ABSTRACT

GREGG, JOHN PATRICK. Herbicide and Nutrient Effects on the Development of Gray Leaf Spot Caused by *Pyricularia grisea* on Tall Fescue.
(Under the Direction of Charles H. Peacock and H. David Shew)

Gray leaf spot, induced by *Pyricularia grisea*, is a disease of increasing importance in tall fescue in the southeastern United States. Previous research has shown that several herbicides may predispose turfgrasses to some diseases and that certain essential nutrients may have antagonistic effects on fungal plant pathogens. The objectives of this research were to evaluate the effects of herbicide and nutrient treatments on gray leaf spot development in tall fescue. Inoculation techniques were also evaluated for establishing gray leaf spot on tall fescue in controlled environments. Field studies revealed that 2,4-D applications resulted in significantly higher quality turf and lower gray leaf spot incidence than the untreated control. Turf treated with 2,4-D amine + mecoprop + dicamba also exhibited significantly less foliar blight symptoms than the untreated control. In vitro experiments revealed the growth-inhibiting effects of 2,4-D on *P. grisea* implicated in the field, as mycelial growth was completely inhibited at concentrations of 500 and 1000 μg ml^{-1}. Colony growth was not affected at 2,4-D concentrations up to 100 μg ml^{-1}. Phosphorous acid treatments resulted in a reduction in turf quality compared to an untreated control, as did manganese and zinc treatments. Foliar blight caused by *P. grisea* was substantially increased in H_3PO_3-treated plots in 2003, where a 40% difference in blighted turf was observed between plots that received H_3PO_3 treatments every 14 days and the untreated control. Area under the disease

progress curve (AUDPC) analysis also revealed the significant detrimental effects of the phosphorous acid treatments. No significant differences in disease incidence or leaf spot size among nutrient treatments were observed in greenhouse treatments. Isolate selection was a significant factor for disease development and leaf spot size following spray inoculation under optimal environmental conditions. In general, disease incidence increased as inoculum density increased. Placing plants in covered containers or plastic bags immediately following inoculation for a 24-h period also appeared to promote disease development. Seeding rate did not have a significant effect on gray leaf spot development. We conclude that herbicide applications do not predispose tall fescue to gray leaf spot development and that applications of nutrients alone do not suppress development of gray leaf spot in tall fescue. Adjusting cultural practices as additional control measures for gray leaf spot does not appear to be a successful approach to managing this increasingly important disease.

INDEX WORDS: *Pyricularia grisea*, gray leaf spot, tall fescue, herbicides, nutrients, inoculation techniques

HERBICIDE AND NUTRIENT EFFECTS ON THE DEVELOPMENT OF GRAY LEAF SPOT CAUSED BY *PYRICULARIA GRISEA* ON TALL FESCUE

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APPROVED BY:

Co-Chair of Advisory Committee	**Co-Chair of Advisory Committee**

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Dedication

I would like to dedicate this thesis to my family and especially to my mother, Sheila Fox. Her emotional and monetary support has allowed me to accomplish all of goals I have desired to achieve, in both education and in life, and I owe a great debt of gratitude to her for all of my success. I would also like to extend special thanks to my aunt and uncle, Angie and Leonard Barefoot, for their outpouring love and support during my years at NC State. Finally, I would like to thank my fiancée, Lauren, for her constant love, understanding, and support over the last four years. Without you all, the completion of this degree would not have been possible. I sincerely thank you for all of your support.

J. Patrick Gregg

Biography

John Patrick Gregg was born on November 8, 1978, in Salisbury, North Carolina to Robert W. Gregg and Sheila F. Fox. He graduated from East Rowan High School in June of 1997. His love for golf and work experiences on golf courses sparked his interest in turfgrass management. He began to pursue an Agronomy degree with a concentration in turfgrass management at North Carolina State University in the fall of 1997 and graduated cum laude in December 2001 with a Bachelor of Science degree.

During his last year as an undergraduate at NCSU, Mr. Gregg worked as a turfgrass research assistant for Dr. Charles Peacock. This research experience, combined with the adverse economic effects following the events of September 11, 2001, persuaded Patrick to pursue a Masters degree in the Plant Pathology and Crop Science Departments at North Carolina State University. His studies in turfgrass pathology were directed by Dr. Charles H. Peacock, Dr. H. David Shew, and Dr. Lane P. Tredway. His degree requirements were completed in June of 2004 with the approval and acceptance of this thesis.

Mr. Gregg currently resides in Raleigh, North Carolina and will be married to Lauren Mims in April of 2005.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Pyricularia grisea (Cooke) Sacc. (teleomorph *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa) is a fungal pathogen of more than 50 species of grasses (Ou, 1987). Small grains, forage grasses, ornamental grasses, and turfgrasses are the primary hosts of this pathogen (Asuyama, 1965; Farr et al., 1989; Ou, 1980). The fungus is primarily known for its worldwide devastation of rice (*Oryza sativa* L.), but it also can severely affect other crops of economic importance (Ou, 1980). Blast of the inflorescence is the common symptom of *P. grisea* infection in rice and wheat (*Triticum aestivum* L.), while leaf spots and foliar blighting are observed in other grasses.

Gray leaf spot, also induced by *P. grisea*, is a disease of increasing importance in the turfgrass industry. The disease was first reported in St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) in 1957 (Malca and Owen, 1957). Since then, epidemics in cool-season turfgrass species, including Italian ryegrass (*Lolium multiflorum* Lam.) (Bain et al., 1972), perennial ryegrass (*Lolium perenne* L.) (Dernoeden, 1996), and tall fescue (*Festuca arundinacea* Schreb.) (Fraser, 1996) have been reported. Epidemics have been documented from several states in the Middle Atlantic region (Uddin et al., 1999), Midwest (Harmon et al., 1999), Northeast (Schumann, 1999), and western United States (Uddin and Viji, 2002) during the past 10 years.

Development of tall fescue varieties or cultivars for use in the southeastern United States has primarily focused on developing cultivars for heat and drought tolerance and resistance to brown patch, induced by *Rhizoctonia solani* Kühn (Morris, 1996). Brown patch is considered the predominant disease of tall fescue in this region (Smiley et al., 1992). Since brown patch-resistant cultivars, in general, are highly susceptible to gray leaf spot and are heavily used in the

Southeast, gray leaf spot has become a more significant problem in the southeastern U.S. (Fraser, 1997). Management of gray leaf spot in turfgrass systems currently consists of the use of commercially available resistant cultivars and preventative fungicide applications. With brown patch-resistant cultivars dominating the market, combined with increasing concerns about pesticides and the environment, alternative management strategies for gray leaf spot need to be developed.

Previous research suggests several herbicides commonly used in turfgrass systems may predispose turfgrasses to gray leaf spot development (Turgeon et al., 1974; Karr et al., 1979; Uddin and Soika, 2000). Additionally, numerous studies have demonstrated the role of nutrients in plant health and disease suppression. The objective of this research was to evaluate the effects of herbicide and nutrient applications on the development of gray leaf spot in tall fescue. This information will be useful in determining the potential for adjusting cultural practices as a component of disease management programs for gray leaf spot, with the goal of reducing the economic and chemical inputs needed to produce high quality turf.

History of Gray Leaf Spot of Turfgrasses

Gray leaf spot has been a problem in several turfgrass species since 1954, when *Pyricularia grisea* was first identified as a pathogen (Malca and Owen, 1957) of St. Augustinegrass, a coarse-textured, warm-season lawn grass grown extensively along the Gulf Coast states (Brecht et al., 2004). Malca and Owen (1957) referred to the disease as "gray leaf spot" rather than blast because it did not result in rapid leaf blighting or plant death (Uddin et al., 2003). Symptoms of gray leaf spot on St. Augustinegrass initially appear on leaves as small, brown or red spots and quickly expand to develop into larger leaf spots that appear tan when dry

and gray and fuzzy when wet. Leaf spot borders are brown or red, sometimes with a chlorotic halo surrounding the outside edges (Brecht et al., 2001). Multiple leaf spots may coalesce into lesions. Severe gray leaf spot damage is rarely reported in this host; however, the disease is a chronic problem. During the long summer months in Florida, the most severe damage has been reported in newly planted sod plugs and regenerating sod fields (Freeman, 1962). Freeman (1962) also reported that gray leaf spot reduced ground coverage of newly sprigged St. Augustinegrass by an average of 36 %. Gray leaf spot in St. Augustinegrass can be managed with the use of resistant cultivars and fungicides, alone or in combination (Colbaugh, 1991; Holcomb, 1995).

Pyricularia grisea was reported as a pathogen on forage ryegrass in the early 1970s in Louisiana and Mississippi (Bain et al., 1972; Carver et al., 1972). The disease was referred to as "ryegrass blast" because of the similarities to the symptoms of pathogen activity in rice blast (Trevathan et al., 1994). *Pyricularia grisea* was first diagnosed on perennial ryegrass turf on golf courses in 1986 (Dernoeden, 1996) and the first serious outbreak was reported on golf course fairways in Pennsylvania in 1991, where extensive damage occurred (Landschoot and Hoyland, 1992). A severe epidemic occurred on perennial ryegrass turf on golf courses and home lawns in 1995 across much of the central United States (Dernoeden, 1996). Since then, outbreaks of gray leaf spot have been sporadic, resulting in serious turf damage in 1998 and 2000 (Uddin et al., 2000).

Symptoms of gray leaf spot on perennial ryegrass initially develop as small, water-soaked leaf spots that expand into gray, grayish-brown, or light brown necrotic spots. Leaf spot borders are purple to dark brown and are often surrounded by a yellow, chlorotic halo (Uddin et al., 2003). Foliar blighting occurs when multiple leaf spots coalesce and become irregular in shape.

Blighted leaves may appear twisted and complete necrosis of the leaves will result in death of the plant. An integrated approach encompassing sound cultural practices, including proper fertilization, irrigation, and fungicide applications, is most effective for controlling gray leaf spot in perennial ryegrass (Uddin, 1999; Uddin et al., 2003). However, resistance to several strobilurin fungicides has been reported for isolates of *P. grisea* in perennial ryegrass (Vincelli, 2000).

Gray leaf spot of tall fescue was first reported in the United States in 1996 when an epidemic occurred in North Carolina (Fraser, 1996). Until then, little information was available on *P. grisea* activity in tall fescue. Similarities of gray leaf spot symptoms and those induced by *R. solani* could have led to inaccurate diagnosis and therefore explain the lack of documentation (Tredway, 2002). Gray leaf spot symptoms on tall fescue are initially located on the upper part of the leaf and appear as tan leaf spots with dark brown borders that turn gray when wet or in humid conditions. Leaf spots may enlarge and coalesce into irregular shaped leaf spots with a chlorotic halo sometimes surrounding the periphery of the spots. As leaf spots expand, leaf girdling will occur, resulting in blight from the tip toward the base of the leaf. In severe instances, patches of blighted turf 7.5 cm to 30.5 cm in diameter will develop.

There is evidence that certain factors play a role in the susceptibility of tall fescue to gray leaf spot. Differences in cultivar susceptibility were first identified among hundreds of tall fescue cultivars in North Carolina in 1995 (Fraser, 1997). Two cultivars, 'Coronado' and 'Coyote,' exhibited no visible gray leaf spot symptoms while several others showed few visible symptoms. These highly-resistant cultivars were identified to be genetically related to 'Coronado,' which indicated a genetic disease resistance mechanism shared among the cultivars (Fraser, 1997). Newly established plants, high nitrogen rates, and irrigation practices promoting

prolonged periods of leaf wetness during weather conditions conducive to disease development also may increase the susceptibility of tall fescue to gray leaf spot, although there are no published reports at present to support this claim.

North Carolina is located within the transition zone, a climatic region positioned between the warm-season and cool-season turfgrass adaptation zones, where both cool- and warm-season species are grown (Emmons, 1995). Tall fescue is a popular species for lawn and landscapes in the transition zone because of its tolerance to drought and temperature extremes. Brown patch, induced by *R. solani*, has been a major factor limiting successful growth of tall fescue in this region (Burpee, 1992). Breeding efforts have focused on developing tall fescue cultivars with improved resistance to brown patch. These new cultivars are, in general, highly susceptible to gray leaf spot (Fraser, 1996) and dominate the tall fescue market in the transition zone. On the other hand, several tall fescue cultivars with resistance to gray leaf spot are commercially available (Fraser, 1997). The use of resistant cultivars and applications of preventative fungicides, combined with proper nitrogen management and irrigation practices, have been shown to be the most successful methods for gray leaf spot control in tall fescue (Burpee, 1997).

Biology of *Pyricularia grisea*

Pyricularia grisea, classified in the Phylum Deuteromycota (Order Moniliales, Family Moniliaceae), is the anamorphic stage of the fungus. The sexual (teleomorph) stage of the fungus, *Magnaporthe grisea*, belongs to Phylum Ascomycota (Order Diaporthales) and has only been observed in vitro (Agrios, 1997). The fungus exhibits broad genetic and host diversity, a combination that makes it a devastating disease of over 22 genera of grass hosts (Farr et al., 1989). *Magnaporthe grisea* isolates from perennial ryegrass are virulent on wheat (*Triticum*

aestivum L.), triticale (*Triticale hexaploide* Lart.), and tall fescue (Viji et al., 2001). Some isolates from perennial ryegrass and wheat cross-infect. Isolates from wheat and triticale were virulent on perennial ryegrass, indicating that these cereal grain crops have the potential to serve as an additional source of inoculum for the spread of the pathogen (Uddin et al., 2003).

Harmon and Latin (2001) successfully induced sporulation of *P. grisea* from dormant mycelium in dead leaves. These conidia apparently serve as the primary inoculum for infections early in the growing season. Conidia are hyaline, pyriform, and septate (1-3 septa) and are produced on simple, gray conidiophores (Ellis, 1993). Conidia are usually produced during periods of warm temperatures (optimum 25 ºC to 28 ºC) and high humidity (minimum 89 %) (Tredway, 2002) and are dispersed primarily by wind, water, and mechanical movement (Uddin et al., 2003). Spores strongly adhere to leaf surfaces by production of a sticky mucilage produced at the spore tip (Hamer et al., 1988). When free moisture is available on the leaf surface, conidia germinate, produce an appressorium, and then penetrate the plant surface directly or through stomata. Production and accumulation of melanins in the appressorium cell wall creates mechanical force for host penetration (Dean, 1997). Enzymatic degradation also is used during host penetration by *P. grisea* (Agrios, 1997; Dean, 1997). Infection of tall fescue requires a minimum of 4 h of leaf wetness at 26 ºC and 28 ºC, but a majority of studies have shown that 12 h of continuous leaf wetness following spore attachment is required for optimal disease development (Moss and Trevathan, 1987; Uddin et al., 1998). Symptom development usually occurs within several hours to several days after inoculation, depending on host species and pathogen isolate (Suzuki, 1975; Tredway et al., 2003).

Nutrients and Plant Disease

Integrated Pest Management (IPM) is a combination of crop protection practices that are designed to keep pests below a designated damage threshold. Plant disease resistance is an induced response to pathogen presence and there is evidence that the mechanisms of disease tolerance are multicomponent (Reuvini, 1995). Resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen, while tolerance refers to the ability of a plant to sustain the effects of a disease without dying or suffering serious injury (Agrios, 1997). Relationships between nutrients and plant health are well documented. Perrenoud (1990) reported results from over 2400 studies concerning the relationship between fertilizer applications, alone or in combination with other elements, and plant resistance or susceptibility. Many studies have evaluated the effects of foliar nutrient applications on plant pathogens in numerous hosts. Although most plant diseases cannot be controlled by the use of fertilizers alone, sound fertilization practices are a vital part of an integrated program for disease management.

Phosphorous (P) is a primary plant nutrient that is a constituent of enzymes and proteins and is involved in nearly all plant metabolic processes (Bennett, 1993). Phosphorous nutrition is provided in various forms of phosphates, the salts of phosphoric acid (Förster et al., 1998). Reuveni et al. (1994, 1996) demonstrated that phosphates effectively suppressed powdery mildew (caused by *Sphaerotheca fuliginea*) in cucumbers (*Cucumis sativus* L.) and common rust (caused by *Puccinia sorghi*) in maize (*Zea mays* L.). Additional research with these crops indicated that resistance to cucumber anthracnose (caused by *Colletotrichum lagenarium*) and powdery mildew can be induced by chemical treatments and phosphates (Gottstein and Kuc', 1989; Mucharromah and Kuc', 1991). Foliar sprays of phosphates on field-grown Chardonnay

grapes (*Vitis vinifera* L.) were shown to inhibit the development of powdery mildew (caused by *Uncinula necator*) and subsequent vine fruit yields were greater than untreated controls (Reuveni and Reuveni, 1998). In many of these cases, alternating phosphate sprays with fungicide applications did not compromise the efficacy of disease control.

Phosphonates are reduced phosphorous compounds with selective effectiveness against oomycetous fungi (Coffey and Ouimette, 1989). Several commercial phosphonate products that are registered as supplemental soil and foliar fertilizers have been shown to have activity against certain plant pathogens. Fenn and Coffey (1989) found that phosphonate anion (HPO_3^{2-}) activity in fosetyl-Al (Signature or Aliette, Bayer Environmental Science, Montvale, NJ), a fungicide commonly used in turf, has a direct mode of action against *Phytophthora* species in tomato (*Lycopersicon esculentum* L.) and tobacco (*Nicotiana tabacum* L.). Phosphonate applications alone, when applied after infection, have been shown to reduce the incidence of downy mildew disease and sporulation of *Plasmopara viticola* in grapes (Wicks et al., 1991).

Phosphite, the salt of phosphorous acid, is the active component in phosphonate materials (Coffey and Bower, 1984). It is readily taken up by the plant and is mobile within both the xylem and phloem. Phytophthora crown rot was found to be significantly reduced in phosphite-treated green pepper (*Capsicum annuum* L.) plants compared with no phosphorous or phosphate-treated plants (Förster et al., 1998). Smillie et al. (1989) observed similar results when assessing development of *Phytophthora* spp. in lupin (*Lupinus angustifolius* L.), paw-paw (*Asimina triloba* L.), and tobacco following phosphite treatments. However, plants supplemented with technical or commercial grade phosphorous acid exhibited reduced growth when compared to plants treated with phosphate.

Potassium (K) is a primary nutrient that plays a major role in water relations within the plant. It is required for turgor buildup and maintenance of osmotic potential in cells (Bennett, 1993). Plant cells deficient in K can lose turgor pressure, which may be a physical factor that facilitates penetration by pathogens. Potassium may affect disease through a direct effect on the pathogen, by promoting antagonistic phylloplane organisms, stimulating host defense reactions, or a combination of these effects (Reuveni and Reuveni, 1998). Kettlewell et al. (1992) showed that rust and powdery mildew disease in barley (*Hordeum distychum* L.) were reduced by foliar applications of potassium chloride (KCl). Large patch (caused by *R. solani*) incidence in St. Augustinegrass was suppressed by applications of K and KCl (Rider, 2001). Goss and Gould (1967) found that K applications were suppressive to take-all patch (caused by *Gaeumannomyces graminis*) on colonial bentgrass (*Agrostis tenuis* L.). Bloom and Couch (1960) suggested that K applications to turfgrasses under high nitrogen management could offset the severity of brown patch. On the contrary, Tredway et al. (2001) found that applications of K in perennial ryegrass resulted in a decline in turf quality and enhanced susceptibility to red thread (caused by *Laetisaria fuciformis* (McAlp.) Burd.).

Manganese (Mn) is a micronutrient identified to play a role in disease resistance in plants. The availability of Mn in the soil (mainly the rhizosphere) and Mn concentrations in the roots influence the severity of many diseases (Huber and Wilhelm, 1988). Graham (1983) noted that the effects of Mn nutrition on disease are normally limited to the deficiency range but there are several indications that the suppressive effects of Mn occur within the sufficiency range. Several researchers have reported successful control of powdery mildew and take-all of wheat with applications of Mn fertilizers (Huber and Keeler, 1977, 1988; Graham, 1983). Increased susceptibility of crops to disease due to Mn deficiency may be related to the role of Mn in cell

wall structural functions as a defense mechanism against invading pathogens (Brown et al., 1984). Hill et al. (1999) found that the severity of take-all patch on creeping bentgrass was reduced by monthly applications of Mn, although foliar symptoms were not entirely eliminated. Uddin et al. (1999) reported significant reductions in both disease incidence and severity of dollar spot (caused by *Sclerotinia homoeocarpa*) on creeping bentgrass (*A. palustris*) after applications of Mn prior to and following inoculation.

Zinc (Zn) is a micronutrient that is important in the integrity and stability of cell membranes (Graham and Webb, 1991). Several research results have suggested that Zn-deficient roots excreted more carbohydrates and amino acids than Zn-adequate roots, thus attracting more pathogens to the root surface (Welch et al., 1982; Loneragan et al., 1987). Thongbai et al. (1993) attributed reductions in Rhizoctonia root rot severity, caused by *R. solani*, in wheat after Zn applications to the role of Zn in cell membrane structure. Other studies suggest that disease reductions are related to toxic effects of Zn directly on the pathogen. Wilkinson and Millar (1981) showed that Zn inhibited sporangium and zoospore formation by *Phytophthora megasperma*. Uddin et al. (1999) also reported significant reductions in both disease incidence and severity of dollar spot (*S. homoeocarpa*) on creeping bentgrass after applications of zinc.

Silicon (Si) is not considered an essential element for plant growth but has been shown to be a major component in biochemical pathways leading to resistance to certain pathogens (Graham and Webb, 1991). These effects have probably been most apparent in rice, where protection against the rice blast fungus (*M. grisea*) and brown spot (caused by *Cochliobolus miyabeanus*) has been demonstrated (Ou, 1987). In Florida, fertilization with calcium silicate in rice production is a common practice for disease management of blast. Silicon has been shown to control several important diseases as effectively as fungicides; therefore, there is the potential

for reduced chemical inputs by substituting Si applications (Datnoff and Snyder, 1994; Datnoff et al., 1997). In the same studies, Si combined with benomyl reduced the incidence of neck blast in upland rice by more than 80 %. A study in eastern Columbia showed that amendment of soil with Si reduced the number of fungicide applications necessary to control rice blast (Seebold, 1998). Brecht et al. (2004) reported applications of calcium silicate, alone and in combination with chlorothalonil, significantly reduced gray leaf spot development in St. Augustinegrass. In soils low or limiting in plant available Si, amendments of soluble Si enhanced the resistance of bermudagrass (*Cynodon dactylon*) against leaf spot caused by *Bipolaris cynodontis* (Datnoff and Rutherford, 2003).

Herbicide Effects on Plant Disease

Herbicides are known to affect the incidence and severity of plant disease in addition to the primary role of reducing weed competition (Campbell and Altman, 1977). Nontarget effects of herbicides on plant pathogens and diseases have been studied and results vary from inhibition to stimulation of the pathogen and/or disease (Altman and Campbell, 1977; Beam et al., 1977; Grinstein et al., 1976). These effects can result in herbicide-induced morphological and physiological changes in host plants, which may alter resistance and susceptibility to diseases. Altman and Campbell (1977) suggested that herbicides affect plant disease by reducing structural host defenses, enhancing exudation from plant cells, stimulating pathogen growth, and inhibiting beneficial microorganisms in competition with potential pathogens.

Many plants exposed to herbicides as early as planting to several weeks after vegetative emergence show signs of herbicide stress. Wheeler (1975) reported that sugar beets (*Beta vulgaris* L.) exhibited symptoms of stress after germination from soil amended with preplant

herbicide applications. The author observed leakage from root cell membranes and slower development of structural components of cells in the presence of chemicals, making the beets more prone to pathogen invasion. Altman and Campbell (1977) showed that damping-off caused by *R. solani* in sugar beets was accelerated when pebulate and PCA were present in soil. Antonopoulos (1969) reported similar results with sugar beets and picloram-treated soil. Applications of EPTC were shown to increase root-rot (caused by *Fusarium solani*) in navy beans (*Phaseolus vulgaris* L.) in both field and greenhouse trials (Wyse et al., 1976).

There is minimal documentation on the effects of herbicides on pathogens and diseases in turfgrasses. In turfgrass systems, herbicides are usually applied to existing stands as either preemergent or postemergent treatments. The effects of seven common preemergence herbicides for selective crabgrass control on Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* spp. *rubra* L.) varieties were studied by Turgeon et al. (1974). The susceptibility of both turf species to strip smut (caused by *Ustilago striiformis* West. Niesel.) was increased when bandane was applied. Various levels of turf injury were evident in Kentucky bluegrass treated with bensulide, terbutol, and bandane. Red fescue plots showed significant injury from DCPA and bandane treatments.

Karr et al. (1979) examined the effects of three herbicides on the growth of several common turfgrass pathogens in greenhouse and laboratory experiments. An experimental herbicide (NC8438) reduced the severity of brown patch, dollar spot, and Pythium blight (caused by *P. aphanidermatum*) in bermudagrass (*C. dactylon*) in greenhouse trials. Brown patch and dollar spot severity was increased when treated with benefin. Growth of all three pathogens in vitro was inhibited to some extent by all herbicides at various label rates, respectively. Herbicide effects on disease development in the greenhouse studies did not always parallel apparent effects

on the pathogens in vitro, however. Direct contact between pathogens and herbicides in vitro would not have been as likely to occur in the complex interactions among plant, pathogen, herbicide, and environment in disease (Karr et al., 1979).

Uddin and Soika (2000) studied the effects of several plant growth regulators (PGR), herbicides, and fungicides, along with combinations among each, on the development of gray leaf spot in perennial ryegrass. They found overall significant interactions of fungicide and PGR/herbicide treatments and disease severity. Specifically, applications of the herbicides ethofumesate (Prograss®) and dithiopyr (Dimension®) significantly increased disease severity when combined with the fungicide flutolanil.

Inoculation Techniques for *Pyricularia grisea*

Artificial inoculation techniques are required to incite gray leaf spot epidemics in controlled environment experiments and often in field studies. Optimal infection conditions include presence of the pathogen, high temperature, high humidity, and a period of prolonged leaf wetness. Since gray leaf spot infection and development in field studies are dependent upon environmental conditions, natural outbreaks are often unpredictable and non-uniform throughout the experimental plots (Han et al., 2003). Artificial inoculation may be necessary to induce gray leaf spot epidemics in these instances. Since consistency between field and controlled environment studies is often difficult to attain, the development and implementation of reliable inoculation methods for both situations is essential.

Trevathan (1982) reported disease development on perennial ryegrass after spray inoculation of 4-week-old plants. Plants were placed in a greenhouse mist chamber under natural light conditions with an average temperature of 25 ºC for 72 h after inoculation. Plants

were removed from the chamber after 72 h and symptoms were first noticed 24-48 h later. Disease severity ranged from no lesions to plant death.

A more in-depth study by Moss and Trevathan (1987) examined the effects of plant age, inoculum density, temperature, and leaf-wetness duration on inoculation efficiency in annual ryegrass. The optimum infection age was estimated to be between 4-5 weeks old, with younger plants being more susceptible to infection than older plants. Total lesion counts increased exponentially with increasing inoculum densities and disease development was consistently higher on older (second, third, and fourth) leaves than the youngest leaf. An inoculum density of 2×10^5 spores ml^{-1} was reported to be adequate for lesion production and development of response curves. The ideal temperature for lesion development was 25 ºC. No lesions were observed at 5 ºC and only a few developed when temperatures were increased to 35 ºC. A minimum constant leaf-wetness period of 24 h was required for adequate infection.

Landschoot and Hoyland (1992) investigated many of the same factors as Moss and Trevathan (1987) and also found that 4-week-old perennial ryegrass plants exhibited significantly more disease symptoms than 20-week-old plants. They also noted that lesion development was greater at 29 ºC than at 22 ºC and reported a significant temperature-age interaction 10 days after inoculation, but not at 14 or 18 days.

Tredway et al. (2002, 2003) reported successful inoculation of several tall fescue cultivars in controlled environment chambers. Plants were inoculated at an inoculum density of 2×10^5 spores ml^{-1} using a CO_2-powered airbrush and placed in covered plastic containers for a 24-h incubation period at 24 ºC, 100 % relative humidity (RH), and no light. Subsequent conditions were 12-h days at 30 ºC and 75 % RH and 12-h nights at 24 ºC and 100 % RH. An incubation period of 5-6 days was reported before gray leaf spot symptoms were observed.

Han et al. (2003) evaluated inoculation methods for gray leaf spot resistance in both controlled environment and field studies. In a greenhouse study, perennial ryegrass plants were inoculated with a conidial suspension of 5 x 10^4 spores ml^{-1} in a plastic mist chamber. Humidifiers maintained desired relative humidity levels. Temperatures were uncontrolled and ranged from 18 ºC to 37 ºC. Symptoms developed within 5-6 days after inoculation. In the corresponding field trial, a plastic wall was built around the test area and plots were inoculated with a conidial suspension of 2.8 x 10^4 spores ml^{-1} three times on two-week intervals. Irrigation was applied twice a day to enhance leaf wetness and encourage disease development. However, few symptoms were observed, possibly due to unfavorable weather conditions. The authors concluded this method was unreliable for screening purposes because of inconsistencies between greenhouse and field trials.

A second inoculation method similar to the one described by Tredway (2002, 2003) was evaluated in a controlled environment growth chamber. This method was then tested on ryegrass tillers in a greenhouse for correlation with field trials. Seed from each of the test plants were harvested and transplanted into field plots where a natural epidemic of gray leaf spot occurred four weeks after seeding. A significant correlation ($r = 0.88$) between growth chamber and field results was reported and this inoculation method was deemed reliable.

In another field experiment, test plots were inoculated using a backpack sprayer and then covered with black plastic film. The covering was removed every morning, plots were irrigated twice during the day, and the plastic was replaced every night after misting. Flutolanil was applied to prevent brown patch development. Gray leaf spot symptoms were observed six days after inoculation. Plots were inoculated a second time one week after the initial inoculation and disease developed into a severe epidemic within a week. A significant correlation ($r = 0.87$) was

reported between results of this test and a recent natural epidemic, indicating this inoculation method was reliable for simulation of natural occurrences of gray leaf spot.

Research Objectives

Integrated pest management strategies that emphasize sound nutrient management and pesticide programs to minimize environmental impacts are continually being revised as a result of ongoing research initiatives. Development and implementation of new approaches to pest management programs will be useful for turfgrass managers in all sectors of the industry. Herbicide applications and fertilization affect turfgrass quality and disease development in several warm- and cool-season turfgrasses. However, minimal attention has been given to these effects on *Pyricularia grisea* and gray leaf spot development in tall fescue. The objectives of this research were:

1. To determine if selected herbicides predispose tall fescue to gray leaf spot development.
2. To assess potential suppression of gray leaf development by nutrient applications.
3. To evaluate inoculation techniques for *Pyricularia grisea* on tall fescue in a controlled environment.

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CHAPTER 2

HERBICIDE EFFECTS ON THE DEVELOPMENT OF GRAY LEAF SPOT CAUSED BY *PYRICULARIA GRISEA* ON TALL FESCUE

(to be submitted to Plant Disease)

Herbicide Effects on the Development of Gray Leaf Spot Caused by *Pyricularia grisea* on Tall Fescue

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ABSTRACT

Gregg, J. P., Peacock, C. H., Shew, H. D., and Tredway, L. P. 2004. Herbicide effects on the development of gray leaf spot caused by *Pyricularia grisea* on tall fescue. Plant Dis. 88:000-000.

Herbicides that are commonly applied to tall fescue were evaluated for their effects on *Pyricularia grisea* and gray leaf spot development from 2002 to 2004. Field studies revealed that 2,4-D applications resulted in significantly higher quality turf and lower gray leaf spot incidence than the untreated control. Turf treated with 2,4-D amine + MCPP + dicamba also exhibited significantly less foliar blight symptoms than the untreated control. Cultivars and environmental conditions played an important role in results from field studies. Greenhouse data revealed no significant differences in disease incidence or leaf spot size as a result of treatments. In culture, 2,4-D totally inhibited mycelial growth of *P. grisea* at concentrations of 500 and 1000 μg ml^{-1}. No inhibition was observed at concentrations up to 100 μg ml^{-1}. Early season applications of herbicides and common postemergent applications did not significantly predispose or promote the development of gray leaf spot in tall fescue.

INTRODUCTION

Pyricularia grisea (Cooke) Sacc. (teleomorph *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa) is a pathogen of more than 50 species of grasses, including small grains, forage, and turfgrasses (2, 10, 16, 17). Gray leaf spot has been a disease of increasing importance in the turfgrass industry since it was first reported on St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) in 1957 (14). *Pyricularia grisea* was identified as a pathogen of annual ryegrass (*Lolium multiflorum* Lam.) in Louisiana and Mississippi in the early 1970s (3, 8) and of perennial ryegrass (*Lolium perenne* L.) in 1992 (13). Tall fescue (*Festuca arundinacea* Schreb.) was reported as a host in 1996 after an epidemic in North Carolina (11). Gray leaf spot sporadically affects all of these turfgrass hosts in their respective growing regions throughout the United States.

Research on gray leaf spot of tall fescue has been fairly limited since the first report less than 10 years ago. Before this time, gray leaf spot probably occurred but was misdiagnosed as brown patch, the predominant disease of tall fescue in the southeastern United States induced by *Rhizoctonia solani* Kühn (5). Several factors that influence the development of brown patch also favor gray leaf spot development. High temperature (25 ºC to 28 ºC), high humidity (>89 %), prolonged leaf wetness, and high nitrogen fertility encourage gray leaf spot development in turfgrasses (21).

Herbicides may affect the susceptibility of many crops to pathogens (6). Several of the phenoxy-type herbicides also inhibit crop growth (9). Morphological and physiological changes in the host induced by herbicides may reduce structural defenses and allow for greater susceptibility to pathogens present at low levels in the environment (1). Certain chemicals stimulate pathogen growth while inhibiting the growth of beneficial microorganisms in

competition with potential pathogens. The effect of the herbicide may be crop specific. For example, 2,4-D increased or decreased disease depending on the crop plant treated (1).

Several preemergent and postemergent herbicides can affect disease development in turfgrass. For example, application of preemergence herbicides for selective crabgrass control in Kentucky bluegrass (*Poa pratensis* L.) and red fescue (*Festuca rubra* spp. *rubra* L.) increased the susceptibility of both turf species to strip smut caused by *Ustilago striiformis*, and reduced overall turf quality (19). The severity of brown patch, caused by *R. solani,* and dollar spot, caused by *Sclerotinia homoeocarpa,* was increased when bermudagrass (*Cynodon dactylon* L.) was treated with benefin (12). Early season applications of the preemergent herbicide dithiopyr were associated with increased severity of gray leaf spot on perennial ryegrass fairways (20). The objective of this study was to evaluate the effects of selected turfgrass herbicides on the development and severity of gray leaf spot in tall fescue.

MATERIALS AND METHODS

Field Study - 2002. This study was conducted at Sandhill Turf in Candor, NC from June to September 2002. Two plots areas (A&B) were located in a stand of 'Rebel III' tall fescue. Plot areas consisted of 1.5 m x 1.8 m individual plots in a randomized complete block design with six treatments and four replications. The experimental areas were irrigated to supplement rainfall and a height of approximately 6 cm was maintained by cutting twice per week. Brown patch development was selectively inhibited in experiment A with applications of flutolanil (Prostar 70WP, Bayer Environmental Science, Montvale, NJ). Applications of 2.9 kg. a.i. ha^{-1} were made on 23 July, 13 August, and 3 September. Experiment B received no fungicide applications.

Five herbicide treatments were applied on 23 July and 20 August: dithiopyr (Dimension 1EC, Dow Agrosciences, Indianapolis, IN) at 0.57 kg a.i. ha^{-1}; fenoxaprop-p-ethyl (Acclaim Extra, Bayer Environmental Science, Montvale, NJ) at 0.10 kg a.i. ha^{-1}; 2,4-D + mecoprop + dicamba (Trimec Classic, PBI/Gordon Corporation, Kansas City, MO) at 1.65 kg a.i. ha^{-1}; dicamba (Banvel, Micro Flo Co., Memphis, TN) at 0.56 kg a.i. ha^{-1}; and 2,4-D (Weedar 64, Nufarm, Burr Ridge, IL) at 1.59 kg a.i. ha^{-1}. Each treatment was applied in 0.14 liter H_2O/m^2 at 276 kPa with a CO_2-powered boom sprayer equipped with flat fan nozzles (Teejet 8004VS, Spraying Systems Co., Wheaton, IL).

Visual turf quality ratings were first recorded on 6 August and were continued bi-weekly until 1 October. Ratings were based on uniformity, density, and color of the turf. A rating scale of 1 to 9 was used, where 9 represents ideal turf and 5 is the minimum acceptable quality. Brown patch was the only disease observed during the studies. Foliar blight incidence ratings began on experiment B when patches were first noticed on 17 September and again on 1 October. Foliar blight incidence was determined visually by estimating the amount of blighted turf in each plot on a scale of 0 to 100 %.

Statistical analyses were performed using SAS v. 8.2 (SAS Institute, Cary, NC). Mean separations for turf quality on individual rating dates, turf quality averages spanning all rating dates, brown patch incidence for individual rating dates, and brown patch averages spanning both rating dates were conducted using the Waller-Duncan *k*-ratio *t*-test (k=100). Analysis of variance (ANOVA) was used to test the significance of herbicide treatments on turf quality and brown patch incidence. A treatment by fungicide interaction was also evaluated for significance on turf quality and brown patch incidence.

Field Study - 2003. This study was conducted at the N. C. State University Lake Wheeler Turfgrass Field Lab in Raleigh, NC. One plot area was established with 'Tarheel' tall fescue by seeding at a rate of 35 g/m^2 in April 2003. Since this cultivar is quite resistant to brown patch, only one plot area was established for the study and no flutolanil applications were applied. A complete fertilizer (18-24-6; Pennington Seed Inc., Madison, GA) was applied at a rate of 24.4 kg ha^{-1} of N, 32.5 kg ha^{-1} of P, and 8.1 kg ha^{-1} of K at time of seeding. The plots were maintained at an approximate height of 7 cm by mowing two or three times a week and turf was irrigated as needed during establishment. A randomized complete block design consisting of 1.5 m x 1.8 m plots was arranged with six treatments and four replications. Herbicide treatments identical to the 2002 study were applied on 1 July and repeated on 29 July. Irrigation frequency was increased to four times per day from July through September to promote gray leaf spot development.

Disease incidence and visual turf quality ratings were first recorded on 14 August when foliar blighting began to form patches in the turf. Foliar blight incidence was measured by placing a 1.5 m x 1.5 m grid over of each plot and counting the number of intersections that overlaid symptomatic turf. The grid consisted of a total of 196 intersections that were spaced 10.2 cm apart. Dividing the number of intersections overlaying symptomatic turf by the total number of intersections on the grid yielded a percentage of blighted turf for each plot. A total of six ratings were recorded approximately every two weeks from 14 August until 1 October, when turf recovery was observed.

Means for turf quality and gray leaf spot incidence were adjusted for field variation using the Papadakis method (4). Linear contrast analysis of means for turf quality and disease incidence for individual rating dates was conducted. Disease development over time was

analyzed and compared using area under the disease progress curve (AUDPC) analysis (7). All disease incidence ratings were used to calculate AUDPC for each treatment using the formula

$$Y = \sum[(X_i + X_{i+1})/2](t_{i+1} - t_i)$$

where Y is AUDPC, X_i is the disease incidence rating for the i^{th} evaluation, X_{i+1} is the disease incidence rating of the $i + 1^{th}$ evaluation, and $(t_{i+1} - t_i)$ is the number of days between two evaluations.

Greenhouse Study - 2004. 'Tarheel' tall fescue was seeded at a rate of 35 g/m^2 into 10 cm diameter plastic pots containing calcined clay (Turface Allsport, Profile Products LLC, Buffalo Grove, IL). The pots were transferred to a greenhouse where they were watered three times per day to promote rapid seed germination. Following germination, the turf was fertilized weekly with 50 ml of 1/2-strength Hoagland's solution containing 105, 16, and 250 μg ml^{-1} of N, P, and K. The turf was maintained at a height of approximately 9 cm by cutting every 14 days.

Five herbicide treatments were initiated eight weeks after seeding on 20 January: dithiopyr at 0.57 kg a.i. ha^{-1}, fenoxaprop-p-ethyl at 0.10 kg a.i. ha^{-1}, 2,4-D + mecoprop + dicamba at 1.65 kg a.i. ha^{-1}, dicamba at 0.56 kg a.i. ha^{-1}, and 2,4-D at 1.59 kg a.i. ha^{-1}. A second application of treatments was made on 17 February. Treatments were applied in 0.14 liter H_2O/m^2 at 276 kPa with a CO_2-powered boom sprayer equipped with flat fan nozzles (Teejet 8004VS, Spraying Systems Co., Wheaton, IL).

Pyricularia grisea isolate 1207-59 was selected from a previous collection of isolates associated with tall fescue cultivars in Georgia (18). Filter paper discs containing the isolate were revived from -80 °C storage by placing the discs on the surface of a 9-cm-diameter Petri dish containing potato dextrose agar (PDA, Difco) amended with 50 μg ml^{-1} each of tetracycline,

streptomycin, and chloramphenicol. After 10 days of incubation at 25 °C in the dark, colonized plugs of the isolate were transferred to two agar media for conidia production. Amended media contained either 150 ml of V8 juice and 3 g $CaCO_3$ per liter or 40 g of single-grain oatmeal per liter. The cultures were placed at room temperature (25 °C) under continuous fluorescent lighting. After 10 days, conidia were harvested from the agar surface of each plate by adding 10 ml of H_2O, lightly brushing with a small paintbrush, and pouring off the conidial suspension into a 500 ml flask. The suspension was then filtered through four layers of cheesecloth into another 500 ml flask. The conidial suspension was adjusted to a concentration of 2×10^5 conidia ml^{-1} using a hemacytometer.

Treatments were arranged in a randomized complete block experimental design with six replications. Seven days following the final treatments, the grasses were randomized by treatment underneath a 3.65 m x 1.22 m x 0.76 m polyvinyl chlorinated (PVC) frame. Each pot was inoculated with 10 ml of the conidial suspension applied with an airbrush (Model 350, Badger Air-Brush Co., Franklin Park, IL). Black plastic was draped over the frame to form an enclosed chamber and two atomizing humidifiers (Herrmidifier 500 Series, Trion Inc., Sanford, NC) were used to provide 100 % relative humidity (RH) during initial incubation period and each nighttime cycle. Mean greenhouse incubation conditions for the first 17 h were 24.5 °C, 100 % RH, and no light. Subsequent conditions averaged 12-h days at 27 °C and 50 % RH and 12-h nights at 24.5 °C and 100 % RH. These conditions were previously shown to be conducive for successful inoculation of *P.* grisea on tall fescue (18).

Disease incidence and leaf spot length were measured six days after inoculation. Disease incidence was measured by counting the number of infected leaves and calculating a percentage of diseased leaves relative to the total number of leaves in each pot. The same total leaf count

was used for all treatments and was determined by calculating the average of the total number of leaves in each of 10 randomly selected pots. Leaf spot length was used as an indicator of disease severity by measuring the length of 10 arbitrarily selected leaf spots per pot using a handheld digital caliper (Series 500, Mitutoyo Corp., Aurora, IL). Data were analyzed using ANOVA and means separations were performed using the Waller-Duncan *k*-ratio *t*-test (k=100).

2,4-D sensitivity study. *Pyricularia grisea* isolates 1207-59 and 1213-77 were revived from -80 °C storage by placing mycelium-coated filter paper discs on the surface of a 9-cm-diameter Petri dish containing PDA amended with 50 μg ml^{-1} each of tetracycline, streptomycin, and chloramphenicol. The isolates were incubated at 25 °C for seven days, transferred to 1.5 % water agar (WA, Difco), and incubated at 25 °C for 14 days.

Solutions were derived from formulated commercial product (Weedar 64, Nufarm, Burr Ridge, IL) and technical grade 2,4-D (98 % minimum, Sigma Aldrich Co., St. Louis, MO) by dissolving each in acetone at concentrations less than < 5 μg ml^{-1}. These suspensions were then added to agar at seven concentrations ranging from 0.01 to 1000 μg a.i. ml^{-1}. Non-amended agar was used as the control medium. Mycelial plugs measuring 6.75 mm in diameter were excised from WA plates and placed at the center of 9-cm-diameter Petri dishes containing herbicide-amended PDA or control medium. Petri dishes were incubated at 25 °C for approximately seven days and colony growth was measured when diameters expanded to greater than 30 mm. The effect of each formulation was assessed twice.

RESULTS

Turf quality. Dicamba-treated plots exhibited significantly lower turf quality when compared to the untreated control in Study A (with flutolanil); however, no other turf quality differences were evident in either study (Tables 1 and 2). Overall turf quality was slightly

greater in Study B (without flutolanil) as opposed to Study A, due to location in the field and proximity to supplemental irrigation. As a whole, decreased turf quality in the 2002 studies was primarily a result of severe drought conditions and inconsistent irrigation.

In the 2003 study, plots treated with 2,4-D exhibited significantly greater turf quality than the untreated check on four of the six rating dates (Table 3). Turf quality steadily declined throughout all plots over the rating dates due to increasing gray leaf spot development. Rainfall and supplemental irrigation were not a limiting factor during this study.

Disease Development. Brown patch was the only disease observed in the 2002 studies, and only turf in Study B (without flutolanil) exhibited symptoms. Minimal disease development occurred due to unfavorable drought conditions and poor turf quality. Consequently, there were no significant differences in brown patch incidence as a result of herbicide treatments when compared to the untreated control (Table 4).

Gray leaf spot developed across the plot area in the 2003 study. Environmental conditions were very conducive for gray leaf spot development. Plots treated with 2,4-D exhibited significantly less foliar blighting than the untreated control on four of the six rating dates, based on linear contrasts (Table 5, Figure 1). Turf treated with 2,4-D amine + MCPP + dicamba also exhibited significantly less foliar blighting than the untreated check on three of the six rating dates. No significant differences were observed among treatments based on AUDPC values (Table 6).

Data from the greenhouse study of 2004 revealed no significant differences in disease incidence or leaf spot size between herbicide-treated turf and untreated controls, although there were differences among herbicide treatments (Table 7). Plants treated with 2,4-D exhibited the highest disease incidence of all treatments and 2,4-D + MCPP + dicamba treatments yielded turf

with the lowest disease incidence. Disease incidence from the 2,4-D-treated plants was significantly higher than that of plants treated with 2,4-D + MCPP + dicamba or dicamba alone. Herbicide treatments resulted in no differences in mean size of leaf spot (disease severity). Ratings on dithiopyr-treated pots were not recorded because of early herbicide injury that resulted in complete plant death in all six replications of this treatment.

Sensitivity to 2,4-D. Mean colony diameters were not affected (range 33-37 mm) at herbicide concentrations up to 100 μg ml^{-1} (Table 8). Concentrations of 500 and 1000 μg ml^{-1} completely inhibited *P. grisea* growth in vitro. Although no general pattern of growth inhibition was observed with increasing 2,4-D concentrations, morphological changes were noticed. Dark, black-colored mycelial growth was consistently observed within a 5 mm radius of the center plugs, compared to more white-colored growth as the colonies expanded to greater than 30mm. However, there was no visible correlation between increasing 2,4-D concentrations and morphological changes of *P. grisea* colonies.

DISCUSSION

Field studies in 2002 and 2003 did not yield similar results in turf quality or disease development. The 2002 studies were affected by severe drought throughout the summer and since the area was on a commercial sod farm, irrigation scheduling was difficult to adjust in order to promote disease development. Turf quality also was adversely affected by the lack of irrigation and few disease symptoms were observed. Although the dicamba-treated plots had lower turf quality than the untreated plots, this effect was not observed in any other studies and was probably the result of the unfavorable growing conditions that prevailed in 2002. No other significant effects were observed. It is also doubtful that artificial inoculation of the plots with *P. grisea* would have been successful due to the lack of rainfall and irrigation.

In the 2003 field study, rainfall and supplemental irrigation were not limiting factors for turf health or gray leaf spot development. The gray leaf spot-susceptible cultivar 'Tarheel' was also used to encourage gray leaf spot development in order to evaluate treatment effects. Turf quality and disease incidence means were adjusted using the Papadakis method to compensate for non-randomized variation within the plot area (4). Turf quality was consistently greater in the 2,4-D-treated plots compared to other herbicide treatments and the untreated control. Foliar blight incidence also was significantly less in plots treated with 2,4-D and the treatment that combined 2,4-D with MCPP + dicamba when compared to the untreated control. The mechanisms for the increased turf quality and reduced disease incidence are unknown. However, since 2,4-D is a growth inhibitor, reductions in pathogen growth might be the result of an effect on the host and not directly on the pathogen. In the colony-growth study, 2,4-D exhibited a very low direct toxicity to *P. grisea*. Mycelial growth was totally inhibited at 2,4-D concentrations greater than 100 $\mu g\ ml^{-1}$, but it had no effect at lower concentrations. The concentration of the herbicide on the leaf surface was not determined.

In contrast to the 2003 field study, 2,4-D treatments did not result in increased turf quality or reduced disease in the greenhouse study. In fact, plants treated with 2,4-D actually exhibited the highest disease incidence among all treatments, although the difference was not significant when compared to the untreated control. The difference was significant, however, when compared to plants treated with 2,4-D + MCPP + dicamba or dicamba alone. This coincides with the results reported by Oka and Pimentel (15), who demonstrated increased growth of the southern corn leaf blight pathogen (*Bipolaris maydis* L.) after 2,4-D applications in corn. Dithiopyr treated-plants were removed from the study due to fatal herbicide injury in all replications. The combination of young plants (eight weeks of age) and applications of the high

label rate might explain this observation. Differences in plant age and growing environments seem to be the only explanations for discrepancies observed between the field and greenhouse studies.

Commonly used preemergent herbicides do not appear to play a predisposing role in gray leaf spot development in tall fescue, nor do applications of certain postemergent herbicides. In fact, applications of 2,4-D actually resulted in significantly greater turf quality and significantly fewer disease symptoms when compared to the untreated control. Growth inhibition of *P. grisea* by high concentrations of 2,4-D may indicate the presence of an antagonistic affect of 2,4-D on *P. grisea*.

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Table 1. Herbicide treatments, application rates, and turf quality means for 2002 study (with flutolanil).

Treatment[y]	Rate (kg a.i. ha^{-1})	6-Aug	20-Aug	3-Sep	17-Sep	1-Oct	TQ Average
Dithiopyr	0.57	5.0a[z]	5.0a	6.0a	7.3a	7.0a	6.0ab
Fenoxaprop	0.10	5.0a	5.0a	6.0a	7.0a	7.3a	6.0ab
2,4-D+MCPP+ dicamba	1.65	5.0a	5.0a	6.0a	7.0a	7.3a	6.0ab
Dicamba	0.56	5.0a	5.0a	6.0a	6.3b	6.8a	5.8b
2,4-D	1.59	5.0a	5.0a	6.0a	7.0a	7.3a	6.0ab
Untreated		5.0a	5.0a	6.0a	7.0a	7.5a	6.1a

[y] Applications of each treatment were applied on 23 July and on 20 Aug
[z] Means followed by the same letter within a column are not significantly different based on Waller-Duncan *k*-ratio *t* test (P=0.05)

Table 2. Herbicide treatments, application rates, and turf quality means for 2002 study (without flutolanil).

Treatment[y]	Rate (kg a.i. ha^{-1})	6-Aug	20-Aug	3-Sep	17-Sep	1-Oct	TQ Average
Dithiopyr	0.57	6.0a[z]	6.0a	6.0a	7.5a	7.0a	6.5a
Fenoxaprop	0.10	6.0a	6.0a	6.0a	7.5a	6.8a	6.5a
2,4-D+MCPP+ dicamba	1.65	6.0a	6.0a	6.0a	7.5a	7.0a	6.5a
Dicamba	0.56	6.0a	6.0a	6.0a	7.5a	6.8a	6.5a
2,4-D	1.59	6.0a	6.0a	6.0a	7.5a	6.8a	6.5a
Untreated		6.0a	6.0a	6.0a	7.5a	7.0a	6.5a

[y] Applications of each treatment were applied on 23 July and on 20 Aug
[z] Means followed by the same letter within a column are not significantly different based on Waller-Duncan *k*-ratio *t* test (P=0.05)

Table 3. Herbicide treatments, application rates, and turf quality means for 2003 study.

Treatment[x]	Rate (kg a.i. ha^{-1})	14-Aug	21-Aug	28-Aug	10-Sep	23-Sep	1-Oct
Dithiopyr	0.57	7.0	6.8	6.2	5.6	5.6	5.9
Fenoxaprop	0.10	7.0	7.0	6.1	5.4	5.3	5.7
2,4-D+MCPP+ dicamba	1.65	7.0	6.8	6.4	5.6	5.6	5.8
Dicamba	0.56	6.4	6.5	5.8	4.9	5.1	5.2
2,4-D	1.59	7.1[y]	7.3[y]	6.4	5.8[y]	5.8	6.3[z]
Untreated		6.4	6.2	5.8	5.2	5.3	5.5

[x] Applications of each treatment were applied on 1 July and on 29 July
[y] Means within a column are significantly different from the untreated control based on linear contrasts (P=0.10)
[z] Means within a column are significantly different from the untreated control based on linear contrasts (P=0.05)

Table 4. Herbicide treatments, application rates, and brown patch incidence means for 2002 study (without flutolanil).

Treatment[y]	Rate (kg a.i. ha^{-1})	16-Sep	1-Oct	Average
Dithiopyr	0.57	3.8a[z]	3.8a	3.8a
Fenoxaprop	0.10	5.0a	5.0a	5.0a
2,4-D+MCPP+ dicamba	1.65	3.8a	3.8a	3.8a
Dicamba	0.56	8.8a	7.5a	8.1a
2,4-D	1.59	7.5a	6.3a	6.9a
Untreated		6.3a	5.0a	5.6a

[y] Applications of each treatment were applied on 23 July and on 20 Aug
[z] Means followed by the same letter within a column are not significantly different based on Waller-Duncan *k*-ratio *t* test (P=0.05)

Table 5. Herbicide treatments, application rates, and gray leaf spot incidence means for 2003 study.

Treatment[x]	Rate (kg a.i. ha^{-1})	14-Aug	21-Aug	28-Aug	10-Sep	23-Sep	1-Oct
Dithiopyr	0.57	9.1	6.7	12.6	19.4	14.3	9.9
Fenoxaprop	0.10	2.4	5.5	10.4	21.3	16.9	14.6
2,4-D+MCPP+ dicamba	1.65	2.2[y]	5.2	5.7[y]	17.1[y]	13.5	9.4
Dicamba	0.56	5.1	7.0	16.3	25.7	23.7	22.0
2,4-D	1.59	0.8[z]	2.7	6.4[y]	16.7[y]	12.3[y]	7.9
Untreated		8.6	8.5	15.6	26.0	20.7	14.9

[x] Applications of each treatment were applied on 1 July and on 29 July
[y] Means within a column are significantly different from the untreated control based on linear contrasts (P=0.10)
[z] Means within a column are significantly different from the untreated control based on linear contrasts (P=0.05)

Table 6. Herbicide treatments, application rates, and area under the disease progress curve (AUDPC) means for 2003 study.

Treatment[y]	Rate (kg a.i. ha^{-1})	AUDPC
Dithiopyr	0.57	819.9a[z]
Fenoxaprop	0.10	625.5a
2,4-D+MCPP+dicamba	1.65	659.9a
Dicamba	0.56	940.0a
2,4-D	1.59	445.2a
Untreated		976.4a

[y] Applications of each treatment were applied on 1 July and on 29 July
[z] Means followed by the same letter within a column are not significantly different based on Waller-Duncan *k*-ratio *t* test (P=0.05)

Table 7. Herbicide treatments, application rates, gray leaf spot incidence means, and leaf spot size means for 2004 greenhouse study.

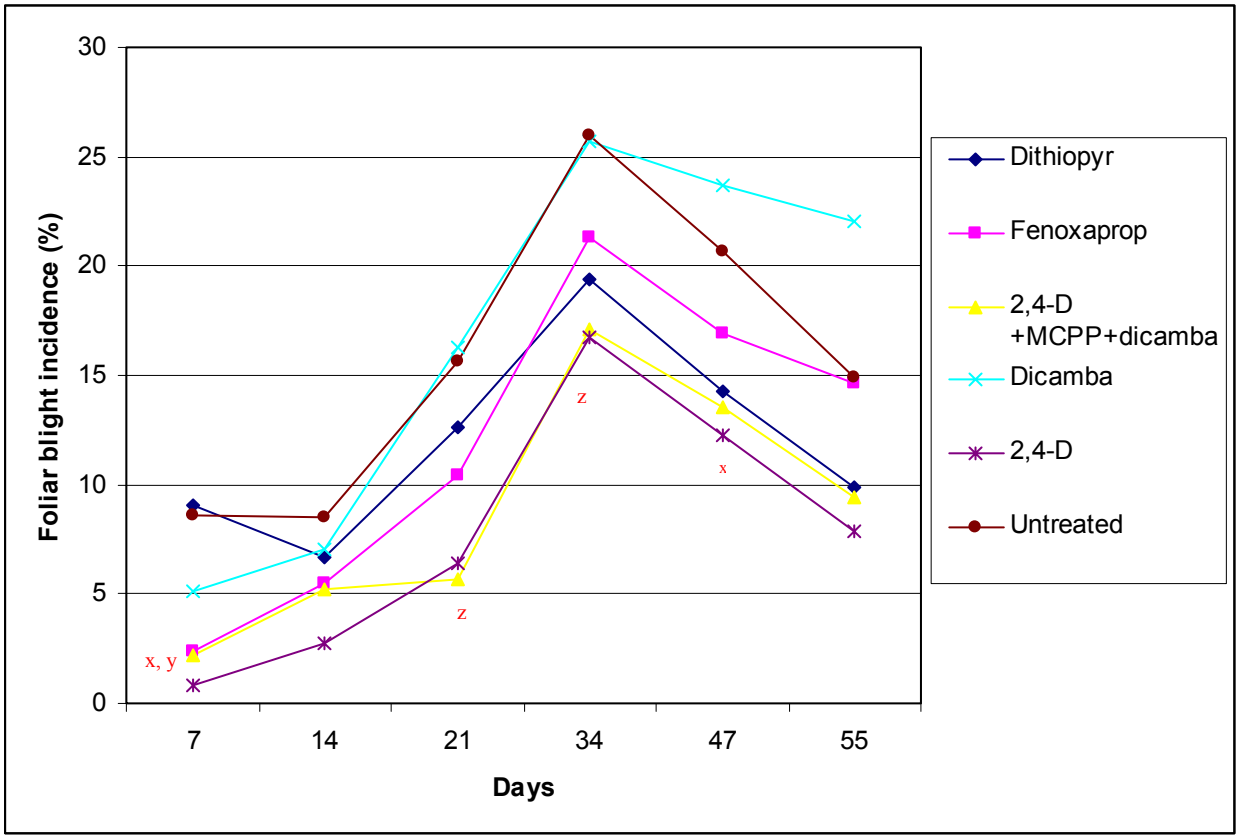
Treatment[y]	Rate (kg a.i. ha^{-1})	Disease incidence (%)	Leaf spot size (mm)
Fenoxaprop	0.10	12.5bcd[z]	3.36a
2,4-D+MCPP+ dicamba	1.65	10.1d	3.46a
Dicamba	0.56	11.4cd	3.29a
2,4-D	1.59	18.8ab	3.17a
Untreated		16.7abcd	3.22a

[y] Applications of each treatment were applied on 20 Jan and on 17 Feb
[z] Means followed by the same letter within a column are not significantly different based on Waller-Duncan *k*-ratio *t* test (P=0.05)

Table 8. Average colony diameters of *P. grisea* isolates 1207-59 and 1213-77 in response to different concentrations and formulations of 2,4-dichlorophenoxyacetic acid in vitro.

Isolate	Formulation	Concentration (μg ml^{-1})	Growth (mm)
1207-59	technical grade	0.00	34.28
		0.01	33.02
		0.10	34.95
		1	34.82
		10	36.13
		100	35.36
		500	0
		1000	0
1207-59	formulated product	0.00	36.33
		0.01	35.24
		0.10	35.42
		1	35.99
		10	35.16
		100	35.69
		500	0
		1000	0
1213-77	technical grade	0.00	36.18
		0.01	35.16
		0.10	35.48
		1	34.39
		10	35.45
		100	34.46
		500	0
		1000	0
1213-77	formulated product	0.00	37.65
		0.01	35.60
		0.10	35.70
		1	35.88
		10	35.85
		100	34.40
		500	0
		1000	0

Figure 1. Foliar blight incidence vs. time in response to herbicide treatments for 2003 study.



[x] Means for disease incidence on the same rating date for 2,4-D treatments are significantly different from the untreated control based on linear contrasts (P=0.05)

[y] Means for disease incidence on the same rating date for 2,4-D+MCPP+dicamba treatments are significantly different from the untreated control based on linear contrasts (P=0.10)

[z] Means for disease incidence on the same rating date for 2,4-D treatments and for 2,4-D +MCPP+dicamba treatments are significantly different from the untreated control based on linear contrasts (P=0.10)

CHAPTER 3

NUTRIENT EFFECTS ON THE DEVELOPMENT OF GRAY LEAF SPOT CAUSED BY *PYRICULARIA GRISEA* ON TALL FESCUE

(to be submitted to Plant Disease)

Nutrient Effects on the Development of Gray Leaf Spot Caused by *Pyricularia grisea* on Tall Fescue

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ABSTRACT

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Applications of nutrients were assessed for potential suppression of gray leaf spot development in tall fescue turf. Severe drought stress was primarily responsible for no significant treatment effects on turf quality or disease incidence in 2002. In 2003, phosphorous acid treatments resulted in lower quality turf than the untreated control, as did manganese and zinc treatments. Foliar blight incidence also was greatly increased in plots in 2003. In plots that received H_3PO_3 treatments on a 14 day schedule, the percentage of blighted turf was 40 % higher than in control plots. Area under the disease progress curve (AUDPC) analysis also revealed significant detrimental effects of the phosphorous acid treatments. Applications of manganous sulfate (on a 14 day interval) and zinc sulfate (on both 14-day and 28-day intervals) significantly reduced turf quality on two of the six rating dates in 2003. No other significant differences in turf quality or disease incidence were observed as a result of the manganese or zinc treatments. Applications of

potassium sulfate and calcium silicate did not significantly affect turf quality or disease incidence, either. Data from a greenhouse study did not agree with findings in the field; no significant differences were observed in disease incidence or size of leaf spots among nutrient treatments. Nutrient applications alone did not suppress development of gray leaf spot in tall fescue and should not be considered an effective alternative management strategy to chemical control.

INTRODUCTION

Pyricularia grisea (Cooke) Sacc. (teleomorph *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa) is a pathogen that affects over 22 genera of grasses of worldwide (10). Several grain crops of global economic importance are severely affected by blast disease caused by *P. grisea*, including rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (17). Common disease management strategies used in the past for these cereal grains have mainly relied on fungicides and resistant cultivars, although the longevity of resistance in cultivars is often short (22). Combinations of fungicides and nutrient applications have shown promise in controlling leaf and neck blast caused by *P. grisea* (18).

Gray leaf spot, induced by *P. grisea*, is a disease that affects several turfgrass species. St. Augustinegrass (*Stenotaphrum secundatum*), a warm-season species grown extensively in the southeastern United States (3), was the first turfgrass species identified as a host of *P. grisea* (16). In the early 1970s, *P. grisea* was reported as a pathogen of annual ryegrass (*Lolium multiflorum*) after a severe epidemic occurred in pastures in Louisiana and Mississippi (1, 7). Perennial ryegrass (*Lolium perenne*) was first identified as a host in 1991, with the first serious

outbreak reported on golf course fairways in Pennsylvania the same year (15). Gray leaf spot of tall fescue (*Festuca arundinacea*) was first observed in 1996 in North Carolina (12).

Integrated Pest Management (IPM) is the use of crop protection practices designed to reduce pest populations while minimizing environmental effects and impacts. Fertilization is a part of the cultural component of IPM that promotes healthy plants and potentially discourages pest invasion. Applications of nutrients, alone or in combination with fungicides, have been shown to be successful in controlling plant pathogens, including *Magnaporthe grisea* (3, 22). Although most plant diseases cannot be controlled by the use of fertilizers alone, sound fertilization practices are a key component of an integrated program for disease management.

Reduced phosphorous compounds, called phosphonates, have shown selective effectiveness against oomycetes (8). Several commercial phosphonate products that are registered as supplemental fertilizers have shown activity against *Phytophthora* sp. (11). Phosphite, the salt of phosphorous acid, has also been demonstrated to reduce the severity of several diseases, including Phytophthora root and crown rot (12, 23). Potassium, another plant macronutrient, can enhance disease control through direct effects on pathogens, by promotion of antagonistic phylloplane organisms, stimulation of host defense mechanisms, or a combination of these effects (21). Several turfgrass diseases, including large patch of St. Augustinegrass, caused by *Rhizoctonia solani*, and take-all patch, caused by *Gaeumannomyces graminis*, of colonial bentgrass (*Agrostis tenuis*), have been suppressed with applications of potassium (2, 13).

Manganese has also been implicated in the suppression of certain plant pathogens. The role of this essential micronutrient in cell wall structural functions may support host defense mechanisms (4). Reductions in disease incidence and severity of dollar spot (caused by *Scletotinia homoeocarpa*) of creeping bentgrass (*A. palustris*) have been reported after

applications of manganese (27). In the same study, applications of zinc also inhibited dollar spot development. Zinc is believed to be a factor in pathogen suppression due to its functions in cell membrane structure (24) and its direct toxicity to the pathogen (28).

Silicon is not considered an essential plant nutrient but has been shown to be a major component in biochemical pathways leading to resistance of plant pathogens (14). These effects have been most apparent in rice, where calcium silicate applications are common practice in controlling blast caused by *P. grisea* (18). Applications of silicon products, alone or in combination with fungicides, also have proven to significantly suppress gray leaf spot in St. Augustinegrass (3). Increases in bermudagrass (*Cynodon dactylon*) resistance to leaf spot, caused by *Bipolaris cynodontis*, have also been observed in response to amending soils with silicon (9). The objective of this study was to assess the effects of nutrient applications on suppression of gray leaf spot development in tall fescue turf.

MATERIALS AND METHODS

Field Study - 2002. This study was conducted at Sandhill Turf in Candor, NC from June to September 2002. Two plots areas (A&B) were established in a stand of 'Rebel III' tall fescue. Both experimental areas consisted of 1.5 m x 1.8 m individual plots in a randomized complete block design with nine treatments and four replications. The plot areas were irrigated to supplement rainfall and a height of approximately 6 cm was maintained by cutting twice a week. Brown patch development was selectively inhibited in experiment A with applications of flutolanil (Prostar 70WP, Bayer Environmental Science, Montvale, NJ). Applications of 2.9 kg. a.i. ha^{-1} were made on 23 July, 13 August, and 3 September. Experiment B received no fungicide applications.

Five nutrient treatments were initiated on 23 July: phosphorous acid (H_3PO_3; Sigma-Aldrich Co., St. Louis, MO) at 1.05 g/m^2, manganous sulfate ($MnSO_4$; Fisher Scientific, Fair Lawn, NJ) at 1.05 g/m^2, zinc sulfate ($ZnSO_4$; Fisher Scientific) at 0.07 g/m^2, potassium sulfate (K_2SO_4; Fisher Scientific) at 1.68 g/m^2, and calcium silicate ($CaSiO_2$; Calcium Silicate Corp., Lake Harbor, FL) at 500 g/m^2. Each treatment was applied in 0.12 liter H_2O/m^2 at 276 kPa with a CO_2-powered boom sprayer equipped with flat fan nozzles (Teejet 8004VS, Spraying Systems Co., Wheaton, IL). Phosphorous acid, $MnSO_4$, and $ZnSO_4$ treatments were each applied on 14- and 28-day intervals. Potassium sulfate was applied only on a 28-day interval and $CaSiO_2$ was applied in a single granular application on 23 July. Plots that received treatments on 14-day intervals received a total of six applications and plots receiving treatments on 28-day intervals received a total of three applications.

Visual turf quality ratings were first recorded on 6 August and continued bi-weekly until 1 October. Ratings were based on uniformity, density, and color of turf. A rating scale of 1 to 9 was used, where 9 represents ideal turf and 5 is the minimum acceptable quality. Brown patch was the only disease observed during the studies. Foliar blight incidence ratings began on experiment B when patches were first noticed on 17 September and were made again on 1 October. Foliar blight incidence was determined visually by estimating the amount of blighted turf in each plot on a scale of 0 to 100 %.

Statistical analyses were performed using SAS v. 8.2 (SAS Institute, Cary, NC). Mean separations for turf quality on individual rating dates, turf quality averages spanning all rating dates, brown patch incidence for individual rating dates, and brown patch averages spanning both rating dates were conducted using the Waller-Duncan *k*-ratio *t*-test (k=100). Analysis of

variance (ANOVA) was used to test the significance of nutrient treatments on turf quality and brown patch incidence.

Field Study - 2003. This study was conducted at the N. C. State University Lake Wheeler Turfgrass Field Lab in Raleigh, NC. One plot area was established with 'Tarheel' tall fescue by seeding at a rate of 35 g/m^2 in April 2003. Since this cultivar is relatively resistant to brown patch, only one plot area was established for the study and no flutolanil applications were applied. A complete fertilizer (18-24-6; Pennington Seed Inc., Madison, GA) was applied at a rate of 24.4 kg ha^{-1} of N, 32.5 kg ha^{-1} of P, and 8.1 kg ha^{-1} of K at time of seeding. The plots were maintained at an approximate height of 7 cm by mowing two or three times a week and were irrigated as needed during establishment. A randomized complete block design consisting of 1.5 m x 1.8 m plots was arranged with nine treatments and four replications. Nutrient treatments identical to the 2002 study were applied from 1 July to 9 September. Irrigation frequency was increased to four times per day from July through September to promote gray leaf spot development.

Disease incidence and visual turf quality ratings were first recorded on 14 August when foliar blighting began to form patches in the turf. Foliar blight incidence was measured by placing a 1.5 m x 1.5 m grid over each plot and counting the number of intersections that overlaid symptomatic turf. The grid consisted of a total of 196 intersections that were spaced 10.2 cm apart. Dividing the number of intersections overlaying symptomatic turf by the total number of intersections on the grid yielded a percentage of blighted turf for each plot. A total of six ratings were recorded approximately every two weeks from 14 August until 1 October, when turf recovery was observed.

Means for turf quality and gray leaf spot incidence were adjusted for field variation using the Papadakis method (5). Linear contrast analysis of means for turf quality and disease incidence for individual rating dates was conducted. Disease development over time was analyzed and compared using area under the disease progress curve (AUDPC) analysis (6). All disease incidence ratings were used to calculate AUDPC for each treatment using the formula

$$Y = \sum[(X_i + X_{i+1})/2](t_{i+1} - t_i)$$

where Y is AUDPC, X_i is the disease incidence rating for the i^{th} evaluation, X_{i+1} is the disease incidence rating of the $i + 1^{th}$ evaluation, and $(t_{i+1} - t_i)$ is the number of days between two evaluations.

Greenhouse Study - 2004. 'Tarheel' tall fescue was seeded at a rate of 35 g/m^2 into 10 cm diameter plastic pots containing calcined clay (Turface Allsport, Profile ProductsLLC, Buffalo Grove, IL). The pots were transferred to a greenhouse where they were watered three times per day to promote rapid seed germination. Following germination, each pot was fertilized weekly with 50 ml of 1/2-strength Hoagland's solution containing 105, 16, and 250 μg ml^{-1} of N, P, and K. The turf was maintained at a height of approximately 9 cm by cutting every 14 days.

Five nutrient treatments were applied to pots eight weeks after seeding: phosphorous acid (H_3PO_3) at 1.05 g/m^2, manganous sulfate ($MnSO_4$) at 1.05 g/m^2, zinc sulfate ($ZnSO_4$) at 0.07 g/m^2, potassium sulfate (K_2SO_4) at 1.68 g/m^2, and calcium silicate ($CaSiO_2$) at 500 g/m^2. Phosphorous acid, $MnSO_4$, $ZnSO_4$, and K_2SO_4 treatments were applied on 20 January, 3 February, and 17 February in 0.12 liter H_2O/m^2 at 276 kPa with a CO_2-powered boom sprayer equipped with flat fan nozzles (Teejet 8004VS, Spraying Systems Co., Wheaton, IL). Calcium silicate treatments were applied in a single granular application on 20 January.

Pyricularia grisea isolate 1207-59 was selected from a previous collection of isolates associated with tall fescue cultivars in Georgia (25). Filter paper discs containing the isolate were revived from -80 °C storage by placing the discs on the surface of a 9-cm-diameter Petri dish containing potato dextrose agar (PDA, Difco) amended with 50 μg ml^{-1} each of tetracycline, streptomycin, and chloramphenicol. After 10 days of incubation at 25 °C in the dark, colonized plugs of the isolate were transferred to two agar media for conidia production. Amended media contained either 150 ml of V8 juice and 3 g $CaCO_3$ per liter or 40 g of single-grain oatmeal per liter. The cultures were placed at room temperature (25 °C) under continuous fluorescent lighting. After 10 days, conidia were harvested from the agar surface of each plate by adding 10 ml of H_2O, lightly brushing with a small paintbrush, and pouring off the conidial suspension into a 500 ml flask. The suspension was then filtered through four layers of cheesecloth into another 500 ml flask. The conidial suspension was adjusted to a concentration of 2 x 10^5 conidia ml^{-1} using a hemacytometer.

Treatments were arranged in a randomized complete block experimental design with six replications. Seven days following the final treatments, the grasses were randomized by treatment underneath a 3.65 m x 1.22 m x 0.76 m polyvinyl chlorinated (PVC) frame. Each pot was inoculated with 10 ml of the conidial suspension applied with an airbrush (Model 350, Badger Air-Brush Co., Franklin Park, IL). Black plastic was draped over the frame to form an enclosed chamber and two atomizing humidifiers (Herrmidifier 500 Series, Trion Inc., Sanford, NC) were used to provide 100 % relative humidity (RH) during initial incubation and each nighttime cycle. Mean greenhouse incubation conditions for the first 17 h were 24.5 °C, 100 % RH, and no light. Subsequent conditions averaged 12-h days at 27 °C and 50 % RH and 12-h

nights at 24.5 °C and 100 % RH. These conditions were previously shown to be conducive for successful inoculation of *P.* grisea on tall fescue (18).

Disease incidence and leaf spot length were measured six days after inoculation. Disease incidence was measured by counting the number of infected leaves per pot and calculating a percentage of diseased leaves relative to the total number of leaves in each pot. The same total leaf count was used for all treatments and was determined by calculating the average of the total number of leaves in each of 10 randomly selected pots. Leaf spot length was used as an indicator of disease severity by measuring the length of 10 arbitrarily selected leaf spots per pot using a handheld digital caliper (Series 500, Mitutoyo Corp., Aurora, IL). Data were analyzed using ANOVA and means separations were performed using the Waller-Duncan *k*-ratio *t*-test (k=100).

RESULTS

Turf quality. No significant differences in turf quality were evident in either of the 2002 studies (Tables 9 and 10). Overall turf quality was noticably greater in Study A (with flutolanil) as opposed to Study B (without flutolanil), due to substantial brown patch development in Study B (Table 11). Turf quality of plots in Study A increased over the last two rating dates, while turf quality of plots in Study B decreased over the same period. Severe drought stress was primarily responsible for reductions in turf quality in 2002.

In the 2003 study, plots treated with phosphorous acid on both 14-day and 28-day intervals displayed significantly lower turf quality than the untreated control on all six rating dates (Table 12). Ratings for H_3PO_3 treatments on 14-day intervals were below the minimally acceptable rating for turf quality of 5 on five of the six rating dates, while ratings for H_3PO_3 treatments on 28-day intervals were below this standard on four of the six rating dates. Plots

treated with manganese ($MnSO_4$) on a 28-day interval exhibited significantly lower turf quality than the untreated control on two of the final three rating dates, as did both zinc ($ZnSO_4$) treatments. Turf quality steadily declined throughout all of the plots over the rating period, primarily due to substantial gray leaf spot development. Rainfall and supplemental irrigation were not a limiting factor during this study.

Disease Development. Considerable brown patch developed in the 2002 nutrient study without flutolanil (Study B). No treatments were significantly different from the untreated control. However, on the first rating date of 16 September, H_3PO_3-treated plots (14-day and 28-day intervals) exhibited a significantly greater brown patch incidence than the untreated control. Differences in brown patch incidence among nutrient treatments were significant in several cases, but none varied significantly from the untreated control.

In the 2003 study, frequent rainfall combined with hot, humid conditions led to a natural gray leaf spot epidemic. Plots treated with H_3PO_3 on both 14-day and 28-day intervals exhibited significantly greater foliar blight incidence than the untreated control, based on linear contrasts (Table 13, Figure 2). Plots treated on 14-day intervals had significantly greater disease than the control plots on all six rating dates and plots treated on 28-day intervals exhibited greater foliar blight incidence on four rating dates. On 10 September, when peak disease incidence was observed in all plots, foliar blighting in H_3PO_3-treated plots on 14-day intervals encompassed greater than 65 % of the plots on average, as compared to 25 % in untreated plots. No other treatment effects were observed. Area under the disease progress curve (AUDPC) data revealed a significant difference between H_3PO_3 plots treated on 14-day intervals and the untreated control (Table 14).

Data from the greenhouse study of 2004 revealed no significant differences in disease incidence or leaf spot size between nutrient-treated turf and untreated controls, or among nutrient treatments themselves (Table 15). Plants treated with zinc ($ZnSO_4$) exhibited the highest disease incidence of all treatments and manganese-treated plants ($MnSO_4$) yielded the lowest disease incidence, although no statistical differences were detected.

DISCUSSION

Environmental conditions and brown patch development were the major factors affecting turf quality in the 2002 field studies. Drought conditions, combined with inadequate supplemental irrigation, affected turf quality in both nutrient studies (A&B). Plots were located in a low area on the sod farm where wind exposure was low and moisture retention was probably greater, which favored disease development. Substantial brown patch was observed in Study B (without flutolanil), but nutrient treatments did affect brown patch incidence. Brown patch incidence was significantly greater in both phosphorous acid treatments compared to the untreated control on one of the two rating dates, indicating some type of chemical/pathogen interaction that enhanced pathogen activity and disease development.

The results from the 2002 field studies somewhat parallel the findings from the 2003 field studies, although cultivars used and disease observed were different. Turf quality was adversely affected by both phosphorous acid treatments throughout the duration of the study. Zinc and manganese treatments also negatively affected turf quality. No phytotoxicities were observed with any of these nutrient treatments, in particular the phosphorous acid treatments, where previous applications of technical grade phosphorous acid negatively affected growth of tobacco (*Nicotiana tabacum*) (23). The adverse effects of the nutrient treatments on turf quality can be explained as a function of treatments on gray leaf spot development affecting overall quality.

Substantial differences in gray leaf spot development between plots treated with phosphorous acid and the untreated control were strikingly evident in the 2003 study. The magnitude of gray leaf spot development in the H_3PO_3-treated plots definitely suggests a specific chemical/pathogen interaction that greatly enhanced *P. grisea* activity and subsequent symptom development. These findings do not agree with previous research that suggests a positive role of phosphorous compounds in suppression of plant pathogens, including *Phytophthora* sp. and *Puccinia sorghi* (8, 12, 19-21, 23). The fact that no other suppressive effects were observed also conflicts with earlier findings that applications of potassium, manganese, and zinc reduced the incidence of the turfgrass diseases dollar spot, take-all patch, and red thread (2, 13, 26, 27). We also saw no effects of a silicon treatment, which is also inconsistent with previous findings (3, 22).

Results from the 2004 greenhouse study did not agree with the results obtained in the field study of 2003. No significant treatment effects on either gray leaf spot incidence or severity were observed. The small range in ratings of disease incidence across all treatment means (14 % to 20 %) gives no indication that any of the nutrient treatments had a suppressive effect on gray leaf spot development under greenhouse conditions after artificial inoculation. Correspondingly, there were no differences in mean leaf spot sizes across treatments. This reveals that the same nutrient treatments did not increase or decrease the severity of leaf spot expansion caused by *P. grisea* in a gray leaf spot-susceptible cultivar of tall fescue.

There was no evidence from this study that nutrient applications have suppressed the development of gray leaf spot in tall fescue. In fact, applications of phosphorous acid appeared to enhance *P. grisea* activity and subsequent symptom expression. The data also indicates that zinc, manganese, potassium, and silicon do not suppress gray leaf spot development.

Applications of nutrients alone do not appear to be a viable option for control of gray leaf spot in tall fescue.

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Table 9. Nutrient treatments, application rates, and turf quality means for 2002 study (with flutolanil).

Treatment[v, w, x, y]	Rate (g/m^2)	6-Aug	20-Aug	3-Sep	17-Sep	1-Oct	TQ Average
H_3PO_3 (14 d)	1.05	5.0a[z]	5.0a	5.0a	6.5a	7.0a	5.7a
H_3PO_3 (28 d)	1.05	5.0a	5.0a	5.0a	6.8a	7.8a	5.9a
$MnSO_4$ (14 d)	1.05	5.0a	5.0a	5.0a	7.0a	7.8a	6.0a
$MnSO_4$ (28 d)	1.05	5.0a	5.0a	5.0a	6.8a	7.5a	5.9a
$ZnSO_4$ (14 d)	0.07	5.0a	5.0a	5.0a	6.8a	7.3a	5.8a
$ZnSO_4$ (28 d)	0.07	5.0a	5.0a	5.0a	6.5a	7.5a	5.8a
K_2SO_4	1.68	5.0a	5.0a	5.0a	6.8a	7.3a	5.8a
$CaSiO_2$	500	5.0a	5.0a	5.0a	7.3a	6.8a	6.1a
Untreated		5.0a	5.0a	5.0a	6.8a	7.5a	5.9a

[v] Applications of each 14-day treatment were applied on 23 July, 6 Aug, 20 Aug, 3 Sep, 17 Sep, and on 1 Oct

[w] Applications of each 28-day treatment were applied on 23 July, 20 Aug, and on 17 Sep

[x] K_2SO_4 applications were made on a 28 day schedule

[y] A single granular application of $CaSiO_2$ was made on 23 July

[z] Means followed by the same letter within a column are not statistically different based on Waller-Duncan *k*-ratio *t*-test (P=0.05)

Table 10. Nutrient treatments, application rates, and turf quality means for 2002 study (without flutolanil).

Treatment[v, w, x, y]	Rate (g/m^2)	6-Aug	20-Aug	3-Sep	17-Sep	1-Oct	TQ Average
H_3PO_3 (14 d)	1.05	5.0a[z]	5.0a	5.0a	4.8a	4.3a	4.8a
H_3PO_3 (28 d)	1.05	5.0a	5.0a	5.0a	4.3a	4.3a	4.7a
$MnSO_4$ (14 d)	1.05	5.0a	5.0a	5.0a	4.8a	5.0a	5.0a
$MnSO_4$ (28 d)	1.05	5.0a	5.0a	5.0a	5.0a	4.5a	4.9a
$ZnSO_4$ (14 d)	0.07	5.0a	5.0a	5.0a	4.5a	4.5a	4.8a
$ZnSO_4$ (28 d)	0.07	5.0a	5.0a	5.0a	4.5a	4.3a	4.8a
K_2SO_4	1.68	5.0a	5.0a	5.0a	5.0a	5.0a	5.0a
$CaSiO_2$	500	5.0a	5.0a	5.0a	5.0a	4.8a	5.0a
Untreated		5.0a	5.0a	5.0a	4.5a	4.5a	4.8a

[v] Applications of each 14-day treatment were applied on 23 July, 6 Aug, 20 Aug, 3 Sep, 17 Sep, and on 1 Oct

[w] Applications of each 28-day treatment were applied on 23 July, 20 Aug, and on 17 Sep

[x] K_2SO_4 applications were made on a 28 day schedule

[y] A single granular application of $CaSiO_2$ was made on 23 July

[z] Means followed by the same letter within a column are not statistically different based on Waller-Duncan *k*-ratio *t*-test (P=0.05)

Table 11. Nutrient treatments, application rates, and brown patch incidence means for 2002 study (without flutolanil).

Treatment[v, w, x, y]	Rate (g/m^2)	16-Sep	1-Oct	Average
H_3PO_3 (14 d)	1.05	40.0a[z]	42.5a	41.3a
H_3PO_3 (28 d)	1.05	37.5a	42.5a	40.0ab
$MnSO_4$ (14 d)	1.05	20.0bc	30.0ab	25.0bcd
$MnSO_4$ (28 d)	1.05	20.0bc	27.5ab	23.8cd
$ZnSO_4$ (14 d)	0.07	27.5ab	27.5ab	27.5abcd
$ZnSO_4$ (28 d)	0.07	27.5ab	35.0a	31.3abc
K_2SO_4	1.68	12.5c	15.0b	13.8d
$CaSiO_2$	500	20.0bc	27.5ab	23.8cd
Untreated		22.5bc	32.5ab	27.5abcd

[v] Applications of each 14-day treatment were applied on 23 July, 6 Aug, 20 Aug, 3 Sep, 17 Sep, and on 1 Oct

[w] Applications of each 28-day treatment were applied on 23 July, 20 Aug, and on 17 Sep

[x] K_2SO_4 applications were made on a 28 day schedule

[y] A single granular application of $CaSiO_2$ was applied on 23 July

[z] Means followed by the same letter within a column are not statistically different based on Waller-Duncan *k*-ratio *t*-test (P=0.05)

Table 12. Nutrient treatments, application rates, and turf quality means for 2003 study.

Treatment[u, v, w, x]	Rate (g/m^2)	14-Aug	21-Aug	28-Aug	10-Sep	23-Sep	1-Oct
H_3PO_3 (14 d)	1.05	6.0[y]	4.7[z]	3.7[z]	3.3[z]	3.3[z]	3.3[z]
H_3PO_3 (28 d)	1.05	5.8[z]	5.6[y]	4.7[z]	3.8[z]	3.7[z]	3.7[z]
$MnSO_4$ (14 d)	1.05	6.9	6.5	5.9	4.7	5.0	5.0
$MnSO_4$ (28 d)	1.05	6.8	6.5	5.9	4.6[y]	4.6[z]	5.0
$ZnSO_4$ (14 d)	0.07	6.9	6.8	5.5	4.7[y]	4.7[z]	4.8
$ZnSO_4$ (28 d)	0.07	6.4	6.1	5.4	4.5[z]	4.5[z]	4.7
K_2SO_4	1.68	6.7	6.7	5.9	5.0	5.5	5.5
$CaSiO_2$	500	6.9	6.8	5.7	5.2	5.3	5.3
Untreated		7.0	7.0	6.0	5.3	5.4	5.4

[u] Applications of each 14-day treatment were applied on 1 July, 15 July, 29 July, 12 Aug, 26 Aug, and on 9 Sep

[v] Applications of each 28-day treatment were applied on 1 July, 29 July, and on 26 Aug

[w] K_2SO_4 applications were made on a 28 day schedule

[x] A single granular application of $CaSiO_2$ was applied on 1 July

[y] Significantly different from the untreated control based on linear contrasts (P=0.10)

[z] Significantly different from the untreated control based on linear contrasts (P=0.05)

Table 13. Nutrient treatments, application rates, and gray leaf spot incidence means for 2003 study.

Treatment[v, w, x, y]	Rate (g/m^2)	14-Aug	21-Aug	28-Aug	10-Sep	23-Sep	1-Oct
H_3PO_3 (14 d)	1.05	16.4[z]	37.7[z]	55.5[z]	65.0[z]	58.6[z]	54.9[z]
H_3PO_3 (28 d)	1.05	9.7	16.8	35.7[z]	47.6[z]	45.6[z]	43.5[z]
$MnSO_4$ (14 d)	1.05	2.6	9.6	13.9	24.0	21.7	19.6
$MnSO_4$ (28 d)	1.05	8.6	12.8	19.7	35.9	27.9	22.7
$ZnSO_4$ (14 d)	0.07	6.7	11.3	21.6	37.2	30.2	23.7
$ZnSO_4$ (28 d)	0.07	7.0	11.8	21.5	34.3	29.5	26.3
K_2SO_4	1.68	7.4	8.6	18.9	28.4	21.9	19.4
$CaSiO_2$	500	5.0	7.2	14.6	21.5	20.0	15.3
Untreated		3.8	7.1	14.5	24.9	21.6	19.7

[v] Applications of each 14-day treatment were applied on 1 July, 15 July, 29 July, 12 Aug, 26 Aug, and on 9 Sep

[w] Applications of each 28-day treatment were applied on 1 July, 29 July, and on 26 Aug

[x] K_2SO_4 applications were made on a 28 day schedule

[y] A single granular application of $CaSiO_2$ was applied on 1 July

[z] Significantly different from the untreated control based on linear contrasts (P=0.05)

Table 14. Nutrient treatments, application rates, and area under the disease progress curve (AUDPC) means for 2003 study.

Treatment[v, w, x, y]	Rate (g/m^2)	AUDPC
H_3PO_3 (14 d)	1.05	2795.7a[z]
H_3PO_3 (28 d)	1.05	2226.0ab
$MnSO_4$ (14 d)	1.05	1289.2ab
$MnSO_4$ (28 d)	1.05	1147.1b
$ZnSO_4$ (14 d)	0.07	956.1b
$ZnSO_4$ (28 d)	0.07	1320.5ab
K_2SO_4	1.68	943.6b
$CaSiO_2$	500	1166.4b
Untreated		722.1b

[v] Applications of each 14-day treatment were applied on 1 July, 15 July, 29 July, 12 Aug, 26 Aug, and on 9 Sep

[w] Applications of each 28-day treatment were applied on 1 July, 29 July, and on 26 Aug

[x] K_2SO_4 applications were made on a 28 day schedule

[y] A single granular application of $CaSiO_2$ was applied on 1 July

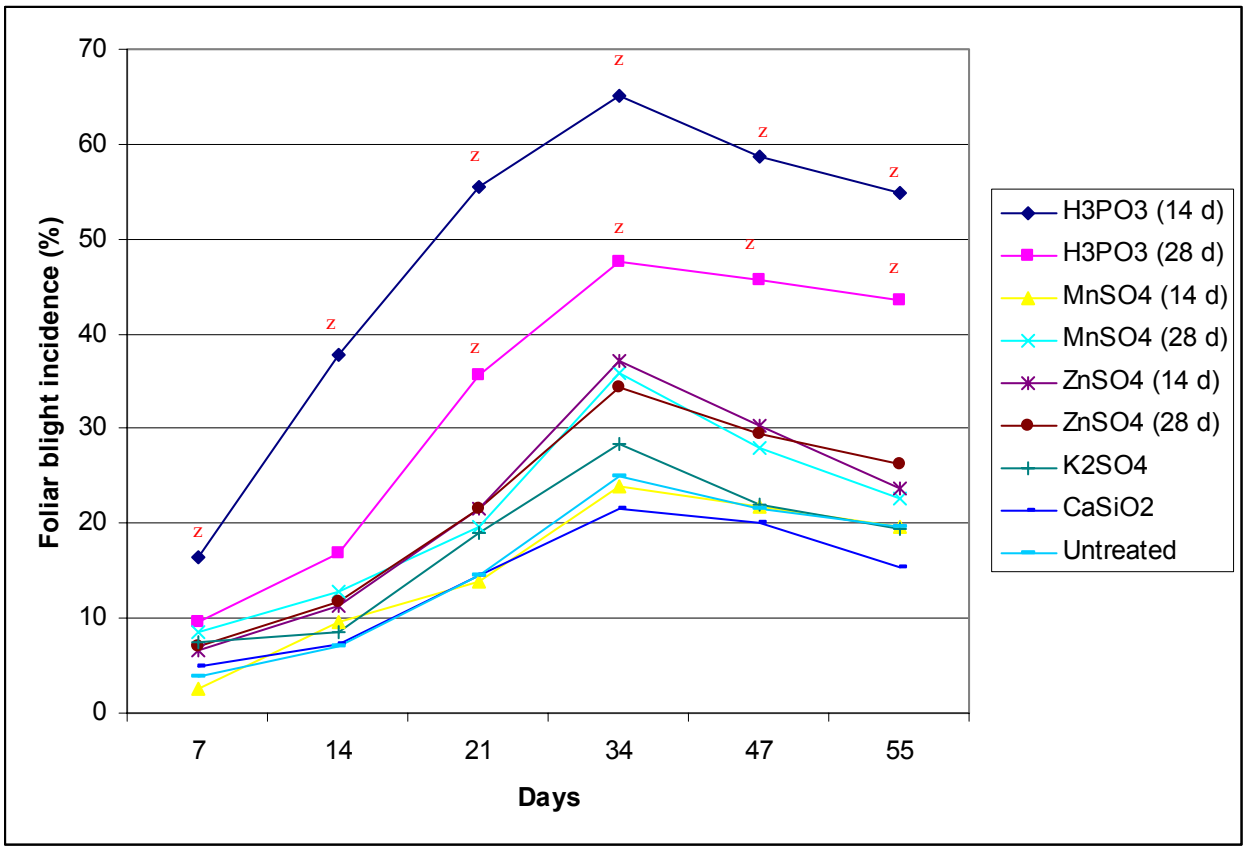
[z] Significantly different from the untreated control based on linear contrasts (P=0.05)

Table 15. Nutrient treatments, application rates, gray leaf spot incidence means, and leaf spot size means for 2004 greenhouse study.

Treatment[x, y]	Rate (g/m^2)	Disease incidence (%)	Leaf spot size (mm)
H_3PO_3	1.05	19.5ab[z]	3.26a
$MnSO_4$	1.05	14.1ab	3.38a
$ZnSO_4$	0.07	20.0a	3.35a
K_2SO_4	1.68	17.8ab	3.46a
$CaSiO_2$	500	18.8ab	3.30a
Untreated		16.7ab	3.22a

[x] H_3PO_3, $MnSO_4$, $ZnSO_4$, and K_2SO_4 treatments applied on 20 Jan, 3 Feb, and on 17 Feb
[y] A single granular application of $CaSiO_2$ was applied on 20 Jan
[z] Means followed by the same letter within a column are not statistically different based on Waller-Duncan *k*-ratio *t*-test (P=0.05)

Figure 2. Foliar blight incidence vs. time in response to nutrient treatments for 2003 study.



[z] Means for disease incidence on the same rating date for H_3PO_3 treatments on 14-day and 28-day intervals are significantly different from the untreated control based on linear contrasts (P=0.05)

CHAPTER 4

INOCULATION TECHNIQUES FOR *PYRICULARIA GRISEA* ON TALL FESCUE UNDER CONTROLLED CONDITIONS

ABSTRACT

Gregg, J. P., Peacock, C. H., Shew, H. D., and Tredway, L. P. 2004. Inoculation techniques for *Pyricularia grisea* on tall fescue under controlled conditions.

Artificial inoculation techniques are required to initiate gray leaf spot epidemics in controlled-environment experiments and are often needed in field studies when natural environmental conditions are not conducive for gray leaf spot development. Seeding rate, isolate selection, inoculum density, and post-inoculation environment (with or without covering with plastic bags) were evaluated for their role in disease incidence and leaf spot development in tall fescue. Isolate selection can also be a significant factor in disease development and leaf spot development. Selection of a virulent isolate on the cultivar of interest is important for successful symptom development. Higher rates of applied conidial suspensions (10 ml/pot) appear to produce significantly greater gray leaf spot symptoms than the low rate (5 ml/pot). Although the conidial concentration (number of spores per ml) was not altered between inoculation rates, increasing the volume of sprayed inoculum from 5 to 10 ml/pot did introduce an overall greater number of spores to the plants. Placing plants in covered containers or plastic bags immediately following inoculation for a 24-h period also appears to promote leaf wetness and subsequent disease development. Seeding rate did not have a significant effect on gray leaf spot development. The methods used for this controlled environment experiment agreed with previous studies and are successful in field applications, as well.

INTRODUCTION

Gray leaf spot, caused by *Pyricularia grisea* (Cooke) Sacc. (teleomorph *Magnaporthe grisea* (Hebert) Yaegashi & Udagawa), is an emerging disease problem in the turfgrass industry. The host range of this pathogen among turfgrass species has expanded over the last three decades. Gray leaf spot of turf was first identified in 1957 on St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze) (7), a coarse-textured, warm-season species grown in subtropical climates along the Gulf Coast states (2). Annual ryegrass (*Lolium multiflorum* Lam.) was first reported as a host after a severe epidemic occurred in Louisiana and Mississippi (1, 3). *Pyricularia grisea* was first diagnosed as a pathogen of perennial ryegrass (*Lolium perenne* L.) in 1986 and now is observed annually throughout the United States wherever perennial ryegrass is grown (4). An epidemic of gray leaf spot was reported in tall fescue (*Festuca arundinacea* Schreb.) in North Carolina in 1996, and the disease continues to be a problem on tall fescue in the transition zone in the southeastern United States (5).

Artificial inoculation techniques are required to incite gray leaf spot epidemics in controlled environment experiments and often are necessary in field studies when natural environmental conditions are not highly conducive for gray leaf spot development. Optimal infection conditions include high humidity and a period of prolonged leaf wetness. Even when favorable conditions are present for pathogen infection and symptom development in field plots, outbreaks resulting from artificial inoculation are often unpredictable and non-uniform in experimental plots. Since consistency in disease development between field and controlled environment studies are desirable, development and implementation of reliable inoculation methods that are successful in both environments is important.

Several inoculation techniques for *Pyricularia grisea* have been evaluated on perennial ryegrass and tall fescue turfgrasses. In a greenhouse study by Trevathan (11), 4-week-old perennial ryegrass plants were spray-inoculated and placed in a mist chamber for 72 hours. Symptoms were apparent 24-48 h after removal from the mist chamber and ranged from no lesions to death of plants. A follow-up study conducted by Moss and Trevathan (8) concluded that 4-5 week-old plants were most susceptible. They found that total lesion counts increased with increasing inoculum densities and that a conidial concentration of 2.0×10^5 spores ml^{-1} was adequate for lesion production. The optimum temperature for symptom development was 25 °C and a minimum of 24 h of continuous leaf wetness was required for infection.

Tredway (9) and Tredway et al. (10) reported successful spray inoculation of tall fescue in controlled environment chambers after a 24-h incubation period at 24 °C, 100 % relative humidity (RH), and no light. Subsequent conditions were 12-h days at 30 °C and 75 % RH and 12-h nights at 24 °C and 100 % RH. A similar method used by Han et al. (6) resulted in successful inoculation of perennial ryegrass in both growth chamber and greenhouse experiments. In a corresponding field study, test plots were inoculated using a backpack sprayer and then covered with black plastic film. The covering was removed every morning, irrigation was applied twice during the day, and the covering was replaced every night after misting. The fungicide flutolanil was applied to prevent brown patch development. Gray leaf spot symptoms were observed six days after inoculation. Plots were inoculated a second time one week after the initial inoculation and disease developed into a severe epidemic within a week. A significant correlation ($r = 0.87$) was reported between results of this test and a recent natural epidemic, indicating this inoculation method is reliable for simulation of natural gray leaf spot epidemics. The objective of this study was to evaluate several factors involved with the inoculation of *P.*

grisea on tall fescue. Seeding rate, inoculation rate, isolate selection, and +/- covering of plants after inoculation were evaluated as factors of disease incidence and leaf spot length.

MATERIALS AND METHODS

This study was conducted from April to June of 2003 in the Southeastern Plant Environment Laboratory at North Carolina State University. 'Rebel III' tall fescue was seeded into 15-cm-diameter plastic pots containing calcined clay (Turface Allsport, Profile ProductsLLC, Buffalo Grove, IL) on 10 April. Twenty-four of the 48 total pots were seeded at 24 g/m^2 and the other half were seeded at 39 g/m^2. Pots were moved into a greenhouse where growing conditions consisted of 12 h at 26 °C and 12 h at 24 °C. Following germination, the turf was fertilized every two weeks with 1/2-strength Hoagland's solution at 105, 16, and 250 $\mu g\ ml^{-1}$ of N, P, and K, respectively. Grasses were watered two times per day and maintained at 8 cm height by cutting every two weeks.

Pyricularia grisea isolates 1207-59 and 1213-77 were revived from -80 °C storage by placing filter paper discs on the surface of a 9-cm-diameter Petri dish containing potato dextrose agar (PDA, Difco) amended with 50 $\mu g\ ml^{-1}$ each of tetracycline, streptomycin, and chloramphenicol. After 10 days of incubation at 25 °C in the dark, colonized plugs of the isolate were transferred to three agar media for conidia production. Amended media contained either 150 ml of V8 juice and 3 g $CaCO_3$ per liter or 40 g of single-grain oatmeal per liter. The third agar media contained 1.5 % water agar (WA, Difco) and was overlaid with 10 sterilized alfalfa stem sections (7-cm-long), with leaves and petioles removed. All cultures were placed at room temperature (25 °C) under continuous fluorescent lighting. After 14 days, conidia were harvested by transferring the alfalfa stem sections to 50 ml conical centrifuge tubes (Corning Inc., Corning, NY) containing 10 ml of H_2O, vortexing for 10 s, then filtering through four layers

of cheesecloth. Conidia from the V8- and oatmeal-amended agar surfaces were removed by gently brushing the agar surface with a small paintbrush after adding 10 ml of H_2O. This suspension was then filtered though four layers of cheesecloth. Conidial suspensions from all three inoculum production methods were combined into one sample for each isolate. The conidial suspensions were adjusted to a final concentration of 2×10^5 conidia ml^{-1} using a hemacytometer.

Eight weeks after seeding, grasses were moved into a growth chamber and arranged in a semi-systematic split plot experimental design. Eight carts were randomly placed into the growth chamber. Each cart consisted of six pots, three of which were seeded at 24 g/m^2 and the other three at 39 g/m^2. Individual carts received the same isolate, inoculation rate, and post-inoculation covering treatment. The whole plot factor consisted of isolate x inoculation rate x covering; seeding rate was the sub-plot factor. The treatment design was a 2 x 2 x 2 x 2 factorial. The grass in each pot was inoculated with either 5 or 10 ml of conidial suspension from a single isolate applied with an airbrush (Model 350, Badger Air-Brush Co., Franklin Park, IL). Immediately following inoculation, one half of the pots were placed in clear plastic bags and sealed at the top with rubber bands. Incubation conditions for the first 24 h were 24 ºC, 100 % RH, and no light. Plastic bags were removed after 24 h. Subsequent growth chamber conditions were 12-h days at 30 ºC and approximately 70 % RH and 12-h nights at 24 ºC and 100 % RH. An automated misting system was used to maintain 100 % RH during incubation and nighttime cycles. The experiment was repeated unsuccessfully once more.

Disease was evaluated seven days after inoculation. Disease incidence was determined by counting the number of leaves per pot with one or more leaf spots present and then dividing by the total number of leaves per pot. The total number of leaves per pot was determined by

calculating an average of the total number of leaves in each of 10 randomly selected pots. The same total leaf count was used for all treatments. Leaf spot length was determined and used as an indicator of disease severity. The length of 10 arbitrarily selected leaf spots per pot was measured using a handheld digital caliper (Series 500, Mitutoyo Corp., Aurora, IL). Statistical analyses were performed using SAS v. 8.2 (SAS Institute, Cary, NC). Data were initially analyzed using ANOVA. Resulting significant factor interactions were identified and treatment means were adjusted for model effects. Treatment means were then analyzed using the LSMEANs procedure and pairwise comparisons were made between significant treatment means.

RESULTS

All of the factors investigated except seeding rate affected the incidence and severity of gray leaf spot. In addition, there also were several significant interactions, including an isolate x inoculum density and an isolate x covering treatment. Isolate 1207-59 had a significantly higher disease incidence at the high inoculum density (10 ml per pot) than at the low inoculum level, whereas, there was no effect of inoculum density on disease incidence with isolate 1213-77 (Table 16). At both inoculum densities disease incidence was higher with isolate 1207-59 than with isolate 1213-77. Average leaf spot length was not affected by inoculum density with isolate 1207-59, but with isolate 1213-77, leaf spot length was greater at the low inoculum density than at the high inoculum density (Table 16). Leaf spot length was greater with isolate 1207-59 ($\geq$ 4.3 mm) than with isolate 1213-77 ($\leq$ 3.5 mm) at both inoculum densities (Table 16).

Disease incidence and leaf spot length were significantly greater for isolate 1207-59 than for isolate 1213-77 regardless of whether plants were covered after inoculation (Table 17). However, isolates reacted differently to the covering treatment. While there were no differences

in disease incidence caused by isolate 1213-77 based on whether plants were covered or not, covering of plants after inoculation reduced disease in plants inoculated with isolate 1207-59 compared to covered plants.

Mean leaf spot length caused by isolate 1207-59 was significantly greater than leaf spot length caused by isolate 1213-77 regardless of cover treatment. Cover treatment had no effect on leaf spot length for isolate 1207-59, but leaf spot length was significantly increased for isolate 1213-77 in the uncovered vs. the covered treatment.

DISCUSSION

The spray inoculation method developed for *P. grisea* in growth chamber studies (6, 9, 10) is successful when environmental parameters can be controlled. Optimal temperatures, relative humidities, moisture, and lighting are imperative for successful infection by *P. grisea* and subsequent gray leaf spot development.

In our study, seeding rates of 24 g/m^2 and 39 g/m^2, alone or in combination with other experimental factors, did not have a significant impact on mean disease incidence or leaf spot length. Plant age was not evaluated in this study, although it has been previously identified as a significant factor for successful infection by *P. grisea* (8, 11).

The two isolates (1207-59 and 1213-77) used in this study differed in aggressiveness and responded differently to the environmental parameters that were varied. Isolate 1207-59 induced significantly more disease and caused larger leaf spots than isolate 1213-77. Very few disease symptoms were observed in pots treated with isolate 1213-77; therefore, conclusions from disease incidence and leaf spot measurements resulting from treatments including this isolate should be made with caution. Selection of an aggressive isolate on the cultivar of interest is very important in obtaining reliable results for determining conditions that favor disease development.

The presence or lack of plastic covering was evaluated in place of the plastic containers used as incubation chambers in previous studies (6, 10). The covered treatments actually resulted in less disease development than uncovered treatments inoculated with one isolate (1207-59); no differences were noticed with the less aggressive isolate (1213-77). Very little gray leaf spot occurred with isolate 1213-77 in both treatments (< 2 %), so the effects of the plastic covering on these treatments should not be strongly considered. It is not known why less disease developed with covering for the more aggressive isolate 1207-59.

Inoculum density was a significant factor in disease development and leaf spot size for the more aggressive 1207-59, but not for the less aggressive 1213-77. This agrees with the findings of Moss and Trevathan (8), where total lesion number increased with inoculum density. Although the conidial concentration (number of spores per ml) was not altered between inoculation rates, increasing the volume of sprayed inoculum from 5 to 10 ml/pot did introduce an overall greater number of spores to the plants.

Isolate aggressiveness, inoculum density, and presence/absence of covering after inoculation, combined with optimal environmental conditions, seem to be the most important factors for successful infection of tall fescue by *P. grisea.* Seeding rate did not have a significant effect on gray leaf spot development following inoculation. Selection of isolates that are virulent and highly aggressive on the cultivar of interest is necessary to initiate epidemics of gray leaf spot.

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Table 16. Effects of isolate and inoculum density on the incidence and severity of gray leaf spot on tall fescue.

Isolate	Inoculation rate (ml)[w]	Disease Incidence (%)[x]	Lesion length (mm)[y]
1207-59	5	9.6b[z]	4.3a
	10	18.3a	4.6a
1213-77	5	1.3c	3.5b
	10	1.3c	3.0c

[w] Inoculation rate is the total amount of conidial suspension (2×10^5 spores ml^{-1}) applied to individual pots

[x] Disease incidence was determined by counting the number of symptomatic leaves per pot and dividing this number by the average number of leaves per pot, which was determined by counting the total number of leaves in each of 10 randomly-selected pots

[y] Leaf spot length was determined by measuring 10 arbitrarily selected leaf spots per pot using a handheld digital caliper

[z] Means followed by different letters within a column are significantly different based on LSMEANs comparisons (P = 0.05)

Table 17. Effects of isolate and covering on the incidence and severity of gray leaf spot on tall fescue.

Isolate	Cover[w]	Disease Incidence (%)[x]	Leaf Spot Size (mm)[y]
1207-59	yes	11.7b[z]	4.3a
	no	16.3a	4.6a
1213-77	yes	1.0c	3.0c
	no	1.7c	3.6b

[w] Cover refers to the presence/absence of a plastic bag over individual pots for a 24-h period following inoculation

[x] Disease incidence was determined by counting the number of symptomatic leaves per pot and dividing this number by the average number of leaves per pot, which was determined by counting the total number of leaves in each of 10 randomly-selected pots

[y] Leaf spot length was determined by measuring 10 arbitrarily selected leaf spots per pot using a handheld digital caliper

[z] Means followed by different letters within a column are significantly different based on LSMEANs comparisons (P = 0.05)

CHAPTER 5

SUMMARY AND CONCLUSIONS

The host range of *Pyricularia grisea* among turfgrasses has expanded greatly over the last three decades. Since gray leaf spot was first reported as a pathogen of St. Augustinegrass in 1957 (Malca and Owen, 1957), this disease has affected annual ryegrass (Bain et al., 1972), perennial ryegrass (Landschoot and Hoyland, 1992), and tall fescue (Fraser, 1997) turfgrasses in their respective growing regions throughout the United States. Previous research has indicated that applications of certain herbicides may enhance disease development in turfgrasses (Turgeon et al., 1974; Karr et al., 1979; Uddin and Soika, 2000). Several studies have also demonstrated suppression effects of nutrient applications on fungal pathogens in turfgrasses (Goss and Gould, 1967; Bloom and Couch, 1960; Uddin et al., 1999). Determining the potential for adjusting cultural practices as additional control measures for gray leaf spot in tall fescue may allow for reduced economic and chemical inputs in pest management programs.

The objectives of this study were to determine distinct herbicide interactions with respect to predisposition to gray leaf spot development, assess potential suppression effects of nutrient applications on gray leaf spot development, and to evaluate inoculation techniques for *Pyricularia grisea* in tall fescue under controlled conditions. The effect of herbicide and nutrient treatments on disease incidence (brown patch and gray leaf spot) and turf quality was determined in field studies conducted in 2002 and 2003. A corresponding greenhouse study was carried out in 2004 to evaluate the same parameters. An in vitro study was conducted in 2003 to demonstrate the growth-inhibiting effects of 2,4-D on *P. grisea*. Several factors were evaluated for their effects on gray leaf spot incidence and leaf spot development in a controlled environment study in 2003.

Herbicide treatments did not significantly enhance gray leaf spot incidence nor negatively affect turf quality in either field study. The 2002 studies were affected by severe drought.

Dicamba-treated plots has statistically lower turf quality than the untreated control during 2002, but this result was not reproduced and should not be considered a significant finding due to unfavorable growing conditions. In the 2003 field study, turf quality was consistently greater in 2,4-D-treated plots than other plots for unknown reasons. Foliar blight incidence was significantly less in plots treated with 2,4-D and 2,4-D + MCPP + dicamba. Considering 2,4-D is a growth inhibitor, reductions in pathogen growth and subsequent symptom development could be explained by a specific, unknown chemical/pathogen interaction.

Results from a greenhouse study in 2004 did not produce similar findings, however. Plants treated with 2,4-D exhibited the highest disease incidence among treatments, although statistically insignificant when compared to the untreated control. There was no effect of herbicide treatment on leaf spot length. Differences in plant age and growing environments seem to be the only explanations for discrepancies in field and greenhouse results. Herbicide applications do not appear to play a predisposing role in gray leaf spot development in tall fescue.

Growth inhibition of *P. grisea* was observed at 2,4-D concentrations greater than 100 μg ml^{-1} in vitro. Increases in 2,4-D concentrations from 0.01 μg ml^{-1} to 100 μg ml^{-1} did not affect mycelial growth, as colony diameters were consistent for all treatments in this range. Morphological changes were noticed in the colonies, with black-colored growth observed with a 5 mm radius of the center plugs compared to more white-colored growth further away from the center. There was no visible correlation between increasing 2,4-D concentrations and morphological changes of colonies, however. The growth-inhibiting effects of 2,4-D on *P. grisea* verified that there is a threshold concentration at which pathogen growth completely ceased.

Nutrient treatments in the 2002 study did not impact turf quality but did affect brown patch incidence. Applications of phosphorous acid significantly increased brown patch incidence. Turf quality and gray leaf spot incidence were negatively affected by phosphorous acid in the 2003 study. No phytotoxicity was observed with these treatments, perhaps indicating some type of chemical/pathogen interaction resulting in increased pathogen activity. Zinc and manganese treatments also negatively affected turf quality. The adverse effects of the nutrient treatments on turf quality can be explained as a function of treatments on gray leaf spot development affecting overall quality. No significant effects in disease incidence or leaf spot size were found among nutrient treatments in the greenhouse study, however. Nutrient treatments alone do not appear to suppress *P. grisea* or gray leaf spot development in tall fescue.

Isolate selection is a significant factor in establishing disease. Disease incidence and leaf spot development were significantly different for the two isolates used in this study. Selection of a virulent and highly aggressive isolate on the cultivar of interest is important for successful symptom development. Inoculum density also affected disease incidence and leaf spot size. Placing grasses in covered containers or plastic bags immediately following inoculation for a 24-h period also appears to promote disease development. Seeding rate did not have a significant effect on gray leaf spot development.

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