centers were present in all plots.
[y] Means followed by the same letter are not statistically different according to Duncan's New Multiple Range Test.
[x] Days After Treatment (DAT).

Table 4. Influence of late-fall (snow mold) treatments on the selection of DMI resistance *S. homoeocarpa* isolates at Hickory Ridge Golf Club, 2013.

Treatments[z]	Rate (kg a.i. ha^{-1})	Number of Isolate		χ^2
		Resistant[y]	Sensitive[x]	P-value[w]
Untreated	-	36	7	-
Untreated	-	20	12	-
Propiconazole	0.44	25	5	0.1902
Propiconazole	0.87	28	6	0.2077
Chl +Ppz +Flu[v]	5.47 + 0.86 + 0.22	33	1	0.0019
Vinclozolin	1.53	19	3	0.1457
Chlorothalonil[u]	8.18	21	5	0.3281
Iprodione	4.26	16	4	0.4165
Fludioxonil	0.37	24	10	0.1000
Propiconazole	0.95	31	1	0.0028
Chlorothalonil[t]	6.59	19	9	0.7786

[z] Treatments represent common name of product.
[y] Represents the number of isolates that showed mycelia growth on Petri dish.
[x] Represents the number of isolates that did not show mycelia growth on Petri dish.
[w] Represents the P-value from the statistical analysis between the fungicide treatments and the two untreated plots that were pooled.
[v] Chl=Chlorothalonil, Ppz=Propiconazole, Flu=Fludioxonil.
[u] Daconil Ultrex.
[t] Daconil Weatherstik.

Table 5. Influence of late-fall (snow mold) treatments on the selection of DMI resistance *S. homoeocarpa* isolates at Hartford Golf Club, 2013.

Treatments[z]	Rate (kg a.i. ha^{-1})	Number of Isolate		χ^2
		Resistant[y]	Sensitive[x]	P-value[w]
Untreated	-	18	4	-
Untreated	-	21	11	-
Propiconazole	0.44	14	7	0.6352
Propiconazole	0.87	16	1	0.0595
Chl +Ppz +Flu[v]	5.47 + 0.86 + 0.22	14	0	0.0254
Vinclozolin	1.53	14	3	0.4024
Chlorothalonil[u]	8.18	9	5	0.5615
Iprodione	4.26	13	15	0.0214
Fludioxonil	0.37	25	0	0.0034
Propiconazole	0.95	16	6	0.9643
Chlorothalonil[t]	6.59	12	4	0.8265

[z] Treatments represent common name of product.
[y] Represents the number of isolates that showed mycelia growth on Petri dish.
[x] Represents the number of isolates that did not show mycelia growth on Petri dish.
[w] Represents the P value from the statistical analysis between the fungicide treatments and the two untreated plots that were pooled.
[v] Chl=Chlorothalonil, Ppz=Propiconazole, Flu=Fludioxonil.
[u] Daconil Ultrex.
[t] Daconil Weatherstik.

Table 6. Influence of late-fall (snow mold) treatments on the selection of DMI resistance *S. homoeocarpa* isolates at Hickory Ridge Golf Club, 2014.

Treatments[z]	Rate (kg a.i. ha^{-1})	Number of Isolate		χ^2
		Resistant[y]	Sensitive[x]	P-value[w]
Untreated	-	32	6	-
Untreated	-	24	11	-
Propiconazole	0.44	25	7	0.8743
Propiconazole	0.87	21	11	0.7184
Chl +Ppz +Flu[v]	5.47 + 0.86 + 0.22	32	3	0.0100
Vinclozolin	1.53	26	4	0.0618
Chlorothalonil[u]	8.18	20	13	0.3808
Iprodione	4.26	34	3	0.0070
Fludioxonil	0.37	29	6	0.1252
Propiconazole	0.95	31	2	0.0047
Chlorothalonil[t]	6.59	32	7	0.1339
Penthiopyrad	0.76	22	6	0.3401

[z] Treatments represent common name of product.
[y] Represents the number of isolates that showed mycelia growth on Petri dish.
[x] Represents the number of isolates that did not show mycelia growth on Petri dish.
[w] Represents the P value from the statistical analysis between the fungicide treatments and the two untreated plots that were pooled.
[v] Chl=Chlorothalonil, Ppz=Propiconazole, Flu=Fludioxonil.
[u] Daconil Ultrex.
[t] Daconil Weatherstik.

Table 7. Influence of early-spring fungicide treatments on dollar spot infection center at Hickory Ridge Golf Club, 2014.

Treatment	Rate (kg a.i. ha^{-1})	Number of Dollar Spot Infection Center[y]			
		5/27	6/6	6/29	7/7
Untreated		6	7	10	19
Propiconazole	0.44	2	3	6	16
Propiconazole	0.87	0	0	0	4
Fluprimidol + Propiconazole	0.42 0.44	0	0	0	8
Fluprimidol	0.84	19	23	20	31
Boscalid	0.38	0	0	4	6
Chlorothalonil	8.17	5	7	8	29
Vinclozolin	1.53	0	3	2	24
P-value		0.1410	0.1463	0.3574	0.3663
DAT[x]		30	39	63	71

[z] Ratings data started once approximately 10 or more infection centers were present in all plots.
[y] Days After Treatment (DAT).

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