

**MANAGEMENT OF DAYLILY RUST WITH DIFFERENT FUNGICIDES, FUNGICIDE
COMBINATIONS, AND SPRAY INTERVALS AND THE DETERMINATION OF
FUNGICIDE SENSITIVITY PROFILES FOR *PUCCININA HEMEROCALLIDIS***

by

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(Under the Direction of JAMES BUCK)

ABSTRACT

Daylily rust, caused by *Puccinia hemerocallidis*, increasingly has become a management issue for growers throughout the southeastern United States. Fungicides remain the most effective tool in managing daylily rust. Foliar sprays of azoxystrobin, propiconazole, thiophanate-methyl, and chlorothalonil were evaluated on field-grown daylilies in 2014. Only treatments containing azoxystrobin provided acceptable rust management and all other treatments were no different than untreated controls. In 2015, foliar sprays of pyraclostrobin, tebuconazole, myclobutanil, flutolanil, pyraclostrobin + boscalid, chlorothalonil, and mancozeb were evaluated. All systemic chemicals provided acceptable levels of management; however, treatments containing tebuconazole outperformed all others. In addition, tebuconazole has the lowest material cost of all the systemic chemicals in this study. Fungicide sensitivity profiles for pyraclostrobin, flutolanil, and thiophanate-methyl were evaluated and isolates were found to be most sensitive to pyraclostrobin and least sensitive to thiophanate-methyl. The broadest range of sensitivity was observed with flutolanil.

INDEX WORDS: Daylily rust, *Puccinia hemerocallidis*, Daylily, Fungicides, Fungicide resistance, Fungicide sensitivity

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
CHAPTER	
1 INTRODUCTION	1
Pathogen Biology	3
Symptoms and Signs on Daylily	5
Plant-Pathogen Interface on Daylily	6
Epidemiology	7
Daylily Rust Management	9
Fungicides and Fungicide Resistance	11
Qualitative and Quantitative Resistance	14
Fungicide Resistance Action Committee	15
Quinone Outside Inhibitors	15
Sterol Biosynthesis Inhibitors	17
Succinate Dehydrogenase Inhibitors	19
Benzimidazoles	21
Multi-Site Inhibitors	22
Fungicide Resistance in the Rust Fungi	22
Fungicide Baselines and Sensitivity Profiles	24
Research Objectives	26

	Literature Cited	27
2	THE MANAGEMENT OF DAYLILY RUST WITH DIFFERENT FUNGICIDE COMBINATIONS AND SPRAY INTERVALS	34
	Materials and Methods.....	38
	Results.....	40
	Discussion.....	41
	Literature Cited.....	47
3	THE MANAGEMENT OF DAYLILY RUST WITH DIFFERENT FUNGICIDES AND FUNGICIDE COMBINATIONS.....	57
	Materials and Methods.....	61
	Results.....	63
	Discussion.....	65
	Literature Cited.....	72
4	THE FUNGICIDE SENSITIVITY PROFILES OF <i>Puccinia hemerocallidis</i> TO PYRACLOSTROBIN, FLUTOLANIL, AND THIOPHANATE-METHYL.....	84
	Materials and Methods.....	87
	Results.....	90
	Discussion.....	90
	Literature Cited.....	97

LIST OF TABLES

	Page
Table 2.1: Daylily rust AUDPC for field one	49
Table 2.2: Daylily rust AUDPC for field two.....	51
Table 2.3: Daylily rust AUDPC for field three.....	53
Table 2.4: Treatment contrasts for all fields	55
Table 3.1: Daylily rust AUDPC for field one	76
Table 3.2: Daylily rust AUDPC for field two.....	78
Table 3.3: Daylily rust AUDPC for field three.....	80
Table 3.4: Treatment contrasts for all fields	82
Table 3.5: Fungicide product application cost comparison	83
Table 4.1: EC ₅₀ values of <i>Puccinia hemerocallidis</i> for selected fungicides.....	101

CHAPTER 1

INTRODUCTION

Daylily (*Hemerocallis* spp.) is an herbaceous perennial plant that is very popular with landscapers, homeowners, and hybridizers. Currently, the American Hemerocallis Society (AHS) lists over 80,000 registered cultivars of daylily, making it one of the most cultivated plants over the last half century (Trotter, 2016). The World Checklist of Selected Plant Families compiled by the Royal Botanic Society recognizes 19 discreet *Hemerocallis* species and two official intra-specific hybrids. All species are native to Southeast Asia where the daylily has been long-valued in both food and medicine (Royal Botanic Gardens, 2014). The popularity of *Hemerocallis* hybrids is due to the extensive breeding efforts of hybridizers and enthusiasts worldwide. There are nearly 600 hybridizers in the U.S. alone (AHS, 2011). Daylilies are available in several flower colors, shapes, and heights. In addition, there are cultivars available for early-, mid-, and late-bloom times. Many cultivars will bloom for extended periods of time or bloom more than once during the growing season. Furthermore, the species has proven itself to be tolerant of a wide range of climates, soil types, and moisture levels. For these reasons, daylilies are one of the most economically important ornamental crops produced in the U.S.

In addition to the outstanding ornamental and cultural characteristics of the *Hemerocallis* spp., until 2000, they were considered relatively pest and disease free in the United States (Williams-Woodward and Buck, 2002). In 2000, daylily rust, caused by the fungus *Puccinia hemerocallidis*, was found in the U.S. in the state of Georgia (Williams-Woodward et al., 2001).

P. hemerocallidis was initially detected in four southeastern states: Florida, Georgia, South Carolina, and Alabama. By 2003, daylily rust had been officially reported in 23 states and unofficially in nine more. Despite federal and state quarantines, daylily rust spread rapidly throughout the country and emerged as an increasing management problem for homeowners, enthusiasts, and nurserymen alike. Currently, daylily rust is considered endemic in the U.S. in areas USDA hardiness zone 7 and greater. These include large portions of North and South Carolina, Georgia, Alabama, Mississippi, Arkansas, Texas, New Mexico, California, Washington, and Oregon. Florida and Louisiana are included completely within this area (Buck and Ono, 2012).

Initially, the detection of daylily rust in the U.S. caused panic among growers. In 2000, *P. hemerocallidis* was placed under quarantine by administrative agencies at both federal and state levels. Stop-sale orders and the destruction of infected plant material proved very costly to growers, all in attempts to eradicate this organism from the U.S. In 2003, the quarantine was lifted because it had been ineffective at containing the spread of *P. hemerocallidis* (North American Plant Protection Organization, 2003).

The failure of quarantine efforts to contain and eradicate *P. hemerocallidis* can be attributed to several factors. Rust infections are, by nature, difficult to detect at low levels and by the time infections are evident, epidemic levels of inoculum may be present. Daylilies are commonly shipped as bare-root plants that have had much of their foliage removed. Infectious lesions or individual spores may be present and unseen, sandwiched between foliage at the crown level. Rust propagules are known to be blown for thousands of miles by the winds of tropical storms. Such is the case for the dispersal of *Phakopsora pachyrhizi* and *Puccinia*

graminis f. sp. tritici, the organisms that cause soybean rust and wheat stem rust, respectively (Rupe and Sconyers, 2008).

One method of inoculum dispersal for *P. hemerocallidis* is the interstate movement of infected plant material in nursery shipments (Buck and Ono, 2012). Large numbers of plants are grown at nurseries and shipped throughout the country. These plants are sold in garden centers and “big-box” retail stores such as Home Depot and Lowe’s. This is the common practice for both susceptible and resistant varieties. Daylilies are very popular among enthusiasts and hybridizers. While the movement of plant material by nurserymen was highly scrutinized during the quarantine period, movement of daylilies by the former two groups was not regulated (Buck and Ono, 2012). From 2000 until 2003, there were few states that had any form of an effective quarantine in place. In addition, in 2000 there were no fungicides labeled for the management of daylily rust and growers had no adequate control measures initially (Buck and Ono, 2012). Currently, there are several fungicides labeled for daylily rust and some provide excellent levels of management (Williams-Woodward, 2015).

Pathogen Biology

Puccinia hemerocallidis is an obligate biotroph and can survive and reproduce only on or in living tissue. *P. hemerocallidis* is a heteroecious, macrocyclic rust. Heteroecious, which in Greek means “different houses”, describes this organism’s utilization of two separate, unrelated hosts to complete its life cycle. Daylily is the primary or telial/uredinial host and *Patrinia* spp. is the alternate, spermagonial/aecial host. *Patrinia* spp. are herbaceous perennials in the Valerianaceae family and are native to the mountain grasslands of Asia. Several species of *Patrinia* including *P. gibbosa*, *P. triloba*, *P. villosa* and *P. rupstris* are sold as landscape plants in

the U.S. and parts of Canada; however, this plant is not common in North American gardens (Celetti et al., 2004). The presence of *Patrinia* is not required for the infection of daylily or survival of the rust pathogen to take place because urediniospores produced on daylily can infect and re-infect the same and surrounding daylilies. This infection paradigm can result in destructive, localized epidemics if management steps are not implemented (Buck and Williams-Woodward, 2003). However, the aecial host is necessary for sexual reproduction to take place and to maintain genetic variability of *P. hemerocallidis*.

Two spore types are produced on *Patrinia*: spermatia (pycnospores) and aeciospores. Three spore types, urediniospores, teliospores, and basidiospores, are produced on daylily. The disease cycle for daylily rust begins in the spring when thick-walled, darkly-pigmented, diploid teliospores that have overwintered on daylily, germinate and go through meiosis to produce haploid basidiospores. Basidiospores are blown or splashed onto *Patrinia* where they germinate to form a haploid mycelium that colonizes the leaf tissue. From this mycelium, a spermogonium is formed and spermatia (pycnospores) are produced within. This spermogonium pushes through the adaxial surface of the leaf. Pycnospores are produced in a sticky substance that attracts insects and insures cross-fertilization. Cross-fertilization results in a dikaryotic mycelium that grows through the leaf tissue and forms an aecium that breaks through the abaxial leaf surface and releases aeciospores.

Haploid aeciospores are blown to daylilies which is the only plant that they can infect. The spores germinate and germ tubes are formed and penetrate the leaf surface. A dikaryotic mycelium is formed which subsequently forms a lesion on the leaf surface known as a uredinium. The uredinium breaks through the leaf surface and releases dikaryotic urediniospores or “repeating spores”. This is the most important stage of the infection for daylily because this

spore is the only one that can infect the same plant on which it is produced. The host plant can be re-infected multiple times in addition to the infection of surrounding plants. Towards the end of the season, environmental cues cause the formation of teliospores (Buck and Ono, 2012; Ono, 2003; Schumann and Leonard, 2000).

Symptoms and Signs on Daylily

Symptoms typically begin as chlorotic areas on the leaf surface 7-10 days after infection (Mueller and Buck, 2003). These areas contain the uredinia and soon after formation they erupt with orange-colored urediniospores which subsequently infect the host and surrounding plants multiple times. A single susceptible plant can produce thousands of lesions, each producing thousands of new spores (Buck and Williams-Woodward, 2003). Heavy infections do not kill plants immediately but foliage does eventually die and render plants unmarketable. This can have a significant impact on the ornamental market in the United States, which in 2014 was valued at \$562 million (United States Department of Agriculture, 2015).

With the onset of freezing temperatures, urediniospores are replaced by the dark-colored teliospores and lesions turn from rusty orange to brown or black. In the spring, teliospores germinate to form basidiospores and the complete life cycle repeats itself, if *Patrinia* is present. In warmer climates, temperatures may not drop low enough to kill all green tissue on daylily. If this is the case, urediniospores will continue to infect plants and serve as both primary and secondary inoculum (Buck and Ono, 2012).

Plant-Pathogen Interface on Daylily

Urediniospores require free moisture on leaf surfaces to infect leaf tissue. When a urediniospore lands on a daylily it forms an adhesion pad that secures it to the leaf surface and it germinates to form a germ tube. The germ tube elongates across the leaf surface, following topographical features of the plant cuticle, until it comes into contact with a stomatal guard cell. It then settles directly over the stomate where an appressorium is formed. The nuclear material and lipid energy reserves packaged in the spore enter the germ tube, leaving behind an empty spore shell. Beneath the appressorium, a single penetration hypha pushes into the sub-stomatal cavity and elongates to form the infection hypha (Li et al., 2007; Mendgen and Hahn, 2002).

The infection hypha grows intercellularly between parenchyma cells until its tip comes into contact with a host cell and forms the haustorial mother cell. It is at this point that the development of the haustorium is initiated. The differentiation of the infection hypha into the haustorial mother cell and subsequent haustorium is essential to plant-pathogen compatibility and is the hallmark of obligate biotrophy (Voegelé and Mendgen, 2011; Perfect and Green, 2001). The haustorial mother cell forms structures known as neckbands on the cell surface that assimilate the two membranes and the new penetration hypha is invaginated within the plant cell. Once inside the plant cell, the hypha enlarges to become the determinate, bulbous structure known as the haustorium, which facilitates nutrient acquisition and host immune suppression (Perfect and Green, 2001).

Although deep within the cytoplasm, the haustorium never comes in direct physical contact with any cellular contents and is separated from the host cytoplasm by multiple layers. The outermost layer, the extrahaustorial membrane (EHM), is believed to be a modified version of the conventional plant cell membrane, differing chemically and structurally and containing

both plant and fungal constituents. It is by way of the EHM that the plant plasma membrane is never physically breached, allowing the fungus to interface biochemically without triggering an immune response (Perfect and Green, 2001). The innermost layer is known as the haustorial plasma membrane and it is surrounded by the haustorial wall. The haustorial wall is separated from the EHM by the extrahaustorial matrix, an apoplastic region that appears to function as another layer of regulation (Mims et al., 2002).

Once the plant-pathogen interface has been established, the haustorium produces multiple proteins and protein complexes essential to nutrient acquisition, such as hexose transporters and amino acid transporters. In addition, the haustorium appears to fulfill multiple biosynthetic functions, such as vitamin B1 synthesis (Voegle and Mendgen, 2011). Furthermore, haustoria have been shown to produce and deliver multiple effector molecules that serve in suppression of the plant's immune system and plant metabolic regulation (Garnica et al., 2014; Koeck et al., 2011).

Essentially, the rust pathogen infects and initiates a parasitic relationship with the plant, commandeers structural and chemical components, suppresses the plant's immune response, redirects nutrient movement and deposition, and reproduces. The former are accomplished beyond host recognition and this cytological paradigm is implemented across multiple plant cells every 10-11 days, forming sporogenous basal cells in the uredinia, which erupt through the leaf epidermis to disseminate urediniospores (Garnica et al., 2014).

Epidemiology

Many daylilies are propagated in the U.S. in production nurseries. Conditions within these facilities can exacerbate the dissemination of and infection by *P. hemerocallidis*.

Typically, thousands of daylilies in 1-gallon (5.7 liter) nursery pots are packed tightly together into blocks of both resistant and susceptible varieties. Irrigation is typically overhead and leaf moisture is excessive. Air movement may or may not be adequate to dry leaf surfaces. Collectively, these factors create the perfect environment for the development and spread of daylily rust: susceptibility, proximity, and free moisture. In addition, plants being packed together into blocks may affect the ability of technicians or nursery workers to spot low levels of infection, and by the time an infection is evident, it has become a local epidemic. Furthermore, close proximity can prevent adequate fungicide coverage that can lead to both application failure and shifts in fungicide sensitivity.

After plants have reached a certain size they are loaded onto trucks and shipped via interstate to any number of ornamental markets across the country. Many large production nurseries will also sell directly to local landscape companies who may buy as needed or buy in bulk and maintain their own plant stock. These landscapers may unknowingly become an entirely new source of infected plants. Enthusiasts and hybridizers are not producing the numbers of plants that nurseries are, but they may still be shipping infected plants to other parts of the country. Thus, long-distance, large scale dispersal of *P. hemerocallidis* is by interstate shipping and the source of initial infection is typically a grower, large or small. This makes the tracking of daylily rust very difficult. Daylilies are not planted across thousands of acres of land, such as soybeans and wheat, and point sources of infection are difficult to identify. By the time an infection is evident in one part of the country, the original source may have already shipped all the infected plants out (Buck and Ono, 2012).

Originally, it was believed that *Hosta* spp. may also be a host for *P. hemerocallidis* (Ono, 2003). This would have been a disaster for the nursery industry because daylilies and hostas are

the two most popular herbaceous perennials in the U.S. Management costs for a serious leaf disease that affected both of these plant species would have been staggering. Fortunately, work that was done in Japan identified *Puccinia funkiae* as the species that infects *Hosta* spp. In many parts of Asia rust is observed on several wild species of *Hemerocallis*; however infections never reach epidemic levels or move to public or private gardens (Ono, 2003).

Daylily Rust Management

An integrated management plan (IPM) is recommended for the effective control of daylily rust. The incorporation of monitoring, removal of infected plant material (roguing), local quarantines, water management, sanitation, use of resistant cultivars, and fungicide use are all essential facets of a successful daylily rust management program. Avoiding *Patrinia* spp. will eliminate the alternate host and sever one link in the disease cycle; however, this is only important in cold climates where herbaceous daylilies are killed out in the winter. Eliminating *Patrinia* spp. does stop sexual reproduction from occurring and genetic variability from being maintained.

Monitoring may be the most time-consuming of the management strategies listed (Dreistadt, 2001). Plants should be inspected thoroughly for signs of disease as often as is possible and local quarantines should be enacted. This refers to quarantines within a nursery, garden center, or home garden. Any plant stock coming into these should be inspected for disease. The incoming stock should be set aside in a separate area away from existing plant material for several days. If disease is present, those plants should be removed and cut back immediately and the foliage should be destroyed. Gloves should be worn and workers should be aware that spores can be moved on clothing and tools.

Leaf wetness is necessary for the infection process to take place. In addition, splashing water droplets move spores to surrounding plants. Overhead watering should be avoided if it is possible. Plants should be irrigated in the morning so that the foliage can dry during the day versus being irrigated in the evening, when infection takes place (Mueller and Buck, 2003). Plant spacing should be maintained to promote maximum air flow.

The use of resistant cultivars is an effective management tool and some research has identified differential resistance. Typically, many of the hybridization efforts for ornamental plants such as *Hemerocallis* have focused on characteristics such as bloom color, size, and duration, while disease resistance is rarely an issue. For this reason, when daylily rust was discovered in the U.S. there was an immediate need to determine the resistance of existing varieties. Given that there are over 80,000 cultivars of *Hemerocallis* spp. available, there is still much work that needs to be done in this field. One study tested the resistance of 84 commercially important daylily varieties over the course of two seasons. Overall, 14 varieties (17%) were considered resistant, 13 (15%) were considered mildly resistant, 22 (26%) were considered mildly susceptible, and 37 (44%) were considered susceptible (Mueller et al., 2003).

In addition to differential resistance expressed by the plants, differential pathotypes (races) of *P. hemerocallidis* with varying levels of virulence have been identified in the southeastern U.S. Races of *P. graminis* f. sp. *tritici*, that express differential levels of virulence against separate varieties of wheat are well-characterized (Singh et al., 2008). A recent study investigated the phenotypical response of 19 daylily varieties to 16 separate isolates of *P. hemerocallidis* that were collected from across Georgia. Fifteen of the varieties expressed differential resistance to the separate isolates. This study supports the hypothesis that this phenomenon also exists within the rust-daylily pathosystem (Buck, 2013).

Fungicides and Fungicide Resistance

Fungicides are principal tools for the management of plant-pathogenic diseases in numerous crops. They are unique among pesticides in that they are not applied to kill established pests but to protect healthy plants from infection. Fungicides are applied to mitigate disease during the crop establishment period, increase crop productivity, reduce fruit discoloration and disfigurement, and elongate storage life and quality of harvested plant products (McGrath, 2004).

Humans have utilized fungicidal products such as arsenic, lime-sulfur mixtures, and mercury chloride in crop protection for hundreds of years. However, the introduction of synthetic fungicides and novel chemistries in the latter half of the 20th century represented an important step forward in plant-disease management. Newer, synthetic active ingredients exhibit an increased level of biological activity, less phytotoxicity, and streamlined application methodologies. Application of fungicides to both ornamental and food crops escalates the question of risks versus benefits. To date, multiple analyses suggest that benefits far outweigh the risks if fungicides are used judiciously and according to label recommendations (Morton and Staub, 2008).

Similar to pharmaceuticals implemented to manage disease in animals, fungicides are subject to resistance by target organisms, rendering chemical therapies ineffective. Fungicide resistance is defined as “a stable heritable trait obtained through evolutionary processes that result in a reduction in sensitivity to a fungicide by an individual fungus” (FRAC 2014). Fungicide resistance develops through a complex interaction of factors including fungicidal mode of action, pathogen biology, fungicide usage pattern, and cropping system.

Fungicides are classified according to similarities in chemical structure and biochemical mode of action. Mode of action (MOA) refers to the biochemical target of a specific active ingredient, such as disruptors of cell division, respiration inhibitors, or sterol biosynthesis inhibitors. Many of the most commercially successful active ingredients marketed today fall within these MOAs and can be referred to as having single-site modes of action. Typically, these products are systemic within the plant and utilized in both a protectant and curative management capacity (Damicone and Smith, 2009). Multi-site inhibitor fungicides have been in the market longer; yet, their modes of action are less understood than the single-site inhibitors. These active ingredients interfere with enzyme activity, leading to overall disruption of metabolism and cell integrity (Gisi and Seirotzki, 2008). Current formulations of these active ingredients are not plant systemic, although some may be locally systemic, and they are utilized as surface protectants in combination and rotation with systemic products. In addition, contact fungicides do not promote resistance development within natural populations of fungi (Schumann and D'Arcy, 2010).

Pathogen biology must also be considered when assessing risk of fungicide resistance. Fungal pathogens with inherently high reproductive rates are most likely to develop fungicide resistance because a higher level of individuals (spores) are produced and exposed to selective pressure. Subsequent reproductive cycles produce resistant individuals that come to dominate the natural population under this selective pressure (fungicide exposure). These organisms complete multiple life cycles within a single growing season and are referred to as polycyclic. Polycyclic organisms typically infect aerial plant parts such as leaves and or fruit (Schumann and D'Arcy, 2010). The uredinial stage of daylily rust is considered polycyclic.

Monocyclic organisms generally produce a single generation per growing season and comparatively low numbers of individuals are exposed to selection pressure. Therefore, the threat of fungicide resistance is less with these organisms, which include soilborne pathogens that produce fewer offspring than their aerial counterparts. Nonetheless, soilborne pathogens such as *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. are among the most destructive organisms to global agriculture annually (Schumann and D'Arcy, 2010).

Fungicide use patterns are determined by the established cropping practices for a specific crop. Many agronomic, vegetable, and fruit and nut crops possess inherently high susceptibility to single or multiple pathogens and therefore require multiple applications of fungicides to remain economically productive. However, total crop losses (% damage + cost of control) of nearly 50% persist even with fungicide implementation (Georgia Crop Loss Estimates, 2013). Repeated fungicide applications, such as with many foliar and fruit pathogens, increases the selection pressure on pathogen populations by exposing individuals to active ingredients multiple times. In addition, the exclusive usage of what are considered high-risk fungicides increases opportunities for resistance development (Damicone and Smith, 2009).

Crop production practices can either increase or decrease the overall frequency of fungicide usage. Modern agricultural practices perpetuate plant diseases by concentrating multiple prospective hosts into localized, genetically homogeneous populations. Plant pathogens will reproduce at lower rates on crop varieties that carry some level of resistance than on susceptible varieties. This may warrant fewer applications and fewer individuals exposed to the active ingredient. Host resistance should be implemented if possible; however, many varieties possessing the most desirable agronomic characteristics, e.g. yield, sugar content, fruit size and color, are often susceptible to disease (Damicone and Smith, 2009). In addition, cultural

practices such as nutrient management, irrigation management, crop rotation, and proper sanitation can all mitigate the development of fungicide resistance by decreasing the need for applications due to reduced inoculum (Damicone and Smith, 2009; McGrath, 2004).

Qualitative and Quantitative Resistance

The mechanisms that confer fungicide resistance are qualitative or quantitative.

Qualitative resistance is an abrupt decrease in fungicide sensitivity by a discrete population of a fungus to a specific mode of action, resulting in catastrophic practical failure of active ingredients within that MOA. Qualitative resistance can typically be correlated with a point mutation (single gene) in the target protein altering the amino acid(s) required for binding of the fungicidal active ingredient. With qualitative resistance even very high fungicide concentrations are ineffective against resistant individuals (Deising et al., 2008). It is believed that small, subpopulations of resistant individuals are present prior to fungicide use and the point mutations occur at low frequencies. Selective pressure subsequently increases the frequency of resistant individuals, that come to dominate the population and chemical control is lost (Damicone and Smith, 2009).

Quantitative resistance refers to the gradual, multi-step resistance due to the accumulation of mutations in multiple genes, leading to reduced sensitivity of the entire population. A slight reduction in fungicidal activity may be observed between sprays or between seasons; however, an abrupt and absolute loss of control is unlikely (Deising et al., 2008). Initially, the overall population is considered sensitive before gradually shifting towards reduced sensitivity under selective pressure (Damicone and Smith, 2009).

Fungicide Resistance Action Committee (FRAC)

The fungicide resistance action committee (FRAC) is an industry consortium and part of CropLife International. It is comprised of industry professionals from many of the largest agrochemical companies. The goal of FRAC is to identify existing and potential resistance issues, provide information that is relevant to research and product registration, and provide guidelines for fungicide resistance management. FRAC assigns all fungicide chemistries a group number based primarily on their market introduction time. In addition, fungicides are grouped according to their biochemical mode of action and determined to have a high, medium, or low risk of resistance development. Furthermore, resistance management guidelines and comments on cross-resistance are included for each group (FRAC; <http://www.frac.info/>).

Quinone Outside Inhibitors-FRAC group 11

The quinone outside inhibitors (QoIs) or strobilurins are one of the most important groups of fungicides in current use. This group was first launched into the market in 1996 and contains multiple active ingredients including pyraclostrobin, trifloxystrobin, kresoxim-methyl, and the world's best-selling fungicide, azoxystrobin. The discovery of these molecules was inspired by a naturally-occurring group of anti-fungal compounds which are derivatives of β -methoxyacrylic acid, the simplest of which being Strobilurin A, Oudemansin A, and Myxothiazole A (Bartlett et al., 2002). These compounds are produced by wood-rotting fungi within the basidiomycota phylum and include the species *Strobilurus tenacellus* and *Oudemansiella mucida*. Hence, the name strobilurins was originally given to this group of chemicals. Since their discovery, their physiological and biomolecular modes of action have

been elucidated and they have been more appropriately renamed the quinone outside inhibitors (Bartlett et al., 2002).

The natural compounds are unsuitable for agricultural use because of their rapid degradation in ultraviolet light. However, the knowledge of their structures and properties provided the starting point for independent research programs at Syngenta and BASF. The commercial products resulting from this initial research have become some of the most effective and successful fungicides currently in use (Fernández-Ortuño et al., 2010). The QoI class of fungicides demonstrate systemic (xylem mobile and acropetal) activity, many show translaminar activity, and trifloxystrobin shows vapor phase redistribution. These chemicals are utilized as protectants, curatives, and eradicants (Latin, 2011; Vincelli, 2002).

The QoI fungicides have demonstrated broad-spectrum activity (Bartlett et al., 2002). Most importantly, all of the commercialized active ingredients in this group show some level of activity against Basidiomycetes, Ascomycetes, and Oomycetes. In addition, this class of fungicides is implemented for disease control on a wide range of crops including turfgrass, ornamentals, cereals, bananas, grapes, cucurbits, tomatoes, potatoes, peanuts, pecans, cotton, soybeans, and several fruit crops. While some active ingredients show phytotoxic effects on some crops, their application to others has been known to enhance yield in a capacity other than disease control (Bartlett et al., 2002). The strobilurin “greening effect” is well documented; however, this phenomenon is poorly understood.

The quinone outside inhibitors share a common, single-site, biochemical mode of action. Group 11 fungicides interfere with the production of energy within the mitochondrial membrane. More specifically, they block the transfer of high-energy electrons at the site of quinol oxidation (the Qo site) of the cytochrome *bc₁* complex (Vincelli, 2002). This interference inhibits

respiration and prevents the production of ATP. Field resistance to the QoI fungicides has been seen in numerous fungal and oomycete species (Sierotzki et al., 2000; Gisi et al., 2000; Gisi et al., 2002).

This resistance, in all cases, correlates to point mutations in the organism's DNA that alter the target gene product and prevent the binding of the fungicide's active ingredient. Three discreet amino acid substitutions have been elucidated: G143A, F129L, and G137R. While all three confer some level of resistance, it is the changing of glycine to alanine at codon position 143 (G143A) that is always correlated with qualitative resistance and the total loss of chemical control (Grasso et al., 2006). It is for this reason that FRAC places the QoIs in the high risk category for resistance and cross-resistance within group 11 is typically complete.

Sterol Biosynthesis Inhibitors-FRAC group 3

This group of anti-fungal compounds is actually composed of four different groups, three of which are used as agricultural fungicides. The fourth group is used in human and veterinary medicine for the management of mycoses (Chambers et al., 2014; FRAC 2016). The largest of these groups is the demethylation inhibitors (DMIs) which includes the triazoles and the imidazoles. The triazoles are the largest and arguably most important group of fungicides on the market today. In 2005 they accounted for nearly 21% of the total world fungicide market (Morton and Staub, 2008). The first triazole, triadimefon (Bayleton), was brought to market in 1973 by Bayer. Since then, the catalog of triazole fungicides has grown to include active ingredients such as myclobutanil, propiconazole, tebuconazole, and prothiaconazole (Morton and Staub, 2008).

Like the QoI fungicides, the DMIs exhibit broad spectrum activity and they are used extensively on numerous agronomic, horticultural, ornamental, and plantation crops. DMI fungicides are so important to wheat production in Europe that economic impact assessments have been generated to predict the effect the loss of these compounds would have on the wheat market (Di Tullio et al., 2012). In addition, the DMIs are the only systemic chemicals that still show activity against many phytopathogenic fungi such as *Zymoseptoria tritici*, the causal agent of Septoria leaf blotch (Cools and Fraaije, 2008).

In contrast to other fungicides with single site modes of action and despite extensive long-term use, the DMIs have experienced relatively few qualitative control failures (Cools et al., 2013). When resistance has been experienced, the resistance is typically moderate to low or quantitative. In addition, cross-resistance within group three is normally incomplete and FRAC considers this group to be a medium risk for the development of resistance (Cools et al., 2013). The DMI fungicides inhibit the sterol C-14 α -demethylation of 24-methylenedihydrolanosterol which is the bio-molecular precursor to ergosterol. The production of, and orientation of the ergosterol molecule within the fungal membrane is essential to maintain fluidity and regulatory in-and efflux of cellular requirements and products (Ma and Michailides, 2005).

While the DMI fungicides exhibit a single-site mode of action, several mechanisms of resistance have been elucidated, none of which confers complete resistance (Cools et al., 2013). This type of resistance has been referred to as quantitative resistance. Four discreet mechanisms have been characterized and correlated with resistance to DMI fungicides; however, few organisms have been found to exhibit all four, with the exception of *Candida albicans*.

Mutations in the target-encoding CYP51 gene: Numerous amino acid substitutions have been found in DMI resistant fungi, including but not limited to Y137F, V136A, I381V, D134G, or

S524T. These substitutions negatively impact the binding affinities of active ingredients within group three to molecular targets (Cools and Fraaije, 2012).

Enhanced active efflux of toxic molecules: Overexpression of genes encoding efflux transporters in the ATP Binding Cassette and Major Facilitator Superfamily families is well-characterized for multi-drug resistant bacteria and pathogenic yeasts (Deising et al., 2008). The role that this mechanism plays in phytopathogenic fungi is unclear and *Botrytis cinerea* is the only plant pathogen known to possess transporters that facilitate the efflux of multiple fungicides and impact efficacy (Cools et al., 2013).

Over-expression of the CYP51 gene: This mechanism of resistance is unique for the DMIs. It is associated with insertions in the predicted promoter regions of resistant species and it has been observed in both animal and plant pathogens (Cools et al., 2012; Cools and Fraaije, 2012).

Multiple CYP51 genes: Multiple target gene paralogues have been identified in several plant pathogens (Cools et al., 2013).

Succinate Dehydrogenase Inhibitors-FRAC group 7

The succinate dehydrogenase inhibitors (SDHIs) are the fastest growing group of compounds currently on the market (Sierotzki and Scalliet, 2013). The first active ingredient in this class, carboxin, was launched in 1966 and it showed a narrow spectrum of activity (Sierotzki and Scalliet, 2013). Several of these chemicals were released from 1971 to 1997; however, they showed only slightly better activity than carboxin. In 2003, boscalid was released and this represented the first true broad-spectrum SDHI. Since then, several “second generation” active ingredients have been launched and there are currently 17 SDHI compounds on the market.

Their spectrum of activity is comparable to the QoI fungicides; however there are currently no SDHI's showing activity against oomycetes (Sierotzki and Scalliet, 2013).

The molecular target of the SDHI fungicides is the succinate dehydrogenase complex in the respiratory chain. This is known as complex two and is upstream of the binding site for the QoI fungicides. Like the QoI fungicides, the SDHIs are respiration inhibitors; however there is no documented cross resistance between these two groups (Avenot and Michailides, 2010; Gudmestad et al., 2013). There are, on the other hand, several documented cases of resistance to the SDHIs carboxin and boscalid (Gudmestad et al., 2013).

The SDH enzyme that binds to complex two, known as succinate ubiquinone oxidoreductase, is composed of four subunits: SDHA, SDHB, SDHC, and SDHD. In addition, SDH inhibitors are known to bind to two sites within complex two: the succinate-binding pocket and the ubiquinone-binding pocket. All active ingredients used in crop protection bind to the latter, corresponding with subunits B, C, and D (Sierotzki and Scalliet, 2013). Since this group has a single-site mode of action, the risk of resistance is considered medium to high and the mechanism is typically a point mutation in the target gene (Avenot and Michailides, 2010). The most common mutation is the changing of histidine (H) to tyrosine (Y) at codon 277 (H277Y); however, at least 27 mutations have been reported in field populations of multiple pathogens (Sierotzki and Scalliet, 2013). Cross-resistance is not necessarily complete within group 7 and varies depending on the binding configuration of the discrete molecules. Negative cross resistance has also been observed with this group (Fraaije et al., 2012).

Benzimidazoles-FRAC group 1

The benzimidazoles were introduced in the late 1960s and they are the oldest major group of systemic fungicides that are still on the market. At the time of their introduction they had unique properties that included broad spectrum and systemic activity, low use rates, and curative capability. These led to their great popularity among growers. It also led to their misuse and this group represents the first case of serious fungicide resistance problems (Morton and Staub 2008). Control failures occurred within a few years of their introduction and currently, more than 100 species of fungi have developed some level of resistance to the benzimidazoles including many key pathogens on economically-important crops (FRAC, 2104).

Despite catastrophic loss of efficacy for many active ingredients in this class, the benzimidazoles are still registered on over 70 crops worldwide and include the active ingredients benomyl, carbendazim, and thiophanate-methyl. They exhibit a single site mode of action and their target molecule is the cytoskeletal component β -tubulin, which is the second of two subunits that comprise microtubules. By inhibiting microtubule assembly, benzimidazoles affect a great number of indispensable cellular functions such as mitosis and meiosis, intracellular molecular and organelle transport, and preservation of cellular shape and mobility (Davidse, 1986).

Qualitative resistance is typically associated with point mutations in this gene; however, different mutations are known to confer different levels of resistance. The most common mutations are found at codons 6, 50, 198, 200, and 240 and those at 198 and 200 are considered to carry zero fitness cost (Ma and Michailides, 2005). Benzimidazole resistance is typically persistent (FRAC, 2014; Ma and Michailides, 2005). Group 1 fungicides are considered to have

a high risk of cross-resistance within their group and negative cross resistance has been observed with the N-phenylcarbamates (FRAC, 2014).

Multi-site inhibitors-Group M

Many of the oldest fungicides that are still in wide use are multi-site inhibitors that are utilized in a protectant capacity. These fungicides belong to several chemical classes such as the dithiocarbamates, phthalimides, and chloronitriles and show activity against a broad range of fungal species (Gisi and Sierotzki, 2008). They typically exhibit no systemic activity and are only effective if applied pre-infection where they inhibit spore germination through the repression of enzymatic activity (Gisi and Seirotzki, 2008). This group includes the active ingredients chlorothalonil, captan, mancozeb, and thiram. While they do not show the same versatility as the aforementioned fungicide groups, require much higher rates, and shorter spray intervals, they should be considered an important part of spray programs as rotation partners. Their multiple modes of action are often poorly understood and the introduction of new multi-site inhibitors is rare in the current market. They are generally considered a low resistance risk and no signs of resistance development have been observed (FRAC, 2013).

Fungicide Resistance in the Rust Fungi

The rust fungi are considered a low risk for developing fungicide resistance even though they exhibit many of the same biological characteristics seen in many of the fungi that are considered high risk, such as *Botrytis* and *Blumeria* spp. The rusts, as a group, produce copious amounts of airborne spores that infect tens of thousands of acres of crops that are intensively cultivated. Exposure to fungicides is high across several generations, exerting selective pressure.

The failure of rust fungi to develop widespread resistance has been ascribed to their inability as a group to express mutant genes (Oliver, 2013).

Certain organisms can express mutations in gene structure without a fitness cost more readily than others (Oliver, 2013). Many of the rust fungi have shown inability to mutate and it is hypothesized that there are two biological phenomena responsible for this: diploidy and gene structure. Of these, only gene structure can sufficiently explain the lack of rust mutants that show decreased sensitivity to fungicides (Oliver, 2013). In addition, resistance management protocols have been successful in the forestalling of fungicide resistance in the rusts. It is hypothesized that gene structure in several genera of rusts makes the mutation that confers resistance to the QoI fungicides lethal to the organism (Grasso et al., 2006). While many phytopathogenic fungi developed resistance to the QoI fungicides within 2-10 years of their introduction, no cases of practical field resistance have been observed in the rusts.

As mentioned above, the most common mutation associated with qualitative resistance to QoI fungicides is the G143A mutation (Oliver, 2013). It has been elucidated that several genera of rusts including *Puccinia*, *Uromyces*, *Phakopsora*, and *Hemileia* contain an intron immediately following the triplet which is the site of the G143A mutation (Grasso et al., 2006). The presence of this intron strongly affects splicing that is carried out during RNA processing, producing a deficient and seemingly lethal gene product. All other pathogenic fungi that demonstrate qualitative resistance to this group of fungicides possess the G143A mutation; however they do not have an intron at this position (Grasso et al. 2006). These fungi include *Alternaria alternata*, *Blumeria graminis*, *Magnaporthe grisea*, *Zymoseptoria tritici*, *Venturia inaequalis*, and *Plasmopara viticola*. It is important to note that *Alternaria solani* also contains an intron at the G143A position, yet a decrease in sensitivity to QoIs has been observed; however not of the

magnitude conferred by this mutation. It is speculated that the F129L mutation is responsible for the decreased sensitivity (Grasso et al., 2006).

Many of the DMI fungicides show efficacy against the rust fungi and their application has become a mainstay over the past thirty years. Unlike the lack of resistant rust populations observed with the QoIs, the DMIs have experienced a gradual shift toward decreased sensitivity in the cereal rusts (*Puccinia* spp.) and soybean rust (*P. pachyrhizi*) (Arduim et al., 2012; Schmitz et al., 2013). The triazoles have been used extensively over the past decade to manage soybean rust in South America and a quantitative loss in efficacy has occurred (Schmitz et al., 2013). This is thought to be due to the multiple mechanisms outlined above. However, studies show that Brazilian populations of *P. pachyrhizi* remain sensitive to the QoI fungicides (Schmitz et al., 2013).

The SDHI fungicides are used to a lesser extent for the management of rust fungi than the other two groups mentioned above. While mutations conferring resistance to the SDHIs have been elucidated in *B. cinerea*, the resistance factors are moderate (Veloukas et al., 2013). A cautious approach has been adopted by the fungicide industry in regards to SDHI usage and no field failures have been observed to this point.

Fungicide Baselines and Sensitivity Profiles

Fungicide resistance detection and management are of great importance to chemical manufacturers and crop protection specialists alike. In the absence of product management protocols, resistance could arise quickly rendering valuable chemical tools ineffective. In order to recognize resistance and correctly evaluate the efficacy of a fungicide, the response of the target fungus to that fungicide before practical exposure must be elucidated (Russell, 2004).

This practice is known as developing a sensitivity baseline for the fungus/fungicide combination. FRAC defines a sensitivity baseline as “A profile of the sensitivity of the target fungus to the fungicide constructed by using biological or molecular biological techniques to assess the response of previously unexposed fungal individuals or populations to the fungicide.”

The term baseline can be used when referring to a new chemistry; however, when assessing the sensitivity of populations that have been exposed to existing chemistries the term “sensitivity profile” is most appropriate (Russell, 2004). This profile is not constructed by evaluating a single data point, but rather by the sampling of numerous individuals and evaluation of the variability between them. This evaluation can then establish a point of reference or baseline, above which an individual can be referred to as less sensitive or resistant (Franke et al., 1998; Gudmestad et al., 2013; Miller et al., 2002; Thomas et al., 2012; Schmitz et al., 2013; Vincelli and Dixon, 2002). This reference point is expressed as a concentration of active ingredient, usually but not necessarily in $\mu\text{g/ml}$. Furthermore, this point is referred to as the EC_{50} , ED_{50} , IG_{50} , or IC_{50} depending on the specific organism and assay methodology. All of these refer to the concentration of fungicide needed to achieve 50% inhibition of maximal growth and may consider mycelial growth, spore production, or lesion formation. Sample size and testing procedure will also vary depending on the organism, e.g. facultative parasites versus obligate biotrophs (Russell, 2004).

Research Objectives

Objective One

To determine the most effective fungicides, fungicide combinations, and application intervals for managing daylily rust on field-grown plants.

Objective Two

To determine the fungicide sensitivity profiles of *Puccinia hemerocallidis* to pyraclostrobin, flutolanil, and thiophanate-methyl.

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CHAPTER 2
MANAGEMENT OF DAYLILY RUST WITH DIFFERENT FUNGICIDE
COMBINATIONS AND SPRAY INTERVALS¹

¹ Emmitt, R.S., Stevenson, K.L., Brenneman, T.B., and Buck, J.W. 2016. Management of daylily rust with different fungicide combinations and spray intervals. *Plant Disease*. 100:188-191.
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ABSTRACT

Daylily (*Hemerocallis* spp.) is a popular herbaceous perennial plant and was considered to be relatively disease free until 2000, when daylily rust, caused by *Puccinia hemerocallidis*, was first detected in the U.S. Management of daylily rust in nurseries is dependent on the use of fungicides, which are typically applied to the foliage of large blocks of plants at 21- or 28-day intervals. The objectives of this study were to determine the most effective fungicides or fungicide combinations and application intervals for managing daylily rust in the field. Foliar sprays of azoxystrobin alone at 14-, 21-, or 28-day intervals, combinations of azoxystrobin + propiconazole, azoxystrobin + chlorothalonil, propiconazole + chlorothalonil, and chlorothalonil + thiophanate-methyl applied at intervals of 21 or 28 days, and a non-treated control were evaluated under high disease pressure, at three locations in Griffin, GA in 2014. In all three fields, all treatments that included azoxystrobin were effective at reducing area under the disease curve (AUDPC) compared to the non-treated control. At two of the three locations, azoxystrobin applied at 14- day intervals had significantly lower AUDPC than when applied at 21- or 28-day intervals. The addition of propiconazole or chlorothalonil to azoxystrobin did not improve rust control. Disease ratings for propiconazole + chlorothalonil and thiophanate-methyl + chlorothalonil applied at 21- or 28-day intervals did not differ from the untreated control. The 21-day treatments resulted in significantly lower disease than 28-day treatments (all fungicides) in the middle and end of the season. Elimination of less efficacious active ingredients and unnecessary applications can help growers maximize profitability by reducing expenses as well as simplifying inventory and storage.

INTRODUCTION

Daylily rust, caused by the fungus *Puccinia hemerocallidis* (Thüm.), has become an increasing problem for daylily growers since its introduction into the U.S. in 2000 (Williams-Woodward et al., 2001). Symptoms on daylily foliage include initial chlorotic spots that become orange, spore-producing lesions followed by foliage dieback. Infected plants are typically unmarketable and daylily rust can cause significant, negative effects on the ornamental market in the U.S. which was valued at \$602 million in 2013 (USDA, 2013).

Daylilies (*Hemerocallis* spp.), are native to Asia and are one of the most widely cultivated plants in the world (Gatlin, 1999). They were considered to be relatively disease- and pest-free in the U.S. until daylily rust arrived (Williams-Woodward and Buck, 2002). *P. hemerocallidis* was initially detected in the southeastern U.S. states of Florida, Georgia, South Carolina, and Alabama (Williams-Woodward et al., 2001). In 2000, *P. hemerocallidis* was placed under state and federal quarantine; the resulting stop-sale orders and destruction of infected plant materials were very costly to growers. In 2003, daylily rust was officially reported in 24 states and unofficially in nine more and the federal quarantine was lifted (Buck and Ono, 2012). *P. hemerocallidis* is currently considered endemic in the U.S. in all U.S.D.A hardiness zones 7 or greater (Wise et al., 2004).

Host resistance to *P. hemerocallidis* has been observed in *Hemerocallis* spp. Of the 84 commercially important daylily varieties assessed for resistance in greenhouse assays, 14 (17%) were resistant, 13 (15%) were mildly resistant, 22 (26%) were mildly susceptible, and 37 (44%) were susceptible (Mueller et al., 2003). There are nearly 80,000 daylily cultivars registered with the American Hemerocallis Society and the phenotype for most cultivars is unknown. Pathotypes (races) of *P. hemerocallidis* were identified in the southeastern U.S., suggesting that host

resistance could be overcome by the fungus (Buck, 2013). Fifteen of the 19 cultivars tested expressed differential resistance to 16 isolates of *P. hemerocallidis*.

Fungicides remain the most effective method for managing daylily rust. Fungicides with active ingredients from several fungicide classes are currently labeled for the management of daylily rust. Products are available for both commercial growers and homeowners to be used in rotation on 7-, 14-, 21-, or 28-day intervals (Buck and Ono, 2012; Williams-Woodward, 2015). Many commercial growers apply fungicides on 21- or 28-day intervals over large blocks of multiple species and many provide excellent rust control (Mueller et al., 2004; Buck and Williams-Woodward, 2003; Buck and Youmans, 2007; Dong et al., 2013). Azoxystrobin or pyraclostrobin (FRAC group 11), chlorothalonil (FRAC group M5), myclobutanil, propiconazole, triadimefon or tebuconazole (FRAC group 3), and flutolanil (FRAC group 7) all give some level of control when applied prior to disease onset. Azoxystrobin, chlorothalonil, myclobutanil, propiconazole, and triadimefon reduced lesion development by *P. hemerocallidis* on daylily when applied as foliar sprays up to 15 days prior to inoculation while azoxystrobin was shown to significantly reduce lesion formation when applied 7 days after inoculation (Mueller et al., 2004). Azoxystrobin was also shown to significantly reduce disease severity for up to 9 weeks post-application when used as a root dip and as a single soil drench treatment (Dong et al., 2013). Azoxystrobin, chlorothalonil, flutolanil, mancozeb, propiconazole, and triadimefon each significantly reduced lesion formation by *P. hemerocallidis* when assessed 15 days postinoculation (Buck and Williams-Woodward, 2003). In this greenhouse study, fungicides were applied as foliar sprays 24 h prior to inoculation and results were consistent across the five trials.

Currently, growers, hybridizers, and homeowners have no definitive chemical combination or application interval for the management of daylily rust. While many chemicals and application intervals are recommended, the elimination of the least effective treatment program can help growers to maximize profitability and decrease applicator exposure. The objectives of this study were to determine the most effective timing intervals and chemical combinations for managing daylily rust on field-grown daylilies.

MATERIALS AND METHODS

Daylily field plantings: Field trials were conducted in 2014 at the Griffin campus of the University of Georgia on Cecil sandy clay loam (pH 6.2, 1.9% organic matter). Three separate fields were used for this study: field 1 is approximately 1.6 km from field 2 and approximately 2.4 km from field 3; field 2 is approximately 0.8 km from field 3. Field 3 and all plants within were used in field trials conducted in 2010 and 2011 (Dong et al., 2013). Fields 1 and 2 were cultivated and planted 2 weeks apart in May, 2014 using bare-root plants of the rust-susceptible cultivar ‘Pardon Me’ (Mueller et al., 2003). All study areas were covered with weed barrier fabric (Greenscapes Inc., Calhoun, GA) prior to planting and covered with 5 to 10 cm of pine bark mulch after planting. Plants were irrigated as needed and fertilized with 10-10-10 soluble fertilizer (Farmer’s Favorite Fertilizer, Evergreen, AL) at 5.7 kg ha⁻¹ every 2 months. Weeds were managed with Gly Star Plus (Albaugh LLC, St. Joseph, MS) non-selective glyphosate herbicide and SedgeHammer, halosulfuron-methyl (Gowan Turf and Ornamental, Yuma, AZ).

Experimental design and fungicide treatments: The experiment was conducted in three fields, each of which consisted of 144 plants, arranged in 12 rows of 12 plants each, spaced 0.6 m within rows and 1.0 m between rows. Each row was divided into 4 replications (experimental

units) consisting of 3 consecutive plants. In total, 432 plants were evaluated in this study. The experimental design was completely randomized with 11 fungicide treatments and one untreated control, each replicated 4 times. Treatments were randomly assigned to experimental units within each field using Agricultural Research Manager (ARM) software (Gylling Data Management Inc., Brookings SD). Thirty six daylily (cultivar Pardon Me) plants in 5.7-liter containers were inoculated with *P. hemerocallidis* isolate Grif 2 (Buck et al., 2010; Buck, 2013) and kept in a greenhouse for 3 weeks. In June, 2014 twelve rust-infected daylily plants were planted into each field. Infected daylilies were spaced evenly throughout each field and planted between rows.

Label rates of azoxystrobin (Heritage 50 WDG, Syngenta Crop Protection Inc., Greensboro, NC) at 0.32 ml liter⁻¹, propiconazole (Banner MAXX 14.3 MEC, Syngenta) at 0.62 ml liter⁻¹, chlorothalonil (Daconil Ultrex 82.5 WDG, Syngenta) at 1.60 g liter⁻¹, and thiophanate-methyl (Clearys 3336 F, Cleary Chemical Corporation, Dayton, NJ) at 0.94 ml liter⁻¹ were applied as foliar sprays. Azoxystrobin alone was applied at 14-, 21-, and 28-day intervals. In addition, azoxystrobin was applied in combination with either propiconazole or chlorothalonil at 21- and 28-day intervals. Propiconazole was applied in combination with chlorothalonil at 21- and 28-day intervals and chlorothalonil + thiophanate-methyl at 21- and 28-day intervals. All fungicide treatments began on 3 Jul, 2014. The 14-day treatment was applied eight times. The 21-day treatments were applied six times and 28-day treatments were applied four times throughout the growing season. All fungicides were applied using a CO₂-pressurized (276 kPa) backpack sprayer using a single flat-fan air-induction nozzle (11002VS; TeeJet Technologies, Wheaton, IL). Initially, a volume of 250 ml of fungicide was applied to each experimental unit of three daylily plants and each plant was sprayed until runoff. The volume of fungicide solution

required to wet the foliage in each block was increased during the experiment as the plants grew larger.

Data collection and analysis: Rust was assessed weekly for 12 weeks starting from the first appearance of widespread symptoms on the non-treated controls (31 Jul), which was approximately 4 weeks after the first fungicide application. Disease intensity was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al. 2103). The youngest third of leaves on the plant were considered new leaves. All remaining leaves were considered old leaves. For each location, disease ratings from all dates were used to calculate area under the disease progress curve (AUDPC) to examine treatment effects through the season. AUDPC values were calculated 4 weeks, 8 weeks and 12 weeks after rust symptoms were first observed. AUDPC values were analyzed using PROC MIXED of SAS, version 9.4 (SAS Institute, Cary, NC) statistical software. Single-degree-of-freedom linear contrasts were calculated to compare specific groups of treatments.

RESULTS

Disease pressure was high in all three fields, with severity ratings on the non-treated control plants reaching 5.0 (>20 lesions on all leaves) on 24 Sep, 17 Sep, and 23 Oct in fields 1, 2 and 3, respectively.

All azoxystrobin treatments were effective at reducing disease severity compared to the non-treated control at 4, 8, and 12 weeks (21 Aug, 24 Sep, and 23 Oct) after the first appearance of rust in all three fields (Table 2.1). AUDPC of the 14-day treatment was significantly lower

than the 28-day treatment in at all assessment dates with the exception of 21 Aug (4 weeks after symptom development) on field 2. AUDPC values of the 21-day azoxystrobin treatments were typically intermediate between those values of the 14- and 28-day azoxystrobin treatments. In general, disease increased with a longer application interval of azoxystrobin. The 21- and 28-day azoxystrobin treatments had significantly lower AUDPC compared to propiconazole + chlorothalonil or thiophanate methyl + chlorothalonil on all assessment dates. The AUDPC for the propiconazole + chlorothalonil and thiophanate methyl + chlorothalonil treatments at 21- and 28-day application intervals did not significantly differ from the non-treated control on the majority of the assessment dates at all locations.

When specific groups of fungicides were compared using linear contrasts, the AUDPC values of plants that received the 14-day azoxystrobin treatment was consistently lower than AUDPC of those that received the 21- and 28-day treatments on all dates with the exception of 21 Aug in field 2 (Table 2.2). AUDPC of 21-day treatments was consistently lower than 28-day treatments with the exception of the 4-week AUDPC (21 Aug) in fields 1 and 2. Treatments containing azoxystrobin had lower AUDPC values than the four treatments not containing azoxystrobin. The AUDPC for treatments that included propiconazole, chlorothalonil, and thiophanate-methyl did not differ from that of the non-treated control (Table 2.2).

DISCUSSION

Several fungicides have are effective in managing daylily rust caused by the fungus *P. hemerocallidis* (Buck and Williams-Woodward, 2003; Buck and Youmans, 2007; Dong et al., 2013; Mueller et al., 2004). The four fungicides used in this study were selected because all are recommended for managing daylily rust when applied on 14-, 21-, and 28-day intervals

(Williams-Woodward, 2015). In the present study, azoxystrobin provided superior season-long rust control, at all three spray intervals, at three study locations.

Container nurseries typically spray large blocks of plant material on 21- or 28-day intervals. The blocks may include daylily cultivars that differ in rust susceptibility and/or multiple plant species. While disease management is of paramount concern for growers, spray practices are matters of economics and convenience. Nevertheless, no definitive fungicide combination or spray interval for the management of daylily rust has been determined. Professional recommendations include 18 different fungicides from six chemical classes: the quinone outside inhibitors (QoIs, FRAC code 11), demethylation inhibitors (DMIs, 3), methyl benzimidazole carbamates (MBCs, 1) succinate dehydrogenase inhibitors (SDHIs, 7), chloronitriles (M5), and dithiocarbamates (M3) (Williams-Woodward, 2015). Application interval recommendations vary from once a week to once a month and are predicated on the expected disease management outcome.

The level of disease tolerated by growers, hybridizers, and homeowners differs. Production nurseries will allow for low levels of disease because, in regards to disease, there are no protocols for determining whether or not a plant should be sold. In addition, it is too labor intensive to monitor low levels of disease given the large inventory of container nurseries (Dreistadt, 2001). Typically, thousands of daylilies in 5.7-liter nursery pots are packed tightly together into blocks of both resistant and susceptible varieties. Overhead irrigation is common and leaf moisture is often excessive. Collectively, these factors create the perfect environment for the development and spread of daylily rust: susceptibility, proximity, and free moisture. After plants have reached a certain size, they are shipped to ornamental wholesale and retail outlets across the country (Buck and Ono, 2012). In the fall, daylily plants are commonly shipped as

bare-root plants with much of their foliage removed. Infectious lesions or individual spores may be present but unseen between overlapping foliage at the base of the plant (Wise et al., 2004).

Given these circumstances and the cost of 7- and 14-day treatment intervals, container nurseries typically apply fungicides on 21- and 28-day schedules. Our field study showed that fungicide azoxystrobin provided a significant disease reduction at 14-, 21-, and 28-day application intervals compared with propiconazole, chlorothalonil, or thiophanate-methyl. Azoxystrobin did not prove as effective in controlling daylily rust when applied at 28-day intervals compared with 14- and 21-day intervals. Nonetheless, disease was found in all treatments and no application interval or spray combination completely eliminated disease. Therefore, given the allowance of low level disease, a 28-day application containing azoxystrobin would be acceptable for container nurseries; however, a 14- or 21-day application would be recommended.

According to the American Hemerocallis Society (www.ahs.org) there are nearly 600 daylily hybridizers in the U.S. Hybridizers operate on a smaller scale (fewer plants, smaller acreage) and typically require a higher level of disease management than container nurseries. Many of their cultivars may be new introductions with unknown levels of rust resistance (Mueller et al., 2003). Hybridizers ship plant selections to homeowners and other hybridizers and require that their plants be free of all disease before shipment. Therefore, hybridizers seek to eradicate daylily rust at their production facilities. No single treatment in our study eradicated daylily rust; even plants treated with azoxystrobin on 14-day intervals displayed some rust. Hybridizers should be advised to spray on 7-day intervals (Mueller et al., 2004). The level of disease management required by a homeowner will vary significantly. Homeowners are not

shipping plants and they are not subject to any professional standards. Some disease, if noticed, will typically be tolerated.

Azoxystrobin provided the highest level of disease management in our study and combining it with other fungicides did not enhance efficacy. While our study examined the use of foliar sprays, prior work has shown azoxystrobin to be effective at managing daylily rust when applied as a soil drench and a root dip (Dong et al. 2013). Azoxystrobin belongs to the quinone outside inhibitor class of fungicides (FRAC group 11), which inhibit cellular respiration. The active ingredients in this group are systemic, broad-spectrum, and are used as protectants, curatives, and eradicants (Bartlett et al., 2002; Vincelli, 2002). Pyraclostrobin is another active ingredient in the same group that is effective at managing daylily rust when applied as a foliar spray (Buck and Youmans, 2007). In addition, azoxystrobin, pyraclostrobin, and two other QoI fungicides labelled for daylily rust, fluoxastrobin and trifloxystrobin, have been shown to significantly reduce urediniospore production by *Puccinia triticina* and *P. hemerocallidis* (Buck et al., 2011).

With AUDPC values similar to the untreated control, propiconazole + chlorothalonil and thiophanate-methyl + chlorothalonil applied at 21- or 28-day intervals failed to protect daylily plants from rust. In addition, the latter fungicide treatments were ineffective when compared with azoxystrobin on each assessment date at all three locations. Propiconazole, a DMI, and thiophanate-methyl, an MBC, were equally ineffective in controlling daylily rust. Previously, Dong et al. (2013) noted that propiconazole was ineffective at managing daylily rust when applied on a 14-day interval, under high disease pressure. Likewise, thiophanate-methyl failed to affect urediniospore production by *P. hemerocallidis* when applied post-inoculation (Buck et al., 2011). Nonetheless, both of these active ingredients are recommended for daylily rust on 21- and

28-day intervals. Our study has shown both of these systemic fungicides applied at these intervals to be ineffective at managing daylily rust under high disease pressure.

Chlorothalonil is a protectant fungicide and is more effective for managing daylily rust than propiconazole when applied as a foliar spray every 14 days (Dong et al., 2013). In addition, inhibition of urediniospore germination by chlorothalonil was similar to that of azoxystrobin and trifloxystrobin (Mueller et al., 2005). Since chlorothalonil has no systemic activity, it must be applied more frequently than systemic chemicals. It is typically used in combination and rotation with systemic fungicides; however, it had no effect on rust development in our study.

There is no definitive spray program for the management of daylily rust. Several active ingredients are recommended to be used from once a week to once a month. Growers could avoid unnecessary exposure and streamline chemical inventories by eliminating ineffective active ingredients. Two of the most commonly recommended fungicide active ingredients for management of daylily rust, propiconazole and thiophanate-methyl, failed to provide acceptable disease reductions when applied at 21- and 28-day intervals. Azoxystrobin provided acceptable disease control when applied at 28-day intervals and excellent disease control when applied at 14- and 21-day intervals. The QoI fungicide exhibited the highest level of management against *P. hemerocallidis*. Utilizing this class of active ingredients in rotation and combination with contact protectants such as chlorothalonil may provide growers with the most effective management of daylily rust. The Fungicide Resistance Action Committee classifies the QoI fungicides as being in the high risk category for development of fungicide resistance in target populations and recommends a rotation with active ingredients with different modes of action. However, no practical control failures have been seen in the QoI group against *Puccinia* spp. (Schmitz et al., 2013). Thus, incorporation of QoI fungicides into management plans for daylily

rust may reduce the development of resistance in *Puccinia hemerocallidis* to other high risk fungicide active ingredients.

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Table 2.1. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 1 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^a	AUDPC (date) ^b		
	21 Aug	24 Sep	23 Oct
Az 14d	0.8 d	6.1 e	16.9 d
Az 21d	1.2 cd	7.2 cde	19.8 cd
Az+Pr 21d	0.6 d	5.9 e	18.3 cd
Az+Cl 21d	0.6 d	6.3 de	17.2 d
Pr+Cl 21d	4.0 a	15.1 a	38.0 a
Th+Cl 21d	4.5 a	15.6 a	39.1 a
Az 28d	2.4 bc	9.7 b	25.8 b
Az+Pr 28d	2.4 bc	8.3 bcd	24.5 b
Az+Cl 28d	1.4 cd	8.4 bc	20.7 c
Pr+Cl 28d	4.3 a	14.4 a	36.2 a
Th+Cl 28d	3.8 ab	15.7 a	38.9 a
Nontreated control	4.5 a	15.4 a	39.6 a

^a Fungicide treatments included azoxystrobin (Az) alone or combinations of azoxystrobin, propiconazole (Pr), chlorothalonil (Cl), and thiophanate methyl (Th) at 14-day (d), 21-day or 28-day applications.

^b Rust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$.

Table 2.2. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 2 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^a	AUDPC (date) ^b		
	21 Aug	24 Sep	23 Oct
Az 14d	4.2 b	6.3 e	21.0 de
Az 21d	4.4 b	9.3 bcd	27.0 bc
Az+Pr 21d	2.1 c	7.1 e	20.3 e
Az+Cl 21d	5.4 b	8.7 cd	27.1 bc
Pr+Cl 21d	7.8 a	16.3 a	43.7 a
Th+Cl 21d	7.7 a	16.3 a	43.2 a
Az 28d	5.5 b	10.8 b	30.6 b
Az+Pr 28d	4.7 b	7.7 de	24.5 cd
Az+Cl 28d	5.3 b	9.7 bc	30.4 b
Pr+Cl 28d	7.5 a	16.5 a	42.7 a
Th+Cl 28d	8.4 a	17.7 a	45.6 a
Nontreated control	8.4 a	17.4 a	45.2 a

^a Fungicide treatments included azoxystrobin (Az) alone or combinations of azoxystrobin, propiconazole (Pr), chlorothalonil (Cl), and thiophanate methyl (Th) at 14-day (d), 21-day or 28-day applications.

^b Rust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$.

Table 2.3. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 3 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^a	AUDPC (date) ^b		
	21 Aug	24 Sep	23 Oct
Az 14d	6.5 de	9.5 e	28.4 f
Az 21d	8.1 bcd	10.5 e	32.8 de
Az+Pr 21d	5.9 e	9.9 e	30.8 ef
Az+Cl 21d	6.1 e	10.2 e	30.8 ef
Pr+Cl 21d	9.3 b	15.2 b	43.2 b
Th+Cl 21d	11.6 a	14.6 bc	44.8 ab
Az 28d	9.3 b	12.5 d	36.6 cd
Az+Pr 28d	6.7 cde	10.7 e	34.3 cde
Az+Cl 28d	8.2 bc	13.4 cd	38.2 c
Pr+Cl 28d	9.6 b	16.1 ab	43.8 ab
Th+Cl 28d	11.8 a	17.0 a	47.3 a
Nontreated control	11.6 a	16.0 ab	46.6 ab

^a Fungicide treatments included azoxystrobin (Az) alone or combinations of azoxystrobin, propiconazole (Pr), chlorothalonil (Cl), and thiophanate methyl (Th) at 14-day (d), 21-day or 28-day applications.

^b Rust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$.

Table 2.4. *P*-values of single-degree-of-freedom linear contrasts of specific fungicide treatments to reduce daylily rust.

Contrasts ^a	Field 1			Field 2			Field 3		
	21 Aug	24 Sep	23 Oct	21 Aug	24 Sep	23 Oct	21 Aug	24 Sep	23 Oct
treatments	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
11 treatments vs. control	0.0005	<0.0001	<0.0001	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
14-day treatment vs. 21-day treatments	0.0283	<0.0001	<0.0001	0.0926	<0.0001	<0.0001	0.0105	0.0002	<0.0001
14-day treatment vs. 28-day treatments	0.0015	<0.0001	<0.0001	0.0090	<0.0001	<0.0001	0.0002	<0.0001	<0.0001
21-day treatments vs. 28-day treatments	0.0529	0.0066	0.0012	0.0792	0.0110	0.0037	0.0132	<0.0001	0.0003
7 Az treatments vs. 4 treatments without Az	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
1 14-day Az vs. 3 21-day Az	0.9573	0.6631	0.3070	0.5185	0.0035	0.0137	0.8076	0.2942	0.0603
Untreated control vs. 4 non-Az treatments	0.5087	0.7653	0.2810	0.7859	0.2528	0.3130	0.1101	0.7039	0.2465

^aContrasts ($P=0.05$) were made between groups of treatments: all 21-day treatments, all 28-day treatments, all treatments including azoxystrobin (Az), all treatments without azoxystrobin

CHAPTER 3
MANAGEMENT OF DAYLILY RUST WITH DIFFERENT FUNGICIDES AND
FUNGICIDE COMBINATIONS²

² Emmitt, R.S. and Buck, J.W. To be submitted to Plant Disease

ABSTRACT

Daylily rust, caused by the fungus *Puccinia hemerocallidis* (Thum), has become a serious management problem for daylily growers in the southeastern United States since 2000, when it was introduced. Production nurseries and hybridizers rely heavily on the use of fungicides to manage daylily rust, which can render plants unmarketable if not controlled. The objectives of this study were to determine the most effective fungicides and fungicide combinations for managing daylily rust on field-grown daylily plants. Foliar sprays of pyraclostrobin, flutolanil, tebuconazole, myclobutanil, chlorothalonil, mancozeb, pyraclostrobin + boscalid, flutolanil + tebuconazole, flutolanil + myclobutanil, flutolanil + chlorothalonil, and flutolanil + mancozeb applied on 14-day intervals, and a nontreated control were evaluated under high disease pressure, at three locations in Griffin, GA in 2015. All treatments were effective at reducing area under the disease progress curve (AUDPC) in comparison to the nontreated control. All treatments containing tebuconazole consistently had significantly lower AUDPC than all other treatments on all but two assessment dates. The combination of tebuconazole + flutolanil resulted in a significantly lower AUDPC than all other treatments on the end-of-season assessment date (5 Nov), in fields two and three. On all but one assessment date, there was no difference observed between treatments containing pyraclostrobin and pyraclostrobin + boscalid. The addition of flutolanil to chlorothalonil and mancozeb did not improve rust control and no difference in disease severity was observed in any treatment containing contact fungicides on all assessment dates. The determination of fungicide performance is paramount to the development of the most effective fungicide rotations for managing daylily rust.

INTRODUCTION

Daylily (*Hemerocallis* spp.) is one of the most widely cultivated plants in the world, with over 80,000 cultivars registered with the American Hemerocallis Society. It is an herbaceous perennial, native to Asia that is used extensively in landscapes throughout the United States (Gatlin, 1999). Much of daylily's popularity is due to the fact that it was considered relatively disease and pest free in the United States until 2000, when daylily rust was first detected (Williams-Woodward et al., 2001).

Daylily rust, caused by the basidiomycete fungus *Puccinia hemerocallidis* (Thum), was initially found in the southeastern states of Georgia, Alabama, Florida, and South Carolina in 2000 (Williams-Woodward et al., 2001). By 2003, daylily rust was reported in 33 states and is currently considered endemic in all U.S. Department of Agriculture hardiness zones 7 or greater (Wise et al., 2004). Daylily rust has become a significant management problem for daylily growers in the southeastern United States given that infected plants are typically unmarketable. This disease can significantly impact the ornamental market in the United States, which in 2014 was valued at \$562 million (United States Department of Agriculture, 2015).

Puccinia hemerocallidis is a macrocyclic, heteroecious rust. The uredinial/telial host is daylily and the alternate, spermagonial/aecial host is *Patrinia* spp., an herbaceous perennial in the Valerianaceae family, native to Asia (Hiratsuka et al. 1992). The presence of *Patrinia* is not required for infection of daylily or survival of the pathogen because urediniospores produced on daylily can infect and re-infect the same and surrounding daylilies. This can result in destructive, localized disease epidemics if management steps are not implemented (Buck and Williams-Woodward, 2003).

The use of resistant cultivars is an effective tool in managing daylily rust (Buck and Ono, 2012); however, the rust reaction phenotype of most of 80,000 cultivars of daylily is unknown. Eighty-four commercially important cultivars were assessed for resistance in greenhouse assays in 2002. Of these, 70% were mildly susceptible, or susceptible to rust (Mueller et al., 2003).

Growers rely heavily on fungicides and they remain the most effective tool for managing daylily rust. Active ingredients from several fungicide classes are currently labeled for application on 7-, 14-, 21-, and 28-day intervals and are available to both commercial growers and homeowners (Williams-Woodward, 2015). Many of these fungicides provide excellent rust management (Buck and Youmans, 2007; Dong et al., 2013; Mueller et al., 2004). Foliar sprays of azoxystrobin (Fungicide Resistance Action Committee [FRAC] group 11) at 14-, 21- or 28-day intervals provided season-long management of daylily rust at three locations in Griffin, GA in 2014 (Emmitt et al., 2016). At two of the three locations, azoxystrobin applied at 14-day intervals provided a higher level of management than 21- and 28-day azoxystrobin treatments. All treatments containing azoxystrobin were effective at reducing area under the disease progress curve (AUDPC) compared to combinations of propiconazole + chlorothalonil (FRAC groups 3 and M5) and thiophanate-methyl (FRAC group 1) + chlorothalonil applied at 21- or 28-day intervals. The combinations mentioned above did not differ from the untreated control. The addition of propiconazole or chlorothalonil to azoxystrobin did not improve efficacy (Emmitt et al., 2015).

There is currently no specific chemical combination and spray interval recommended for the management of daylily rust; however, prior work has shown that application intervals greater than 14 days may not provide an acceptable level of efficacy under high disease pressure (Emmitt et al., 2016). The objectives of this study were to determine the most effective

chemicals other than azoxystrobin and chemical combinations applied at 14-day intervals for managing daylily rust on field-grown daylily plants.

MATERIALS AND METHODS

Daylily field plantings: Field trials were conducted in 2015 at the Griffin campus of the University of Georgia on Cecil sandy clay loam (pH 6.2, 1.9% organic matter). Three separate fields used in our previous study (Emmitt et al., 2016) were used for this study. The three locations had weed barrier fabric (Greenscapes Inc., Calhoun, GA) covered with 5 to 10 cm of pine bark mulch. Plants were irrigated as needed and fertilized with 10-10-10 soluble fertilizer (Farmer's Favorite Fertilizer, Evergreen, AL) at 5.7 kg ha⁻¹ every 2 months. Weeds were spot-treated with SedgeHammer (halosulfuron-methyl; Gowan Turf and Ornamental, Yuma, AZ) and Gly Star Plus (Albaugh LLC, St. Joseph, MS) non-selective glyphosate herbicide.

Experimental design and fungicide treatments: The experimental design was the same as outlined in Emmitt et al. (2016). Briefly, each experimental field had 144 plants, arranged in 12 rows of 12 plants each, spaced 0.6 m within rows and 1.0 m between rows. Each row was divided into four replications (experimental units) consisting of three consecutive plants. The experimental design was completely randomized with 11 fungicide treatments and one untreated control, each replicated 4 times. Treatments were randomly assigned to experimental units within each field using Agricultural Research Manager (ARM) software (Gylling Data Management Inc., Brookings SD). Isolate Grif2 (Buck, 2013) of *P. hemerocallidis* was maintained on 'Pardon Me' daylilies in a greenhouse. Fresh urediniospores were collected from sporulating lesions using a vacuum spore collector as described by Mueller et al. (2003). Urediniospores were suspended in sterile water at a concentration of 1×10^5 urediniospores ml⁻¹ with 0.05% Tween

20(J.T. Baker; Phillipsburg, N.J.). This solution was used to inoculate existing spreader plants (Emmitt et al., 2016) in all three fields in June 2015.

Fungicides at label rates were applied on 14-day intervals. Flutolanil was applied alone or in combination with tebuconazole, myclobutanil, chlorothalonil, or mancozeb. Pyraclostrobin (Insignia SC, BASF Corporation, Research Triangle Park, NC) at $0.46 \text{ g liter}^{-1}$, flutolanil (Prostar 70 WP, Bayer Cropsience LP, Research Triangle Park, NC) at $0.46 \text{ g liter}^{-1}$, tebuconazole (Torque, Cleary Chemicals LLC, Dayton, NJ) at $0.53 \text{ ml liter}^{-1}$, myclobutanil (Eagle 20 EW, Dow AgroSciences LLC, Indianapolis, IN) at $0.94 \text{ ml liter}^{-1}$, chlorothalonil (Daconil Ultrex 82.5 WDG, Syngenta Crop Protection Inc., Greensboro, NC) at $1.60 \text{ g liter}^{-1}$, mancozeb (Dithane, Dow AgroSciences) at $1.80 \text{ g liter}^{-1}$, and pyraclostrobin + boscalid (Pageant, BASF Corporation) at $0.94 \text{ g liter}^{-1}$ were applied as foliar sprays. All fungicide treatments began on 7 July, 2015 and were applied eight times throughout the growing season. All fungicides were applied to runoff using a CO_2 -pressurized (276 kPa) backpack sprayer using a single flat-fan air-induction nozzle (11002VS; TeeJet Technologies, Wheaton, IL).

Data collection and analysis: Disease intensity was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103, Emmitt et al., 2016). The youngest third of leaves on the plant were considered new leaves. All remaining leaves were considered old leaves. Rust was assessed on individual plants weekly for 12 weeks starting from the first appearance of widespread symptoms on the non-treated controls (6 August), which was approximately 4 weeks after the first fungicide application. For each location, disease ratings from all dates were used to calculate area under the disease progress curve (AUDPC) to examine treatment effects through

the season. AUDPC values were calculated 4 weeks, 8 weeks and 12 weeks after rust symptoms were first observed. AUDPC values were analyzed using PROC MIXED of SAS, version 9.4 [statistical software] (SAS Institute, Cary, NC). Single-degree-of-freedom linear contrasts were calculated to compare specific groups of treatments.

RESULTS

Disease pressure was high in all three fields, with severity ratings on untreated control plants reaching 5.0 (>20 lesions on all leaves) on 1 October in all three fields. Due to significant ($P<0.05$) field-treatment interactions on AUDPC, data from individual fields are presented separately.

All treatments were effective at reducing disease severity compared with the untreated control in fields two and three (Tables 3.2 and 3.3), at 4, 8, and 12 weeks (26 August, 8 October, and 5 November) after first appearance of symptoms, with the exceptions of 26 August in fields two and three (Tables 3.2 and 3.3). The 26 August assessment date in field one was compromised by deer herbivory and not included in analyses. AUDPC for all treatments containing tebuconazole was significantly lower than all other treatments on all but two assessment dates: 8 August and 5 November in field two (Table 3.2). In addition, the tebuconazole + flutolanil combination had the lowest AUDPC on all assessment dates and was significantly different from all other treatments on the final assessment date (5 November), in fields two and three (Tables 3.2 and 3.3). No difference was observed between pyraclostrobin alone and the combination of pyraclostrobin + boscalid on any assessment date. On all but one assessment date (5 November) in field three, flutolanil alone was no different than the combination of flutolanil + myclobutanil (Table 3.3). In addition, no difference was observed

between myclobutanil alone and flutolanil + myclobutanil except on two assessment dates (26 August and 8 October) in field two, when the combination product had significantly lower AUDPC (Table 3.2). No difference was observed among chlorothalonil alone, mancozeb alone, flutolanil + chlorothalonil, and flutolanil + mancozeb, in all fields.

When specific groups of fungicides were compared using linear contrasts, the AUDPC of tebuconazole alone was significantly lower than all other treatments containing a single fungicide in all three fields (Table 3.4). The AUDPC for the combination of flutolanil + tebuconazole was consistently lower than all other treatments; however, when compared with tebuconazole alone, a difference was only seen in two of the three final assessment dates (5 November) (Tables 3.2 and 3.3). In addition, the AUDPC of flutolanil + tebuconazole was consistently lower than all other treatments containing flutolanil in all fields. No difference was observed between pyraclostrobin alone and pyraclostrobin + boscalid or between chlorothalonil alone and mancozeb alone.

The increase in AUDPC was consistently higher for all treatments between the eighth week and twelfth week (8 October and 5 November, respectively), in fields two and three, compared to the increase in AUDPC between the fourth week and eighth week (26 August and 8 October) (Tables 3.2 and 3.3). In field two, the flutolanil + chlorothalonil treatment resulted in the greatest increase in AUDPC of 16.0 to 35.4. The combination of flutolanil + mancozeb resulted in a similar increase in AUDPC of 19.1. Both treatments had greater increases in AUDPC than the control (18.8), during this time period. In field three, the greatest increase in AUDPC was observed with the combination of flutolanil + chlorothalonil (18.0), with the exception of the control (18.8) (Table 3.3). Once again, of the fungicide treatments, the combination of flutolanil + mancozeb showed the second greatest increase of 17.2 in AUDPC

during this time. The combination of flutolanil + tebuconazole exhibited the smallest increase in AUDPC of 10.2 and 2.2, in fields two and three, respectively (Tables 3.2 and 3.3).

DISCUSSION

The use of fungicides is necessary for the effective management of daylily rust and fungicides remain the most consistent tool available for growers, hybridizers, and homeowners. Several fungicides are recommended and many provide excellent rust control (Buck and Williams-Woodward, 2003; Buck and Youmans, 2009; Dong et al., 2013, Emmitt et al., 2016; Mueller et al., 2004). However, our previous work has shown that application intervals greater than 14 days may be ineffective at managing rust under high disease pressure (Emmitt et al., 2016). Therefore, this study focused on treatments on 14-day application intervals. The seven fungicides used in this study are all recommended for managing daylily rust when applied at a 14-day interval (Williams-Woodward, 2015). In the current study, treatments containing tebuconazole provided the highest level of season-long rust control at all three study locations.

Disease managers choose fungicide combinations, fungicide rotations, and application intervals based on expected disease management outcomes. In addition, product value must also be considered. Typically, low levels of disease will be tolerated at production nurseries and it is common practice to spray large blocks of plant material on 14- and 21- day intervals. The spray areas may include *Hemerocallis* cultivars that differ in rust susceptibility or multiple plant species. Plants in nursery pots are often packed tightly together into groups of both resistant and susceptible cultivars (Buck and Ono, 2012; Dreistadt, 2001). *Hemerocallis* hybridizers have less tolerance than production nurseries for disease and require a higher level of rust management. Hybridizers are seeking to eradicate rust at their facilities and plant shipments must be free of all

disease. Homeowners exhibit the greatest variability in regards to the level of disease that will be tolerated and low to medium levels of rust, if noticed, may not warrant fungicide application.

Tebuconazole provided the highest level of disease management in our study.

Treatments containing tebuconazole consistently had the lowest AUDPC values and the smallest increases in AUDPC between assessment dates, at three study locations. The combination of flutolanil + tebuconazole had the lowest AUDPC value on all assessment dates; however, it was significantly different from tebuconazole alone on only two dates: 5 November in fields two and three (Tables 3.2 and 3.3). Therefore, it is unclear if flutolanil enhanced efficacy. All other flutolanil treatments provided inferior rust management to those containing tebuconazole.

Tebuconazole belongs to the demethylation inhibitor, DMI, group of fungicides (FRAC group 3), which target sterol 14 α -demethylase. This P450 enzyme is essential to the biosynthesis of ergosterol, a prerequisite to fungal membrane integrity (Cools et al., 2013). The active ingredients in this group are broad spectrum and exhibit varying degrees of systemic activity. In addition to tebuconazole, the DMI fungicides myclobutanil, propiconazole, and triadimefon are also recommended for daylily rust (Williams-Woodward, 2015). Myclobutanil, propiconazole, and triadimefon have all been shown to reduce lesion formation by *P. hemerocallidis* when applied as foliar sprays prior to inoculation (Buck and Williams-Woodward, 2003; Mueller et al., 2004). Prior work has shown propiconazole to be ineffective at reducing disease severity when applied as a foliar spray to field-grown plants, on a 21-day interval (Emmitt et al., 2016). In our current study, myclobutanil on a 14-day interval was effective at reducing disease severity and provided an intermediate level of rust management. It is common for fungicides in the same chemical group to provide differential activity spectra to a specific fungal pathogen. This is thought to be due to the structure of discrete molecules (Fraaije et al., 2011; Hutson and

Miyamoto, 1998; Latin, 2011; Mueller et al., 2013). This phenomenon exists with the DMI fungicides in wheat leaf and stem rust, *Puccinia triticina* and *P. graminis*, respectively (Hershman, 2011; Osbourne and Stein, 2009; Martinez et al., 2014; Wise, 2015). It appears to exist for the DMI fungicides and *P. hemerocallidis*.

No difference was observed in AUDPC values between the treatments containing pyraclostrobin alone and the combination of pyraclostrobin + boscalid. Therefore, the addition of boscalid, a succinate dehydrogenase inhibitor fungicide (SDHI; FRAC group 7) did not improve efficacy. Pyraclostrobin and pyraclostrobin + boscalid provided excellent rust management in our current study and their AUDPC values were second lowest to treatments containing tebuconazole. Pyraclostrobin is a member of the quinone outside inhibitors, QoI group of fungicides (FRAC group 11). Active ingredients in this group are systemic respiration inhibitors, broad spectrum, and used as protectants, curatives, and eradicants (Bartlett et al., 2002; Vincelli, 2002). Azoxystrobin, another QoI fungicide, has been shown to be effective at managing daylily rust when used as a foliar spray, soil drench, and root dip (Dong et al., 2013; Emmitt et al., 2016). This group of active ingredients has consistently provided a high level of efficacy against *P. hemerocallidis*.

Flutolanil (FRAC group 7) was applied in our current study alone and in combination with both systemic and contact fungicides. AUDPC values for flutolanil treatments were greater than treatments containing tebuconazole and pyraclostrobin on all assessment dates. Flutolanil alone provided an intermediate level of rust management. The addition of flutolanil to myclobutanil (FRAC group 3), chlorothalonil (M5) and mancozeb (M3) did not improve efficacy. Currently, there is a paucity of research in regards to SDHI fungicides and the management of daylily rust and subsequent research is needed.

Chlorothalonil and mancozeb are contact fungicides that exhibit no systemic activity and must be applied more frequently than systemic chemicals. However, chlorothalonil has proven to be more effective for managing daylily rust as a foliar spray than propiconazole when applied at 14-day intervals (Dong et al., 2013). Both chlorothalonil and mancozeb are commonly applied in combination and rotation with systemic fungicides. In our study, no difference was observed between chlorothalonil and mancozeb alone or in combination with the systemic fungicide flutolanil. In addition, prior work shows that chlorothalonil did not improve efficacy of azoxystrobin, propiconazole, or thiophanate-methyl (FRAC group 1) in management of daylily rust (Emmitt et al., 2016).

An integrated disease management (IPM) plan is recommended for the control of daylily rust. The incorporation of monitoring, removal of infected plant material (roguing), and the use of resistant cultivars are all key facets of an IPM program. Nonetheless, the use of fungicides remains the most consistent tool for maintaining low levels of daylily rust inoculum (Mueller et al., 2004). The decision-making process for applying foliar fungicides should include several considerations. Fungicide efficacy, timing and frequency of applications, and product value are all paramount to successful disease management. In addition, the mitigation of fungicide resistance must be considered in the choice of active ingredients and application schedule (Mueller et al. 2013; FRAC 2016).

Daylily rust is difficult to track, and currently no forecasting methodologies exist. Nonetheless, in much of the southeastern U.S., where *P. hemerocallidis* is endemic, fungicides will be most effective when applied preventatively. Daylily rust symptoms typically manifest in early to late summer or early fall, depending on environmental conditions such as temperature and relative humidity (Mueller and Buck, 2003). Azoxystrobin, chlorothalonil, myclobutanil,

propiconazole, and triadimefon were more effective at reducing lesion formation on daylilies when applied prior to inoculation when compared to post inoculation (Mueller et al., 2004).

In our current study, the greatest increase in AUDPC values was observed from 8 October to 5 November (the eighth and twelfth assessment date). Mean increases in AUDPC of 17.5 and 14.2 were observed across all treatments at two study locations in comparison to 11.0 and 11.5, respectively, between 26 August and 8 October (the fourth and eighth assessment dates), at these locations (Tables 3.2 and 3.3). Emmitt et al. (2016) observed mean AUDPC increases of 17.2, 21.5, and 25.2, across all treatments at three study locations between 24 September and 23 October. This is in comparison to mean increases of 8.1, 6.0, and 4.2 between 21 August and 24 September at the same locations.

Fungicide value is a function of product price and product performance. The fungicides used in our study range from \$10.32 to \$104.88 per application, when applied at the highest label rate (Table 3.5). The tebuconazole product (Torque) provided the highest level of rust management overall and had the lowest product cost (\$14.00/application), of the systemic products. Pyraclostrobin (Insignia SC) had the highest product cost among the systemic products at \$104.88/application. This product provided a high level of rust management; however, the combination product of pyraclostrobin + boscalid (Pageant) provided a similar result with an application cost of \$77.16. Flutolanil (Prostar 70 WP) provided an intermediate level of rust management similar to that of myclobutanil (Eagle 20 EW). The costs were \$25.68 and \$19.68/application for flutolanil and myclobutanil, respectively. The product application cost for the contact fungicides was similar. Mancozeb (Dithane 75 DF) had a cost of \$10.32/application which was similar to that of chlorothalonil (Daconil Ultrex) at \$14.34/application. Both products provided the lowest levels of disease management.

Currently, there is no definitive spray program for the management of daylily rust. Growers can increase profits, streamline chemical inventories, and avoid unnecessary applications with a greater understanding of which active ingredients are most effective, when to apply them, and at what frequency. No single management practice is effective at controlling daylily rust and an integrated approach is recommended. Likewise, no single active ingredient can provide effective long-term control in the presence of fungicide resistance (Brent, 1995; FRAC, 2015). Tebuconazole provided the highest level of rust management and had the lowest product cost. Myclobutanil, another DMI fungicide, provided an intermediate level of rust management at an intermediate product cost. Current and prior studies have observed differential levels of rust management within the DMI fungicides (Dong et al., 2013; Emmitt et al., 2016). FRAC classifies the DMI fungicides as being in the medium risk category for developing fungicide resistance.

The QoI fungicides are classified as high risk for developing fungicide resistance; however, no practical control failures have been seen in this group against *Puccinia* spp. worldwide (Schmitz et al., 2014). Both products containing QoI fungicides provided a high level of rust management and both have a high application cost. Active ingredients within this group consistently provide excellent rust management. Flutolanil provided an intermediate level of rust management at an intermediate application cost. Currently, flutolanil and boscalid are the only active ingredients within the SDHI fungicides labeled for daylily rust. Additional research is needed for this chemical group and future label expansion should be considered.

The objective of this study was to determine the most effective fungicides for managing daylily rust and ascertain greatest product value. While all fungicides were applied as individual treatments to determine individual performance, the use of fungicide rotations and combinations

including active ingredients with different modes of action, is paramount to managing fungicide resistance. The incorporation of all of these fungicides into management plans for daylily rust can decrease disease severity and mitigate the development of fungicide resistance in *P. hemerocallidis*.

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Table 3.1. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 1 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^b	AUDPC (date) ^c				
	26 Aug	8 Oct	5 Nov		
Pyr	_a	8.7	c	25.8	f
Flu	_	11.3	bc	28.3	def
Teb	_	1.6	d	6.3	g
Myc	_	11.7	bc	31.5	bcd
Chl	_	11.9	bc	31.8	bc
Man	_	12.0	bc	30.1	bcde
Pyr+Bos	_	9.1	c	27.4	ef
Flu+Teb	_	1.1	d	5.0	g
Flu+Myc	_	10.4	bc	29.1	cdef
Flu+Chl	_	13.5	b	33.0	b
Flu+Man	_	13.1	b	32.0	bc
control	_	17.8	a	37.8	a

^aThe first assessment date in field 1 was compromised by deer herbivory and not considered in analyses

^bFungicide treatments included pyraclostrobin (Pyr), flutolanil (Flu), tebuconazole (Teb), myclobutanil (Myc), chlorothalonil (Chl), mancozeb (Man) and pyraclostrobin + boscalid (Pyr+Bos) at 14-day applications.

°Rust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20 lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$ (SAS, Cary N.C.).

Table 3.2. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 2 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^a	AUDPC (date) ^b					
	26 Aug		8 Oct		5 Nov	
Pyr	2.3	bcde	11.0	e	25.8	ef
Flu	2.6	bcd	12.8	de	30.4	cd
Teb	0.5	ef	4.5	f	21.8	f
Myc	4.3	ab	18.6	b	37.2	b
Chl	3.4	bc	16.7	bc	35.5	b
Man	2.3	cdef	15.0	cd	33.7	bc
Pyr+Bos	1.3	def	10.3	e	27.9	de
Flu+Teb	0.3	f	3.4	f	13.6	g
Flu+Myc	2.0	cdef	14.2	cd	33.3	bc
Flu+Chl	2.8	bcd	16.0	bc	35.4	b
Flu+Man	3.1	bcd	16.3	bc	35.4	b
control	5.6	a	23.6	a	42.4	a

^aFungicide treatments included pyraclostrobin (Pyr), flutolanil (Flu), tebuconazole (Teb), myclobutanil (Myc), chlorothalonil (Chl), mancozeb (Man), and pyraclostrobin + boscalid (Pyr+Bos) at 14-day day applications.

^bRust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20

lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$ (SAS, Cary N.C.).

Table 3.3. *Puccinia hemerocallidis* area under the disease progress curves (AUDPC) for plants in field 3 treated at 14-day applications intervals with different fungicides and fungicide combinations

Fungicide Treatment ^a	AUDPC (date) ^b					
	26 Aug		8 Oct		5 Nov	
Pyr	3.1	cd	12.0	e	25.9	e
Flu	5.0	b	16.8	d	31.8	d
Teb	0.9	ef	2.7	f	6.1	f
Myc	4.3	bc	17.5	cd	34.3	cd
Chl	5.6	ab	20.1	bc	37.2	bc
Man	5.1	b	19.0	bcd	35.5	bc
Pyr+Bos	2.2	de	13.7	e	27.8	e
Flu+Teb	0.0	f	0.7	f	2.9	g
Flu+Myc	4.7	bc	18.0	bcd	35.0	c
Flu+Chl	4.9	bc	20.3	b	38.3	b
Flu+Man	4.3	bc	19.2	bcd	36.4	bc
control	7.3	a	25.9	a	44.7	a

^aFungicide treatments included pyraclostrobin (Pyr), flutolanil (Flu), tebuconazole (Teb), myclobutanil (Myc), chlorothalonil (Chl), mancozeb (Man), and pyraclostrobin + boscalid (Pyr+Bos) at 14-day day applications.

^bRust was assessed on a 0-5 rating scale, where 0 = no lesions, 1 = 1-20 lesions per leaf on old leaves, 2 = >20 lesions per leaf on old leaves, 3 = 1-20 lesions per leaf on new leaves, 4 = >20

lesions per leaf on new leaves, 5 = >20 lesions on every leaf (Dong et al., 2103). Data were then converted to area under the disease progress curves (AUDPC). Each datum set was analyzed using PROC MIXED at $P=0.05$ (SAS, Cary N.C.).

Table 3.4. *P*-values of single-degree-of-freedom linear contrasts of specific fungicide treatments to reduce daylily rust.

Contrasts ^a	Field 1			Field 2			Field 3		
	26 Aug	8 Oct	5 Nov	26 Aug	8 Oct	5 Nov	26 Aug	8 Oct	5 Nov
Treatments	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
11 treatments vs. control	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Teb vs. all other single treatments	–	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Teb alone vs. Flu+Teb	–	0.0048	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Teb+Flu vs. all other Flu combinations	–	0.8636	0.4775	0.0003	0.3322	0.1510	0.0436	<0.0001	0.0003
Teb vs. Myc	–	0.0061	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Pyr vs. Pyr+Bos	–	0.0005	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	0.2942	0.0603
Chl vs Man	–	0.2870	0.6730	0.2929	0.2889	0.2273	0.2246	0.7039	0.2465

^aThree letter abbreviations for active ingredients are same as in tables 3.1, 3.2, and 3.3.

Table 3.5. Product cost range of fungicides used in this study

^a Fungicide	Activity	FRAC Group	Recommended spray interval	Rate/100 ga. (low-high)	^{bc} Cost/oz.	Cost/100 ga. (low-high)
Insignia SC	Systemic	11	7-14 days	4-8 oz	\$13.11	\$52.44-\$104.88
Prostar 70 WP	Systemic	7	14-21 days	3-6 oz	\$4.28	\$12.84-\$25.68
Torque	Systemic	3	14-21 days	4-10 oz	\$1.40	\$5.60-\$14.00
Eagle 20 EW	Systemic	3	10-14 days	6-12 oz	\$1.64	\$9.84-\$19.68
Pageant	Systemic	11 + 7	7-14 days	6-12 oz	\$6.43	\$38.58-\$77.16
Daconil Ultrex	Contact	M5	7-14 days	1.4 lbs	\$0.64	\$14.34
Dithane 75DF	Contact	M3	7-10 days	1.5 lbs	\$0.43	\$10.32

^aFungicides are listed by trade name. Insignia SC = pyraclostrobin, Prostar 70 WP = flutolanil, Torque = tebuconazole, Eagle 20 EW = myclobutanil, Pageant = pyraclostrobin + boscalid, Daconil Ultrex = chlorothalonil, and Dithane 75 DF = mancozeb

^bProduct prices were collected from four online sources. Prices reflect lowest product cost found.

^cPrices are product cost only and do not include ancillary costs such as labor, equipment, or overhead.

CHAPTER FOUR

**FUNGICIDE SENSITIVITY PROFILES OF *Puccinia hemerocallidis* TO
PYRACLOSTROBIN, FLUTOLANIL, AND THIOPHANATE-METHYL³**

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ABSTRACT

Fungicides are the most effective tools for managing daylily rust, caused by *Puccinia hemerocallidis*. Eighteen different active ingredients from seven chemical classes are recommended as foliar sprays on intervals ranging from weekly to monthly. Active ingredients in the quinone outside inhibitors (QoI), succinate dehydrogenase inhibitors (SDHI), and methyl benzimidazole carbamates (MBC) are frequently used. The objective of this study was to determine the sensitivity profiles of *P. hemerocallidis* to pyraclostrobin, flutolanil, and thiophante-methyl. Forty isolates were used to determine the effective concentration of active ingredient to inhibit 50% spore germination. Isolates were most sensitive to pyraclostrobin with EC₅₀ value ranges of 0.00013 to 0.00049 µg ml⁻¹ and 0.00013 to 0.00052 µg ml⁻¹ with mean EC₅₀ values of 0.000284 µg ml⁻¹ and 0.000285 µg ml⁻¹ in experiments one and two, respectively. Isolates were least sensitive to thiophanate-methyl with value ranges of 0.00084 to 0.0204 µg ml⁻¹ and 0.00082 to 0.0211 µg ml⁻¹ with mean values of 0.0096 and 0.0094 µg ml⁻¹. The widest range of variation in sensitivity was observed with flutolanil, having ranges of 0.00043 to 0.01539 µg ml⁻¹ and 0.00047 to 0.01858 µg ml⁻¹. The detection and management of shifts in fungicide sensitivity are paramount to forestalling fungicide resistance and prolonging the efficacy of active ingredients.

INTRODUCTION

Daylilies are popular herbaceous perennials that are native to Asia and marketed extensively throughout the U.S. (Gatlin, 1999). Daylily rust, caused by *Puccinia hemerocallidis* (Thum), is one of the most significant diseases affecting daylilies (*Hemerocallis* spp.) in the southeastern U.S. and infections can render plants unmarketable (Buck and Ono, 2012). Since

its introduction to the U.S. in 2000, daylily rust has become an increasing problem for daylily growers (Williams-Woodward et al., 2001).

An integrated disease management approach incorporating host resistance, cultural practices and preventative fungicide sprays is most effective for managing daylily rust (Mueller, 2004). However, there are more than 80,000 registered cultivars of daylily (Trotter, 2016), and little is known about the rust reaction phenotype for most (Mueller et al., 2003). In addition, cultural practices such as irrigation management, roguing, and sanitation are not sufficient alone to control disease on daylilies. Fungicides remain the most effective tool for managing daylily rust and can be applied at intervals ranging from weekly to monthly. Prior to 2000, there were no fungicides labeled for daylily rust in the U.S. (Buck and Ono, 2012). Currently, 18 different active ingredients from seven chemical classes are recommended for managing daylily rust (Williams-Woodward, 2015).

The intensive use of fungicides for plant disease management has led to the development of fungicide resistance in several phytopathogenic fungi to many of the most economically successful chemical groups, including the methyl benzimidazole carbamates (MBC), the demethylation inhibitors (DMI), the quinone outside inhibitors (QoI), and the succinate dehydrogenase inhibitors (SDHI) (FRAC, 2013). The mitigation and management of fungicide resistance is imperative to delay the possibility of sensitivity shifts in target populations and more importantly, the development of resistance and control failures. Therefore, it is the objective of resistance management to forestall the development of resistance altogether rather than to manage fungal pathogens once resistance has developed (McGrath, 2004).

Monitoring for resistance using established fungicide sensitivity profiles or baseline fungicide sensitivities is the best method for determining if resistance is developing in fungal

populations. Fungicide baselines should be established prior to product market launch, in advance of fungal exposure (Russell, 2004). However, with existing chemicals that have been in use for decades and emergent diseases such as daylily rust, determining whether a population has been exposed is less than absolute.

The rust pathogens are considered low risk for developing resistance to fungicides and in some cases, e.g. the QoIs, no practical field resistance has been observed (Schmitz et al., 2014). However, a shift towards resistance has been reported for the DMIs in *Phakopsora pachyrhizi* and *Puccinia triticina*, the organisms causing soybean rust and wheat leaf rust, respectively (Arduim, 2012; Schmitz et al., 2014). In addition to the QoI and the DMI chemical classes, the MBCs and SDHIs are recommended for the management of several rusts on ornamental plants (Williams-Woodward, 2015). Nonetheless, there is a paucity of research on the fungicide sensitivities of most rust species to QoI, DMI, MBC and SDHI fungicides. No research has been conducted to determine the sensitivity of *Puccinia hemerocallidis* to the active ingredients labeled for its management and it is unclear if resistance is present in natural populations. The objective of this study was to determine the fungicide sensitivity profiles for *P. hemerocallidis* to pyraclostrobin (QoI), thiophanate-methyl (MBC), and flutolanil (SDHI). Propiconazole was included in our study; however, no dose response was observed and results were omitted from the analyses.

MATERIALS AND METHODS

Isolate collection and maintenance: Forty isolates of *P. hemerocallidis* were collected from Georgia (31), Alabama (2), Louisiana (1), Florida (2), Arkansas (1), Tennessee (1), Virginia (1), and Kentucky (1) from 2003 until 2014. Isolates were collected from nurseries, hybridizers,

public gardens, and residential landscapes. A single sample was collected from each location and designated as a discrete isolate. The history of fungicide use for some locations is well documented and unknown for other locations.

Urediniospores from a single lesion from each sample were collected by vacuum and transferred to healthy 'Pardon Me' variety daylilies in Metro-Mix 400 (The Scotts Company; Marysville, OH), in 5.7 liter nursery pots (Mueller et al. 2003). Plants were then misted with water, sealed in plastic bags (Poly-America; West Prairie, TX), and placed in darkness for 24 h at 22° C. After 24 h, plants were removed from bags and placed in clear plastic columns (0.46 m diam., 0.76 m height; Plasto-O-Mat; Warp Bros. Chicago, IL). Plants were then maintained in a greenhouse on the Griffin campus of the University of Georgia, at an average day/night temperature of 26 and 22°C, respectively. Plants with each individual isolate were maintained in plastic columns (0.46 m diam., 0.76 m height) on greenhouse benches. . Plants were irrigated as needed and fertilized with Osmocote Plus 15-9-12 controlled release fertilizer (The Scotts Company). Arthropod pests were managed using standard practices.

After 10-14 days, plants were inspected for lesion formation and urediniospore production. Plants were removed separately from plastic enclosures and urediniospores were collected from leaf surfaces by vacuum in 20-ml vials. This process was repeated as necessary to obtain enough urediniospores for each assay described below. Urediniospores were dessicated and maintained in storage at 4° C.

Fungicides: Technical grade propiconazole, pyraclostrobin, thiophanate-methyl (Sigma Aldrich, St. Louis, MO) and flutolanil (ChemService, West Chester, PA), were used to make stock solutions of 100 ppm in acetone. All solutions were stored at 4° C. .

Sensitivity Assays: The sensitivity of *P. hemerocallidis* isolates was tested using spore germination assays on potato dextrose agar (Becton, Dickinson, and Company; Sparks, MD). Potato dextrose medium was amended with fungicides at concentrations of 0, 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1 $\mu\text{g ml}^{-1}$ after it was autoclaved and cooled to approximately 50°C. Acetone was adjusted to 0.1% for all treatments including the nonamended control. Media was allowed to sit for 24 h at 22°C.

Spore solutions for each isolate were prepared in 0.05% Tween solution (J.T. Baker, Phillipsburg, NJ), at 1×10^5 urediniospores ml^{-1} . One hundred μl of spore solution for individual isolates was pipetted onto fungicide amended media. Each plate was divided into four quadrants, each containing an isolate, laid out at 12:00, 3:00, 6:00, and 9:00 positions, according to increasing numeric designation. After incubation in darkness at 22°C for 24 h, spore germination was determined through microscopic examination, based on a minimum of 150 spores for each isolate. Spores with germ tubes twice as long as spore width, were considered germinated. There were three replications of each isolate at each fungicide concentration. This experiment was conducted twice. Less than 25% germination was observed in the untreated controls of five isolates and those isolates were omitted from the analyses.

Data Analysis: The 50% effective concentration (EC_{50}), value for each replication of each isolate was estimated by linear regression of the probit-transformed relative inhibition value on \log_{10} -transformed fungicide concentration (Miller et al., 2002; Thomas et al., 2010). The frequency distribution of EC_{50} for each fungicide was tested for normality using Shapiro-Wilk tests (PROC UNIVARIATE) for normality in SAS (Version 9.4; SAS Institute, Cary, NC).

RESULTS

Frequencies of EC₅₀ values for pyraclostrobin ([Pr < W] = 0.1706 and 0.1518), flutolanil ([Pr < W] = 0.0806 and 0.0560), and thiophanate-methyl ([Pr < W] = 0.1675 and 0.1256) were normally distributed for experiments one and two, respectively. EC₅₀ values for pyraclostrobin ranged from 0.00013 to 0.00049 µg ml⁻¹ and 0.00013 to 0.00052 µg ml⁻¹ with means of 0.000284 µg ml⁻¹ and 0.000285 µg ml⁻¹, in two experiments, respectively. The median EC₅₀ values were 0.000293 µg ml⁻¹ and 0.000294 µg ml⁻¹ (Table 4.1). For flutolanil, the EC₅₀ values ranged from 0.00043 to 0.01539 µg ml⁻¹ and 0.00047-0.01858 µg ml⁻¹. The means were 0.0056 and 0.0060 µg ml⁻¹ and the medians were 0.0052 and 0.0050 µg ml⁻¹, in experiments one and two, respectively (Table 4.1). For thiophanate-methyl, EC₅₀ values ranged from 0.00084-0.0204 µg ml⁻¹ and 0.00084-0.0208 µg ml⁻¹ with means of 0.0096 and 0.0094 µg ml⁻¹ and median values of 0.0089 and 0.0091 µg ml⁻¹ for experiments one and two, respectively (Table 4.1).

DISCUSSION

In this study, we determined the sensitivity profiles of *P. hemerocallidis* for three commercially available fungicides that are recommended for managing daylily rust. A sensitivity profile is important to establish a basis for future comparison in monitoring for shifts in pathogen sensitivity to fungicides, which, in turn can predict the effectiveness of management programs. Baseline sensitivity distributions are typically determined before a product is marketed, based on a population of unexposed individuals (Russell, 2004). Before a new fungicide active ingredient (a.i.) can be launched, it must be tested on a wide range of plant diseases on several crops. If that a.i. is uniformly efficacious over several seasons it may be considered for commercial development (Brent, 1995).

When the exposure history of a collection of fungal isolates is unknown or uncertain, a sensitivity profile is considered adequate. The fungicides labeled for ornamental rusts have been commercially available for several decades. However, no fungicides were labeled for daylily rust in 2000, when it was first detected in the U.S. (Buck and Ono, 2012). Currently, 18 active ingredients from seven chemical classes are labeled for use on daylilies (Williams-Woodward, 2015).

The isolates in our study were most sensitive to pyraclostrobin, having mean EC_{50} values of $0.000284 \mu\text{g ml}^{-1}$ and $0.000285 \mu\text{g ml}^{-1}$ and ranges of 0.00013 to $0.00049 \mu\text{g ml}^{-1}$ and 0.00013 to $0.00051 \mu\text{g ml}^{-1}$ for experiments one and two, respectively. In addition, the isolates exhibited the lowest variability in sensitivity to pyraclostrobin, based on the ranges. Field studies have shown that azoxystrobin, another QoI fungicide, provided excellent control of daylily rust when applied on a 14-day interval to field-grown plants (Emmitt et al., 2016). Pyraclostrobin provided a high level of rust management on field-grown plants in a 2015 study (unpublished). In addition, azoxystrobin and a second QoI fungicide trifloxystrobin, were toxic to urediniospores at all label rates in fungicide toxicity assays (Mueller et al., 2005).

Pyraclostrobin is in the QoI group of fungicides (FRAC 11), which are broad spectrum respiration inhibitors. Group 11 fungicides interfere with the production of energy within the mitochondrial membrane. More specifically, they block the transfer of high-energy electrons at the site of quinol oxidation (the Qo site) of the cytochrome *bc₁* complex (Vincelli, 2002). This interference inhibits respiration and prevents the production of adenosine triphosphate (ATP). Field resistance to the QoI fungicides has been seen in numerous fungal and oomycete species (Sierotzki et al., 2000; Gisi et al., 2000; Gisi et al., 2002). This resistance, in all cases, correlates to point mutations in the organism's DNA that alter the target gene product and prevent the

binding of the fungicide's active ingredient. Three discrete amino acid substitutions have been elucidated: G143A, F129L, and G137R. While all three confer some level of resistance, it is the changing of glycine to alanine at codon position 143 (G143A) that is always correlated with qualitative resistance and the total loss of chemical control (Grasso et al., 2006). It is for this reason that FRAC places the QoIs in the high risk category for resistance. Nonetheless, no QoI resistance has been observed in the rust species (Oliver, 2014).

Rust species that have been studied, including *Puccinia* spp., have an intron that disrupts the codon at the G143 position, making the most common and agronomically important point mutation lethal (Grasso et al., 2006). Mutations conferring a lower level of resistance, such as F129L may still occur; however, these have not been observed in *Puccinia* spp. (Leiminger et al., 2013). It is unknown whether *P. hemerocallidis* possesses an intron at the G143 codon. There is a possibility that intron-free isolates exist and could develop resistance to the QoI fungicides. Therefore, it is important to continue to monitor for shifts in QoI sensitivity in *P. hemerocallidis* populations (Oliver, 2014).

The second group of fungicides in our study is the SDHIs (FRAC group 7), which includes the a.i. flutolanil. Similar to the QoIs, the SDHIs are respiration inhibitors only with a different target site; however, no cross resistance has been observed (Avenot and Michailides, 2010). The molecular target of the SDHI fungicides is the succinate dehydrogenase complex in the respiratory chain. This is known as complex two and is upstream of the binding site for the QoI fungicides. This group has a single-site mode of action. The risk of resistance is considered medium to high and the mechanism is typically a point mutation in the target gene (Avenot and Michailides, 2010). The most common mutation is the change from histidine (H) to tyrosine (Y) at codon 277 (H277Y); however, at least 27 mutations have been reported in field populations of

multiple pathogens (Sierotzki and Scalliet, 2013). No practical failures have been observed in the rust species to the SDHIs; however, that may be due to the cautious approach taken by the fungicide industry in resistance management recommendations.

Two SDHI fungicides are currently labeled for daylily rust: boscalid and flutolanil. Flutolanil is labeled for individual use while boscalid is labeled only as a combination product with pyraclostrobin (Williams-Woodward, 2015). In our study, mean EC_{50} values of $0.0056 \mu\text{g ml}^{-1}$ and $0.0060 \mu\text{g ml}^{-1}$, in experiments one and two, respectively, were observed for flutolanil. These EC_{50} values are higher than those observed in pyraclostrobin indicating that our isolates were more sensitive to pyraclostrobin overall. The ranges of EC_{50} values observed for flutolanil were 0.00043 to $0.01539 \mu\text{g ml}^{-1}$ and 0.00047 to $0.01858 \mu\text{g ml}^{-1}$ suggesting that a wide range of sensitivity to flutolanil was present in our isolate collection. The high end of the range in experiment one ($0.01539 \mu\text{g ml}^{-1}$) was approximately 36 times greater than the low end of the range. A similar result was observed in experiment two with the high end of the range being approximately forty times greater than the low end of the range. The same values for pyraclostrobin were approximately four for both experiments. In addition, the lowest EC_{50} for flutolanil is within the ranges observed for pyraclostrobin. This wide variation in sensitivity may be due to the presence of fungicide resistance; however, it is more likely that inherent or natural resistance is responsible. There is currently a paucity of research on the sensitivities of rust species to the SDHI fungicides.

The third fungicide in our study was thiophanate-methyl which is in the MBC (1) fungicide class. The MBCs or benzimidazoles were first introduced in the 1960s, making them the oldest group of systemic chemicals on the market. Control failures occurred within a few years of their introduction and currently, more than 100 species of fungi have developed some

level of resistance to the benzimidazoles including many key pathogens of economically-important crops (FRAC, 2014). The benzimidazoles exhibit a single site mode of action and their target molecule is the cytoskeletal component β -tubulin, which is the second of two subunits that comprise microtubules. By inhibiting microtubule assembly, benzimidazoles affect a great number of indispensable cellular functions such as mitosis and meiosis, intracellular molecular and organelle transport, and preservation of cellular shape and mobility (Davidse, 1986).

It is unclear if the genetic mechanisms conferring resistance to benzimidazoles, found in a wide range of fungal organisms, are present in the rust fungi (Oliver, 2013). No field resistance has been documented but that may be due a low usage frequency. In addition, rust fungi may exhibit an inherent resistance to the benzimidazoles. Thiophanate-methyl is the only benzimidazole labeled for daylily rust (Williams-Woodward, 2015). This active ingredient exhibited the highest mean EC_{50} values of all chemicals evaluated in our study at $0.0096 \mu\text{g ml}^{-1}$ and $0.0094 \mu\text{g ml}^{-1}$, in experiments one and two, respectively, suggesting that our isolate collection was least sensitive to thiophanate-methyl. In addition, the isolates exhibited lower variation in sensitivity when compared to flutolanil. Emmitt et al. (2016) observed no reduction of daylily rust with thiophanate-methyl when applied at 21- and 28-day intervals, in combination with the contact chloronitrile fungicide chlorothalonil, compared to the non-treated controls.

There is small body of research concerning the fungicide sensitivities of the rust fungi. Typically, these studies are conducted for agronomic crops before an active ingredient is marketed. Currently, the QoI and DMI fungicide groups are the most heavily sprayed for the management of rust diseases on wheat and soybeans (Schumann and Leonard, 2011; Rupe and Sconyers, 2008), and other fungicide groups are utilized to a lesser extent. While no resistance

has been observed for the QoIs in those pathosystems, a shift toward decreased sensitivity in the DMIs has been documented for both (Arduim, 2012; Schmitz et al., 2014). Arduim (2012) observed low IC_{50} values of 0.0034 mg L^{-1} and 0.0025 mg L^{-1} for pyraclostrobin in two experiments investigating *Puccinia triticina*. Five isolates were used in this study. Schmitz et al. (2014) reported a median ED_{50} value of 0.95 mg L^{-1} for pyraclostrobin on *Phakopsora pachyrhizi* for 38 isolates.

Both studies used detached leaf assays and neither investigated the response to a MBC fungicide. In addition, neither study could conclude that resistance to QoI fungicides was present in the respective pathosystems, based on reference baseline isolates. Values in both studies were greater than those found for pyraclostrobin in our study. However, the values in our study are much closer to Arduim's results than are Aduim's to Schmitz et al. This indicates that there is great variation in sensitivity to pyraclostrobin between rust species. There is currently no sensitivity data available for SDHI fungicides and MBC fungicides in rust pathosystems.

Recommendations for the management of rusts on ornamental plants such as gladiolus rust (*Uromyces* spp.), geranium rust (*Puccinia pelargonii-zonalis*), iris rust (*Puccinia iridis*), and the cedar apple and cedar hawthorne rusts (*Gymnosporangium* spp.), are similar to recommendations for *P. hemerocallidis* (Williams-Woodward, 2015). There are multiple products from several fungicide classes recommended; however, they are sprayed with less frequency over a smaller area than with agronomic crops. This and an overall lack of documented rust resistance are two reasons for the paucity of research on rust pathosystems.

The objective of this study was to determine the fungicide sensitivity of *P. hemerocallidis* to three commonly recommended fungicides, in the southeastern U.S. The elucidation of EC_{50}

values will provide the initial point from which to evaluate fungicide performance and monitor for shifts in fungicide sensitivity by *P. hemerocallidis*.

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Table 4.1 EC₅₀ values for *P. hemerocallidis* to selected fungicides

Fungicide	Experiment	Range	Mean	Median
Flutolanil	1	0.00043-0.01539	0.0056	0.0052
	2	0.00047-0.01858	0.0060	0.0050
Pyraclostrobin	1	0.00013-0.00049	0.000284	0.000293
	2	0.00013-0.00052	0.000285	0.000294
Thiophante-methyl	1	0.00084-0.0204	0.0096	0.0089
	2	0.00082-0.0211	0.0094	0.0091