EVALUATION OF RACE POPULATION DISTRIBUTION, FUNGICIDE SENSITIVITY, AND FUNGICIDE CONTROL OF *EXSEROHILUM TURCICUM*, THE CAUSAL AGENT OF NORTHERN LEAF BLIGHT OF CORN

BY

JAPHETH DREW WEEMS

DISSERTATION

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Doctoral Committee:

Professor Carl Bradley, Chair
Professor Mohammad Badadoost
Associate Professor Martin Bohn
Professor Andrew Miller
Assistant Professor Maria Villamil

ABSTRACT

Northern leaf blight (NLB) of corn, caused by *Exserohilum turcicum*, is a yield reducing foliar disease common across the north central U.S. Previous race population distribution studies identified five physiological races present in the U.S., prior to 1995. For this study, 156 *E. turcicum* isolates were screened on corn differential lines containing *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* resistance genes. Isolates were collected from fields in Illinois, Indiana, Iowa, Minnesota, North Carolina, Ohio, and Wisconsin, including: 143 isolates collected between 2007 and 2014; and 13 isolates collected between 1979 and 1985. Twenty different physiological races were observed based on the resistance response of the differential corn lines. *Exserohilum turcicum* race 0, 1, 1mn were the most prevalent races, comprising 21%, 27%, and 13% of the 156 isolates, respectively. Race populations were diverse within states and years. Virulence to multiple *Ht* resistance genes within individual isolates was observed in 47% of those tested, with 3% of the isolates conferring virulence to all *Ht* resistance genes. Virulence to the *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* resistance genes was present in 64%, 20%, 18%, 32%, and 27% of the *E. turcicum* isolates, respectively. Virulence to *Ht* resistance genes was fairly evenly distributed across states in isolates collected after 2008. *Ht2*, *Ht3*, *Htm1*, and *Htn1* virulence decreased after 2010. Variations in race population diversity are difficult to explain without knowing the level of selection pressure present in fields, and information regarding *Ht* resistance gene deployment in commercial varieties is not publicly available. While virulence was observed for all *Ht* resistance genes, qualitative *Ht* resistance genes could be used in conjunction with quantitative resistance to increase NLB control.

Demethylation inhibitor (DMI) fungicides have been labeled for corn use since the early 1990s; in this dissertation a study was conducted to measure *E. turcicum* baseline sensitivity to

DMI fungicides and monitor shifts in sensitivity over years. Metconazole, propiconazole, and prothioconazole are DMI fungicides commonly used to control NLB. Monitoring for shifts in DMI sensitivity in *E. turcicum* populations is important for making management decisions and maintaining fungicide efficacy. Sensitivity to metconazole, propiconazole, and prothioconazole was determined for *E. turcicum* isolates collected prior to DMI use on corn (baseline group) and *E. turcicum* isolates collected in 2009, 2010, 2011, 2012, and 2014. An in vitro mycelial growth assay was used to determine the effective fungicide concentration at which 50% of the fungal growth was inhibited (EC_{50}) for each isolate-fungicide combination. Baseline EC_{50} value lsmeans for metconazole, propiconazole, and prothioconazole were 0.032 µg/ml, 0.060 µg/ml, and 0.254 µg/ml, respectively. When lsmeans of EC_{50} values for 2009, 2010, 2011, 2012, 2013, and 2014 *E. turcicum* isolates were compared to the lsmean of the baseline *E. turcicum* EC_{50} values, no significant ($P \leq 0.05$) shift towards reduced sensitivity was observed in metconazole, propiconazole, or prothioconazole. Three isolates had EC_{50} values significantly higher ($P \leq 0.05$) than the least sensitive baseline isolate for metconazole, and one isolate had an EC_{50} value significantly higher ($P \leq 0.05$) than the least sensitive baseline isolate for propiconazole. These isolates will require further evaluation to determine if they demonstrate reduced field sensitivity. Small but statistically significant ($P \leq 0.05$) positive correlations were found between metconazole and propiconazole ($r = 0.3269$), as well as metconazole and prothioconazole ($r = 0.0.0295$) but not between propiconazole and prothioconazole. Positive correlations between metconazole and the other fungicides suggest the potential for cross-resistance between these DMI fungicides. To date, no loss of NLB control has been observed with the use of metconazole, propiconazole, and prothioconazole in the field.

Fungicides containing quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) active ingredients alone or in combination are frequently applied to control NLB. Field trials were conducted in Illinois at DeKalb, Monmouth, and Urbana in 2012 and Dixon Springs and Urbana in 2013 to evaluate NLB control of DMI, QoI, and QoI + DMI fungicides applied at the solo label rates and the reduced rates present in QoI + DMI premixed fungicides. A moderately susceptible field corn hybrid (Pioneer 33W84) was planted at all site locations across years. Trials were inoculated at the 4-leaf growth stage and fungicides were applied at silk emergence. The mean NLB percent leaf infection for the ear leaf, leaf above the ear, and below the ear and the plot NLB percent severity were evaluated at corn reproductive stages R1, R2, R3, R4 and R5. Stalk rot severity, plant maturity, and yield data were collected. Sweet corn trials were conducted in Urbana in 2012 and 2013 using the same methods. NLB leaf and plot severities were evaluated at reproductive stages R1, R2, and R3 and mean ear weight was calculated at harvest. In the greenhouse, trials were conducted to evaluate NLB percent leaf severity on plants inoculated with *E. turcicum* up to seven days before and after fungicide application with azoxystrobin, propiconazole, prothioconazole, or pyraclostobin. In field trials with low disease severity, no significant differences in treatments were observed for NLB severity ratings, stalk rot severity, plant maturity, or yield. In field corn trials with moderate disease severity, label rates of metconazole and azoxystrobin + propiconazole significantly ($P \leq 0.05$) reduced NLB leaf and plot disease severity compared to the non-treated control across reproductive stages. DMI fungicides at high rates and QoI + DMI premixes offered greater NLB control than other treatments. Fungicide treatments did not significantly affect stalk rot, plant maturity, or yield in field trials with moderate disease severity. In sweet corn trials, metconazole, propiconazole, and azoxystrobin + propiconazole significantly ($P \leq 0.05$) reduced plot disease severity compared to

the non-treated control at R2 and R3. DMI fungicides controlled NLB better than other treatments when compared by fungicide chemical group and rates. Ear weight was not significantly affected by treatments in sweet corn trials. In greenhouse trials, all fungicides significantly ($P \leq 0.05$) reduced disease severity when applied 3 days, 1 day, and 3 hours before inoculation and 3 days and 7 days after inoculation. QoI and DMI fungicides can control NLB when applied prior and post infection; however, products containing DMI fungicides offered better NLB control in the field.

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CHAPTER 1: LITERATURE REVIEW

Introduction

Corn (*Zea mays*) is a major crop produced in the United States and has increased in market value in recent years. In 2014, the U.S. planted over 36 million hectares of corn valued at over $52 billion (United States Department of Agriculture, National Agricultural Statistics Service; USDA-NASS). From 2001 to 2005, the average price of corn in the U.S. was $0.08/kg ($2.14/bushel) compared to an average of $0.14/kg ($3.61/bushel) from 2006 to 2010 (USDA-NASS). The average price from 2011 to 2014 was $0.23/ kg ($5.74/bushel) (USDA-NASS). The price inflation has led to an increase in the overall area planted to corn and an increase in the practice of planting corn back into the same field without rotating to alternative crops. Increased corn prices also have prompted producers to select corn hybrids based on high yield potential with less emphasis on disease resistance. These trends teamed with no-till and reduced-tillage practices have led to an increase in many foliar pathogens that overwinter in corn residue (Pedersen and Oldman, 1992; Wise and Mueller, 2011). *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs (syn., *Setosphaeria turcica* (Luttrell) K.J. Leonard & E.G. Suggs, *Helminthosporium turcicum* Pass., *Bipolaris turcica* (Pass.) Shoemaker, *Drechslera turcica* (Pass.) Subramanian and P. C. Jain, and *Trichometasphaeria turcica* Luttrell), the causal agent of northern leaf blight of corn (NLB), is one such pathogen that can cause major yield reductions (Bowen and Pedersen, 1988; Fisher et al., 1976; Raymundo and Hooker, 1981; Ullstrup and Miles, 1957). According to a recent survey of corn growers and crop consultants from Illinois, Iowa, Ohio, and Wisconsin, a third of the corn growers and certified crop advisers listed NLB as one of the most prevalent foliar diseases observed in fields (C. Bradley, personal communication).

NLB can be found across corn producing regions around the world, in temperate climates and the tropics and subtropics (Carson, 1999). It was first identified in Perma, Italy in 1876 by Passerini (Drechsler, 1923). In the U.S., NLB was first reported in New Jersey in 1878 (Drechsler, 1923). When conditions are favorable for NLB, epidemic levels of disease have been observed in corn producing regions across the U.S. (Drechsler, 1923; Carson, 1999; Levy and Pataky, 1992). In the Midwest, where a majority of the nation's corn is produced, NLB appears annually and is one of the predominant diseases (Brewster et al., 1992; Pataky, 1992). In 2014, NLB was the primary economic disease, reducing yields by an estimated nine million tonnes in the major corn producing regions of the U.S. and Ontario, Canada (Wise, 2015).

Disease cycle and corn yield effects

Exserohilum turcicum is a polycyclic, heterothallic, facultative parasite of corn and sorghum (*Sorghum bicolor*) crops. While there is some evidence suggesting the sexual stage of the pathogen may occur in nature (especially in tropical and subtropical regions), the asexual phase is considered more significant in the disease cycle (Carson, 1999; Ferguson and Carson, 2004). The pathogen primarily overwinters in infected host residue as mycelia, conidia and chlamydospores (Boosalis et al., 1967; Levy, 1984; Robert and Findley, 1952; Takan et al., 1994), but conidia also can be carried long distances by wind (Ferguson and Carson, 2004). Primary inoculum levels can be elevated by the use of no-till and conservation tillage practices resulting in increased surface residue, especially following years of high disease severity (de Nazareno et al., 1993; Pedersen and Oldham, 1992; Takan et al., 1994). Initial inoculum is wind- and rain-dispersed onto the lower host leaves. In the presence of free water on the leaf and

a dark environment, conidial germination and direct infection can occur in as little as 5 hours (Levy and Cohen, 1983a). Increased light intensity can reduce conidial germination and increase incubation time, as observed by Levy and Cohen. Light, at a photon flux of 150 $\mu E.s^{-1}m^{-1}$ during the wet-leaf incubation period, inhibited conidial germination by 85% when compared to the dark control (Levy and Cohen, 1983b). Disease development is more aggressive on young, susceptible plants and favored by warm (near 20˚C) and humid conditions (Levy and Cohen, 1983a). Initial NLB lesions usually appear as elliptical or cigar-shaped, water-soaked, spots that are grayish-green in color (Ullstrup, 1966; Ullstrup and Miles, 1957). As lesions progress they increase in size, typically 12 mm wide and 3-15 cm long, and become necrotic, appearing tan or gray and may have red or brown margins (Ullstrup, 1966; Ullstrup and Miles, 1957). Conidia produced on necrotic lesions act as secondary inoculum and are wind and rain dispersed to the upper canopy (Ullstrup, 1966; Ullstrup and Miles, 1957). Severely affected fields may have a scorched appearance (Ullstrup, 1966; Ullstrup and Miles, 1957). Heavily diseased plants have decreased grain yield due to reductions in the photosynthetic potential of infected leaves and defoliation. Root and stalk infection and lodging may increase as carbohydrates are translocated to the ear for kernel fill as carbohydrate production from leaves is decreased (Dodd, 1980a; Dodd, 1980b; Fisher et al., 1976; Fajemisin and Hooker, 1974; Pedersen and Oldham, 1992; Raymundo and Hooker, 1981).

Yield reductions due to NLB can be significant depending on disease severity, timing, and plant susceptibility. Raymundo and Hooker (1981) reported an increasing positive correlation between decreased yield and the area under disease progress curve (AUDPC) as NLB severity increased. Fisher et al. (1976) estimated that corn yield was reduced 0.2% for each 1% increase in NLB severity when measured 3 to 4 weeks after mid-silk. Ullstrup and Miles (1957)

observed yield reductions ranging from 40% to 70% when disease was severe 2-3 weeks after pollination; however, yield was not affected if disease was not severe until 6-8 weeks after pollination. Differences in host susceptibility have been identified and shown to affect yield reductions due to disease (Bowen and Pedersen, 1988; Raymundo and Hooker, 1981). Raymundo and Hooker (1981) compared NLB effects on yield for an early-maturing, susceptible hybrid, a hybrid with polygenic resistance to NLB, and a hybrid with a dominant *Ht* resistance and polygenic resistance, and reported yield reductions of 63%, 43%, and 17%, respectively. Similar results were observed when Bowen and Pedersen (1988) measured 5% to 44% yield reduction in a susceptible inbred with NLB severities ranging from 52% to 100% at dent stage, but no significant yield reductions in the moderately resistant and highly resistant inbreds.

Pathogen taxonomy and biology

Exserohilum turcicum is a fungal pathogen that was first identified in 1876 and has undergone several changes in nomenclature. *Exserohilum turcicum* is currently classified in the family *Pleosporaceae* in the order *Pleosporales* in the class *Dothideomycetes* in the phylum *Ascomycota* (Alcorn, 1988; Drechsler, 1923). With the adoption of 'one fungus = one name'convention, *Exserohilum turcicum* (Pass.) K.J. Leonard and E.G. Suggs is the accepted name (Rossman et al., 2015); however, it was initially placed in the genus, *Helminthosporium* as *Helminthosporium turcicum* Pass. (Alcorn, 1988; Drechsler, 1923). *Helminthosporium* was later divided into three separate genera: *Drechslera, Bipolaris* and *Exserohilum* (established in 1930, 1959, and 1974, respectively) based on differences in morphology. *Exserohilum turcicum* is the type species of the *Exserohilum* genus (Alcorn, 1988). Introduction of the *Exserohilum* genus occurred years

after initial species identification and adoption of the taxonomic changes varied resulting in the anamorphic phase of *E. turcicum* having multiple synonyms, including: *Exserohilum turcicum* (Pass.) K. J. Leonard and E. G. Suggs, *Bipolaris turcica* (Pass.) Shoemaker, *Drechslera turcica* (Pass.) Subramanian and P. C. Jain, and *Helminthosporium turcicum* Pass (Alcorn, 1988). Furthermore, the *E. turcicum* teleomorphic phase was identified in 1957 and named *Trichometasphaeria turcica* Luttrell and later changed to *Setosphaeria turcica* (Luttrell) K. J. Leonard and E. G. Suggs) (Alcorn, 1988; Leonard and Suggs, 1974; Luttrell, 1964).

The anamorphic phase of *E. turcicum* is most often observed in nature. Brown conidiophores emerge individually or in groups of two to four from stomata in necrotic leaf lesions (Luttrell, 1964). Single conidia form at the tip of conidiophores. Following conidia formation, the conidiophore proliferates laterally below the initial conidia to form a new terminal growing point for the formation of conidia (Luttrell, 1964). Conidia are large (20 × 105 μm), olive gray to brown and cylindrical to spindle-shaped with three to eight transverse septa and a protruding hilum (Alcorn, 1988; Luttrell, 1964). Spore germination commonly occurs at one or both polar cells, with the basal germ tube growing semi-axial, close to the hilum (Alcorn, 1988). Direct penetration into the host is achieved through formation of an appressorium and a penetration peg.

The teleomorphic phase of *E. turcicum* has been observed in the laboratory but typically not in nature. *Exserohilum turcicum* is a heterothallic fungus with three distinct mating types found in nature: *MAT 1*, *MAT 2* and *MAT 1, 2* (Yongshan et al., 2007). Isolates of different mating types are able to undergo sexual recombination forming dark globose pseudothecia with cylindrical, bitunicate asci containing one to eight fusoid, three septae, hyaline ascospores (Luttrell, 1964). Near equal proportions of mating types and near gametic phase equilibrium

found in populations across several states in the U.S. suggest the occurrence of sexual recombination in nature (Ferguson and Carson, 2004). Research has shown sexual recombination of *E. turcicum* can increase pathogenicity and host range (Nelson, 1965; Rodriguez and Ullstrup, 1962), as well as lead to the production of new races (Fallah and Pataky, 1994).

Studies of population genetics have indicated high genetic diversity in *E. turcicum* (Borchardt et al., 1998a; Ferguson and Carson, 2004; Ferguson and Carson, 2007; Yongshan et al., 2007). Greater genotypic diversity and gametic phase equilibrium was observed in *E. turcicum* populations from tropical regions (Kenya, Mexico, and southern China) than populations from temperate regions (Europe and northern China) (Borchardt et al., 1998a). The results support a higher frequency of sexual recombination in tropical climates, while populations in temperate regions appear to be more clonal. Borchardt et al. (1998a) also found extensive migration within agroecological zones and concluded that *E. turcicum* populations were highly adaptable in both temperate and tropical climates. Ferguson and Carson (2004) also found similar evidence to support *E. turcicum* exhibiting high genetic diversity, clonal and sexual reproduction, and long distance migration in the United States.

Host resistance

Host resistance has been the most effective and prevalent form of NLB control in the U.S. Quantitative (polygenic) and qualitative (monogenic *Ht* genes) sources of NLB resistance can be utilized in corn breeding (Ferguson and Carson, 2004; Schechert et al., 1999; Welz and Geiger, 2000). Some of the qualitative resistant genes, especially *Ht1*, were used in commercial corn

lines when first discovered; however, virulent races have since been observed for most *Ht* genes (Jordan et al., 1983; Keller and Bergstrom, 1990; Leath et al., 1990; Lipps and Hite, 1982; Ogliari et al., 2005; Raymundo and Hooker, 1982; Thakur et al., 1989a; Turner and Hart, 1975; Turner and Johnson, 1980; Welz and Geiger, 2000; Winders and Pedersen, 1991). Corn breeders currently select for quantitative resistance to *E. turcicum*, which is more durable and less affected by environmental conditions (Carson, 1995; Welz and Geiger, 2000).

Polygenic resistance was identified in breeding programs in the 1950s after producers suffered severe NLB epidemics on highly susceptible hybrids (Welz and Geiger, 2000). Quantitative resistance is expressed as reductions in disease severity and progression during grain-fill. Reduced disease development is accomplished in a variety of ways, including: reduced infection efficiency, increased incubation and latent periods, reduced lesion size and expansion rates, and reduced sporulation (Adipala et al., 1993; Brewster et al., 1992; Ullstrup, 1970). *Exserohilum turcicum* isolates vary in their sensitivity to polygenic resistance (Levy and Pataky, 1992; Robert and Sprague, 1960). Most commercial breeding programs in the U.S. utilize quantitative resistance for control of NLB (Carson, 1995; Carson and Van Dyke, 1994; Jordan et al., 1983; Welz and Geiger, 2000).

The first single gene resistance, *Ht1*, was identified by Hooker in 1963 (Hooker, 1963). Hooker also identified the *Ht2* gene in 1977 and *Ht3* gene in 1981 (Hooker, 1977; Hooker, 1981). The genes *Ht1*, *Ht2*, and *Ht3* confer a resistance response by causing chlorotic lesions with reduced sporulation, with *Ht2* and *Ht3* displaying slightly more necrosis than *Ht1* (Hooker, 1963; Hooker, 1977; Hooker, 1981; Welz and Geiger, 2000). During the same decade, Gevers (1975) identified *HtN* (syn. *Htn1*) which confers a resistance response resulting in a delay in lesion development and decreased lesion number (Gevers, 1975; Leonard et al., 1989; Welz and

Geiger, 2000). Other single resistance genes that have been identified include recessive *ht4*, *Htm1*, *HtNN, HtP*, recessive *rt*, and another with unidentified genetic action, though not known to be deployed in commercial lines (Carson, 1995; Maize Data Base, 1999; Ogliari et al., 2005; Robbins and Warren, 1993; Welz and Geiger, 2000). The recessive *ht4* gene confers a chlorotic halo, while *Htm1* and *HtNN* confer complete resistance (Welz and Geiger, 2000). Ogliari et al. (2005) observed resistance responses varying from complete resistance to smaller necrotic lesions with chlorotic halos for the *HtP*, recessive *rt* gene, and unidentified resistant gene. More single resistance genes are likely but have not been identified (Welz and Geiger, 2000). Most research has focused on *Ht1*, *Ht2*, *Ht3* and *Htn1* genes which have been backcrossed into widely used inbred lines (Ferguson and Carson, 2004; Ferguson and Carson, 2007; Jordan et al., 1983; Pataky et al., 1986; Welz and Geiger, 2000). Qualitative genes have proven effective against *E. turcicum* and their efficacy can be improved when incorporated into lines with quantitative resistance (Lipps et al., 1997; Pataky et al., 1986).

Qualitative resistant *Ht* genes have successfully been used in breeding programs; however, limitations have been discovered. The first report of *E. turcicum* virulence on corn containing the *Ht1* gene came from Hawaii in 1972 and first reported in the U.S. grain belt in 1979 (Bergquist and Masias, 1972; Turner and Johnson, 1980). *Exserohilum turcicum* isolates collected from Estill, SC in 1976 were found to be virulent on corn containing the *Ht2* and *Ht3* genes (Smith and Kinsey, 1980). It was later determined that virulence to *Ht2* and *Ht3* was conferred by the same single gene (Welz, 1998). Isolates collected from Texas in 1986 caused symptoms on corn containing the *Ht2*, *Ht3*, and *Htn1*, which was the first observed virulence to the *Htn1* gene (Thakur et al., 1989a). The recessive *ht4* gene conferred resistance to the isolates tested in the U.S.; however, was not effective against isolates from Kenya tested in 1996 (Welz

and Geiger, 2000). Virulence to the *rt* gene and the unidentified resistant gene were observed when the resistance genes were first identified (Ogliari et al., 2005). Interestingly, environmental conditions can affect the expression of virulence to some of the *Ht* genes. Isolates virulent on corn with *Ht1* at a 22°/18°C (day/night) temperature regime, may trigger the *Ht1* resistant response at a 26°/22°C (day/night) temperature regime depending on the host genetic background (Thakur et al., 1989). Similarly, virulence to *Ht3* and *Htn1* were best expressed at a 22°/18°C (day/night) temperature regime and light intensities of 324 μmol m^{-2} s^{-1} and lower (Leath et al., 1990; Thakur et al., 1989a).

Populations of *E. turcicum* are designated by physiological races based on virulence to host *Ht* gene(s), for example: race 1 is virulent against the *Ht1* gene; race 23N is virulent against the *Ht2*, *Ht3*, and *Htn1* genes; while race 0 is avirulent against all of the *Ht* genes (Leonard et al., 1989). In the U.S., five major races of *E. turcicum* have been reported to overcome specific *Ht* resistance (Fallah and Pataky, 1994; Ferguson and Carson, 2007; Windes and Pedersen, 1991). Races 0 and 1 are most prevalent, while races 23, 2N, and 23N are rare (Fallah and Pataky, 1994). In a temporal analysis conducted with isolates collected from the U.S. between 1974 and 1994, Ferguson and Carson (2007) identified a shift in race populations as race 0 declined in frequency from 83% in 1974 to 50% in the 1990s, most likely due to selection pressure from wide utilization of the *Ht1* gene in corn hybrids. In several states, race 1 made up the largest proportion of isolates (Ferguson and Carson, 2007). Ferguson and Carson (2004) also were able to identify populations with high genetic diversity, nearly equal mating type frequencies, and gametic phase equilibrium in the asexual stage samples of *E. turcicum* collected from several states, which suggested the likelihood of sexual recombination occurring. Similar findings in population studies across the world support the notion of the occurrence of sexual recombination

(Borchardt et al., 1998a; Borchardt et al., 1998b). Studies also have shown that *E. turcicum* migration over long distances is possible, which could transfer virulence to new regions (Borchardt et al.b, 1998; Ferguson and Carson, 2004). Selection pressure, sexual recombination within the pathogen, and ability to migrate long distances could produce more virulent populations and lead to spatial and temporal race population shifts. Alterations in race population distributions could be partially responsible for the increased presence of NLB in recent years.

Management with foliar fungicides

Since 2007, the use of foliar fungicides on corn in the U.S. has increased (Munkvold et al., 2008; Wise and Mueller, 2011). Increased fungicide application is the result of multiple factors, including: increased availability of fungicides labeled for corn, elevated corn prices making fungicide application economically feasible, and higher disease levels due to cultural practices previously described (Wise and Mueller, 2011). Most foliar-applied fungicide products used on corn contain a fungicide from the quinone outside inhibitor (QoI) class, a.k.a. strobilurins, as the only active ingredient, a demethylation inhibitor (DMI) class, a.k.a. triazoles, or a QoI fungicide and a DMI fungicide mixture. QoI fungicides function by targeting the cytochrome *bc1* enzyme complex (complex III) at the quinone outer binding site in the mitochondrial respiration pathway of fungi, disrupting electron transport, which interferes with spore germination and reduces mycelial growth (Gisi et al., 2002; Fernandez-Ortuno et al., 2008; Ma and Michailides, 2005; Wise and Mueller, 2011). Shah and Dillard (2010) reported that the QoI fungicides, azoxystrobin and pyraclostrobin, reduced NLB severity in sweet corn. The DMI fungicides bind

to the cytochrome P450 lanosterol 14α-demethylase (*CYP51*), which interferes with ergosterol production in fungi (Yoshida, 1993). Without ergosterol, fungi cannot develop functional cell membranes leading to reduced mycelial growth and eventual death (Ma and Michailides, 2005; Mueller et al., 2013). The DMI fungicide, propiconazole, has demonstrated effective control of NLB in field corn and sweet corn (Bowen and Pedersen, 1988; Harlapur et al., 2007; Pataky, 1992; Raid, 1991). NLB control also has been achieved using QoI + DMI products, such as pyraclostrobin and epoxiconazole (Da Costa and Boller, 2008). Which fungicide products (QoI, DMI, or QoI + DMI) are the most effective in controlling NLB is unclear.

QoI fungicides were first introduced to the marketplace in 1996 (Bartlett et al., 2002; Gisi et al, 2002). Since then, resistance to QoI fungicides has been observed in multiple phytopathogenic fungi on multiple crops (FRAC, 2013). Frequently, QoI resistance is the result of one of two possible point mutations in the mitochondrial cytochrome b (*cyt b*) gene. An amino acid substitution from glycine to alanine at codon 143 (G143A mutation) or from phenylalanine to leucine at codon 129 (F129L mutation) confers QoI resistance with no fitness penalty (Bartlett et al., 2002). G143A confers a higher level of resistance than F129L (Kim et al., 2003). Another point mutation at codon 137, recognized as G137R, substitutes arginine for glycine conferring QoI resistance levels similar to F129L; however, this mutation is much rarer than either G143A or F129L (Sierotzki et al., 2007). Miguez et al. (2004) reported reduced QoI sensitivity of *Mycosphaerella graminicola* resulting from the activation of an alternative oxidase (AOX), especially at low azoxystrobin dose rates. The fungicide resistance action committee (FRAC, 2013) considers QoI fungicides to be high risk for pathogen resistance development due to the presence of single gene mutation conferring near-complete resistance with no fitness

penalty, potential for cross-resistance to chemicals in the QoI group, and the speed at which resistance developed in the field following the initial use.

DMIs were introduced during the mid-1970s and resistance has developed in multiple pathogens that affect many crops (De Waard et al., 1986; Karaoglanidis, 2000; Morton and Staub, 2008; Spolti et al., 2014). Shifts of sensitivity to the DMI fungicides have been tied to three main resistance mechanisms: i) mutations in the 14α-demethylase (*CYP51*) gene (Leroux et al., 2007; Ma and Michailides, 2005), ii) overexpression of the *CYP51* gene, and iii) overexpression of ABC (ATP-Binding Cassette) transporters (Ma and Michailides, 2005; Steffens et al., 1996). All of the resistance mechanisms confer partial resistance (decreased sensitivity), often allowing DMIs to remain relatively effective for disease management (Leroux et al., 2007). Cross-resistance to different chemistries within the DMI class can vary but are common (De Waard el al., 1986; Hsiang et al., 1997; Leroux et al., 2007). FRAC (2013) considers DMI fungicides to be medium risk for pathogen resistance development due to the presence of multiple mechanisms of resistance and potential for cross-resistance to chemicals in the DMI group; however, high levels of resistance would require multiple mechanisms within an organism.

QoI and DMI fungicide-associated risk of resistance has been assessed from various fungal responses already observed; however, species vary in resistance response to fungicides (Brent and Hollomon, 2007; Gisi et al., 2002). QoI resistance is unlikely to be a significant problem in *E. turcicum* since it is unable to develop the highly resistant G143A mutation due to the presence of an intron sequence directly after codon 143 (Stammler, 2012). Codon 143 plays a role in the removal of the intron, which is necessary for a functioning complex II, and the G143A mutation has never been observed when the intron is present (Stammler, 2012).

Exserohilum turcicum could develop the F129L, G137R, or AOX mutations; however, these mutations confer low resistance, and the QoIs still offer control (Kim et al., 2003; Sierotzki et al., 2007; Stammler, 2012). *Exserohilum turcicum* could become resistant to DMI fungicides as previously discussed; however, resistance is observed as a gradual reduction in sensitivity over time. This stepwise loss of DMI sensitivity over time is the result of resistance being conferred by multiple mutations instead of a strong single mutation. Selection pressure for resistance is applied by continued exposure to DMIs, which eventually shift the population (Leroux et al., 2007; Ma and Michailides, 2005). *Exserohilum turcicum* is a polycyclic disease with relatively high genetic diversity, limited in its host range by the agricultural practices in the U.S. grain belt which could increase the risk of resistance (Ferguson and Carson, 2004; USDA-NASS, 2014; Carson, 1999). FRAC (2013) categorizes *E. turcicum* at medium risk of developing fungicide resistance since it has not been a major problem thus far; however, the lack of observed resistance could be the result of limited fungicide use in corn prior to the mid-2000s (Bradley and Ames, 2008; Wise and Mueller, 2011).

The probability of selecting for decreased fungicide sensitivity in the *E. turcicum* population greatly increases with the adoption of foliar fungicide application as a management tool in field crops across the U.S. Corn Belt (Bradley and Ames, 2008; Wise and Mueller, 2011; Mallowa et al., 2015). Corn hectares that receive foliar fungicide applications fluctuate with field conditions annually. In 2007, approximately 18% of corn hectares planted in major corn producing states received foliar fungicide applications (Munkvold et al., 2008). In 2010, the corn hectarage sprayed with foliar fungicides was estimated to be approximately 10% (Mallowa et al., 2015; Wise and Mueller, 2011). Large populations of *E. turcicum* across millions of hectares in major corn producing regions of the U.S. are exposed repeatedly to selection pressure

for QoI and DMI fungicide resistance (Wise and Mueller, 2011; Mallowa et al., 2015). Monitoring resistance progression can aid in making management decisions and maintain fungicide efficacy (Brent and Hollomon, 2007).

Objectives

NLB, caused by *E. turcicum*, has recently increased in prevalence in the U.S. due to changes in cultural practices. Research to determine fungicide efficacy and establish baseline sensitivity for monitoring fungicide resistance, as well as, determining race population distribution in the north central U.S. will ultimately aid producers in making sound disease management decisions. The objectives of this research are to:

1) Determine races of *E. turcicum* present in the North Central U.S.
2) Establish a baseline sensitivity of *E. turcicum* to DMI fungicides.
3) Determine which foliar fungicides from different chemistry classes are the most effective for control of NLB.

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CHAPTER 2: *EXSEROHILUM TURCICUM* RACE POPULATION DISTRIBUTION IN THE NORTH CENTRAL UNITED STATES

Abstract

Northern leaf blight (NLB) of corn, caused by *Exserohilum turcicum*, is a yield reducing foliar disease common across the north central U.S. Previous race population distribution studies identified five physiological races present in the U.S., prior to 1995. For this study, 156 *E. turcicum* isolates were screened on corn differential lines containing *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* resistance genes. Isolates were collected from fields in Illinois, Indiana, Iowa, Minnesota, North Carolina, Ohio, and Wisconsin, including: 143 isolates collected between 2007 and 2014; and 13 isolates collected between 1979 and 1985. Twenty different physiological races were observed based on the resistance response of the differential corn lines. *Exserohilum turcicum* races 0, 1, 1mn were the most prevalent races, comprising 21%, 27%, and 13% of the 156 isolates, respectively. Race populations were diverse within states and years. Virulence to multiple *Ht* resistance genes within individual isolates was observed in 47% of those tested, with 3% of the isolates conferring virulence to all *Ht* resistance genes. Virulence to the *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1* resistance genes was present in 64%, 20%, 18%, 32%, and 27% of the *E. turcicum* isolates, respectively. Virulence to *Ht* resistance genes was fairly evenly distributed across states, in isolates collected after 2008. *Ht2*, *Ht3*, *Htm1*, and *Htn1* virulence decreased after 2010. Variations in race population diversity are difficult to explain without knowing the level of selection pressure present in fields, and information regarding *Ht* resistance gene deployment in commercial varieties is not publicly available. While virulence was observed for all *Ht* resistance genes, qualitative *Ht* resistance genes could be used in conjunction with quantitative resistance to increase NLB control.

Introduction

Northern leaf blight (NLB) of corn (*Zeae mays* subsp. *mays*), caused by *Exserohilum turcicum* (syn. *Bipolaris turcica*, *Drechslera turcica*, *Helminthosporium turcicum*, *Setosphaeria turcica*, and *Trichometasphaeria turcica*), is a common disease found in corn-producing areas worldwide (Dong et al., 2008; Ferguson and Carson, 2004; Muiru et al., 2010; Scherchert et al., 1999). NLB is a foliar disease that initially presents as green-gray, elliptical lesions which turn necrotic (Carson, 1999; Ullstrup and Miles, 1957). In cool, moist field conditions, lesions expand and coalesce, which reduce host photosynthetic potential (Levy and Cohen, 1983). Secondary infections occur from conidia formed on the surface of necrotic lesions spread to the upper canopy by wind and rain (Carson, 1999; Ullstrup and Miles, 1957). Disease stress during seed set and fill can result in yield reductions greater than 50%, while potentially increasing the incidence of stalk rots and lodging (Dodd, 1980a; Dodd, 1980b; Fisher et al., 1976; Pedersen and Oldham, 1992; Raymundo and Hooker, 1981).

Host resistance has been effectively used to control NLB in the U.S. through the deployment of qualitative and quantitative resistance genetics. The first qualitative *Ht* resistance gene, designated *Ht1*, was discovered in 1963 (Hooker, 1963). The resistance response presents as chlorotic streaking surrounding reduced necrotic lesions with decreased sporulation. The *Ht2* and *Ht3* genes were discovered in 1977 and 1981, respectively, and display similar resistance responses as *Ht1*, but with more necrosis (Hooker, 1977; Hooker, 1981). In 1975, a single gene conferring delayed latent period and decreased lesion number was identified, and later designated as the *Htn1* (syn. *HtN*) gene (Gevers, 1975; Leonard et al., 1989; Welz and Geiger, 2000). In 1993, another resistance gene conferring complete resistance to *E. turcicum* was observed and later designated *Htm1* in analogy to *Htn1* (Robbins and Warren, 1993; Welz and

Geiger, 2000). The *Ht1* gene often was utilized as the primary form of NLB control in corn breeding lines until virulent *E. turcicum* populations were observed across corn producing areas of the U.S. (Ferguson and Carson, 2004; Jordan et al., 1983; Lipps and Hite, 1982). The *Ht2*, *Ht3*, and *Htn1* genes have not been heavily utilized in field corn breeding due to presence of *E. turcicum* virulent populations, as well as variations in resistance under different light intensities and temperatures (Ferguson and Carson, 2004; Leath et al., 1990; Thakur et al., 1989a; Thakur et al., 1989b). Furthermore, a dominant gene, designated *Sht1*, suppresses expression of *Ht2*, *Ht3*, and *Htn1*, which makes backcrossing more difficult (Ceballos and Gracen, 1989; Simcox and Bennetzen, 1993). The *Htm1* gene also has limited deployment in field corn breeding, and little *E. turcicum* race screening has been done using this resistance gene. Quantitative resistance breeding has become the primary method for NLB control due to the durability of polygenic resistance across environments and *E. turcicum* races (Carson, 1995; Carson and Van Dyke, 1994; Jordan et al., 1983; Welz and Geiger, 2000).

NLB has increased in prevalence in the U.S. since 2000 (Munkvold et al., 2008; Wise and Mueller, 2011). Adoption of reduced tillage and no-tillage practices, as well as increased hectares in continuous corn has led to increased field residue. *Exserohilum turcicum*-infected corn residue generally serves as the primary inoculum that causes infections and NLB development (Mallowa et al., 2015; Wise and Mueller, 2011). Currently, producers tend to select corn hybrids based on high yield potential over those with high disease resistance, which has also led to increased disease severity (Wise and Mueller, 2011). This suggests that some high-yielding lines may lack adequate quantitative resistance genetics to control NLB in some regions. Single resistance genes can be backcrossed into corn lines fairly quickly, relative to breeding for polygenic resistance. The qualitative *Ht* genes could increase NLB control if used

in conjunction with partial resistance or provide protection while quantitative resistance is improved in high-yielding lines. Lipps et al. (1997) reported that crosses of lines containing *Ht* genes and lines with moderate partial resistance controlled NLB better than crosses of the same lines without *Ht* genes and lines with moderate partial resistance when inoculated with *E. turcicum* race 0 (avirulent to all *Ht* genes). In addition, Pataky et al. (1986) reported that qualitative resistance genes and high partial resistance were equally effective in limiting spread and development of NLB in the presence of a race avirulent against *Ht* genes.

To determine if any of the *Ht* genes could be widely deployed to aid in NLB control, the distribution of *E. turcicum* race populations in corn-producing regions must be assessed. *Exserohilum turcicum* races are determined by inoculating differential corn lines containing the individual *Ht* genes and assessing the presence or absence of the host resistance response (Berguist and Masias, 1974). Physiological races are designated based on virulence to host *Ht* gene(s). For example, race 1 of *E. turcicum* is virulent against the *Ht1* gene; race 23N is virulent against the *Ht2, Ht3,* and *Htn1* genes; while race 0 is avirulent against all of the *Ht* genes (Leonard et al., 1989). Previous studies, conducted with various isolates collected prior to 1995, showed that five races occur in the U.S. Races 0 and 1 were found to be dominant, while races 23, 2N, and 23N occurred at much lower frequencies (Fallah and Pataky, 1994; Ferguson and Carson, 2004; Ferguson and Carson, 2007; Windes and Pedersen, 1991). These race-typing studies were performed with isolates collected prior to 1995. Little is known about the present *E. turcicum* race population distribution in the central U.S. or the presence of selection pressure. It is widely believed that *Ht* genes are not being deployed in private, commercially-developed hybrids; however, that information is not publicly available (Carson, 1995; Carson and Van Dyke, 1994; Jordan et al., 1983; Welz and Geiger, 2000). The purpose of this study was to

determine the race population diversity and distribution of *E. turcicum* present in several states in the North Central U.S.

Materials and Methods

Isolate collection. The *E. turcicum* collection consisted of 156 isolates obtained from corn. Thirteen isolates collected prior to 1986 were from a collection originally compiled by Dr. Martin Carson and later curated by Dr. Peter Balint-Kurti (United States Department of Agriculture, North Carolina State University, Raleigh, NC) (Ferguson and Carson, 2004; Ferguson and Carson 2007). In addition to these 13 "historical" isolates, isolates from corn leaf samples from Illinois, Indiana, Iowa, Ohio, and Wisconsin were collected between 2007 and 2014. Either leaf samples or isolate cultures collected between 2009 and 2014 from states other than Illinois were provided by Drs. Alison Robertson (Iowa State University, Ames, IA), Paul Esker (University of Wisconsin, Madison, WI), Pierce Paul (The Ohio State University, Wooster, OH), and Kiersten Wise (Purdue University, West Lafayette, IN). NLB symptomatic leaf tissue were received and placed in humidity chambers (sealed plastic bags containing a damp paper-towel) to induce sporulation. Conidia were rinsed off the leaf surface with 200 to 500 µl of water, and the conidial suspension was spread onto Petri plates containing potato dextrose agar (PDA) (Fisher Scientific, Pittsburgh, PA) amended with rifampicin (25 mg/L) (Fisher Scientific, Pittsburgh, PA) to inhibit bacterial growth. Conidia were allowed to germinate, and single germinated *E. turcicum* conidia were collected and transferred to new PDA the following day. Isolates were allowed to grow at 20 to 25°C with 12 hour light/dark conditions for 7 to 14 days before mycelia samples were cut from plates and placed in 1.5 ml

tubes containing 850 µl of 15% glycerol solution. Tubes were placed in a -80°C freezer for long term storage until isolates were screened for race-type. The final collection contained isolates collected between 1979 and 2014 from Illinois, Indiana, Iowa, Minnesota, North Carolina, Ohio, and Wisconsin (Table 2.1).

Race determinations. To determine race, virulence was assessed on differential corn lines containing *Ht1*, *Ht2*, *Ht3*, *Htm1* or *Htn1* qualitative resistance genes or no *Ht* qualitative resistance gene. Unfortunately, limitations in the quantity of seed of lines that contained *Ht1*, *Htm1*, or *Htn1* required the use of multiple lines to test all isolates. The corn lines containing the *Ht* resistance genes were: A619 (no *Ht* genes), A619*Ht1*, B37*Ht1*, A619*Ht2*, A619*Ht3*, H102*Htm1*, H117*Htm1*, Mayorbela*Htm1*, Mayorbela(R)C3*Htm1*, A509*Htn1*, A632*Htn1*, B37*Htn1*, FRS*Htn1*, MLS*Htn1*, and MRS*Htn1*. The majority of seed for the A619 near isogenic lines and all of the seed for the B37 near isogenic lines came from Dr. Don White's collection (Emeritus Professor, University of Illinois, Urbana, IL). A portion of the seed for the A619 lines, H102*Htm1*, A632*Htn1*, and A509*Htn1* were provided by Dr. Krishan Jindal (Agriculture and Agri-Food Canada, Ottawa, ON, Canada) and Albert Tenuta (Ontario Ministry of Agriculture, Food and Rural Affairs, Ridgetown, ON, Canada). The remaining *Htm1* and *Htn1* screening lines were procured from the U.S. National Plant Germplasm System (NPGS, http://www.ars-grin.gov/npgs). Pots (30.2 cm wide x 27.9 cm tall, C2000, Nursery Supplies Inc., Chambersburg, PA) were filled with a 1:1:1 (soil:peat:perlite) steam-sterilized soil mix. All pots were planted with 18 seeds (3 seeds of each *Ht* gene type and 3 seeds of the A619 control with no *Ht* genes). All plants in each pot were inoculated with a single *E. turcicum* isolate. Plants were watered as needed and fertilized with Osmocote Classic 13-13-13 (N-P-K) controlled release fertilizer (The Scott's Company, Marysville, OH) to maintain plant health and growth.

The greenhouse was maintained at 21°C ± 3° daytime and 18°C ± 3° nighttime temperatures. Supplemental lighting was used at 25 to 50 klux (325 to 650 µE m^{-2} s^{-1}) with 15 hour daytime and 9 hour nighttime. Greenhouse temperature and light intensity were consistent with those used by Leonard et al. (1989) and Ferguson and Carson (2004), since virulence to *Ht1*, *Ht2*, and *Ht3* can be affected by varying temperature and light intensity (Leath et al., 1990; Thakur et al., 1989). Plants were inoculated at the four leaf growth stage.

Exserohilum turcicum isolates were grown on lactose casein agar (LCA) (Tuite, 1969). Cultures were maintained at 20-25°C under a 12 hour light and dark regime for 7 to 14 days to promote sporulation (Ferguson and Carson, 2004). Pieces (approximately 5 mm^2) were cut from the sporulating cultures, placed in 1.5 ml tubes containing 800 µl of water, and vortexed to dislodge conidia. Conidial suspensions were quantified with a hemocytometer and diluted with water containing Tween 20 (10 µl/liter) to approximately 1,000 conidia/ml. Plants were inoculated in the whorl with 150 µl of the conidial suspension using a pipette. To promote infection, plants were placed in a humidity chamber in the greenhouse for 20 to 24 hours following inoculation. The humidity chamber consisted of an opaque plastic tent constructed in the greenhouse with 2 humidifiers that maintained relative humidity at 100%.

Plants were inspected for symptoms of infection 14 and 21 days after inoculation. Symptoms caused by virulent *E. turcicum* isolates initially appeared as gray-green elliptical lesions in the first week, generally turning necrotic by the 14 day evaluation and continuing to increase in size by the 21 day evaluation. Host resistant responses (avirulence) provided by *Ht1*, *Ht2*, and *Ht3* appeared as chlorotic streaking surrounding reduced necrotic lesions. Host resistant responses provided by *Ht2* and *Ht3* usually displayed more necrosis than *Ht1*. The host resistance response provided by *Htm1* appeared as slight necrotic flecking at the point of

infection, with minimal necrotic lesions. The *Htn1* host resistance response presented as delayed lesion development and reduced lesion size. If the appropriate *Ht* host resistance response was observed on any of the plants containing the *Ht* gene, the isolate was considered avirulent against that gene. Races were assigned based on plant resistance responses following the race scheme outlined by Leonard et al. (1989).

Results

Twenty races of *E. turcicum* were observed when the isolates were tested on the corn differential lines containing the various *Ht* resistance genes (Table 2.1). Races observed were not consistent across years within states. Race 0 (avirulent against all *Ht* genes) was found in 50% of the counties sampled in Iowa, 65% of the counties in Illinois, 80% of the counties in Indiana and Minnesota, 50% of the counties in North Carolina, and 100% of the counties in Ohio. Races containing virulence to *Ht1* were observed in 100% of the counties sampled in Iowa, 41% of the counties in Illinois, and 80% of the counties in Indiana. None of the "historical" isolates collected from Minnesota or North Carolina were virulent to *Ht1*, while 100% of the counties sampled in Ohio and Wisconsin had isolates with *Ht1* virulence. Races with virulence to *Ht2* were identified in 100% of the counties in Iowa, 35% of the counties in Illinois, 40% of the counties in Indiana, 100% of counties in North Carolina, and 50% of the counties in OH. Virulence to *Ht3* was observed in all of the same locations in which virulence to *Ht2* was observed, except that virulence to *Ht2* was not observed in Piatt County, IL but was observed in Grant County, WI. Virulence to *Htm1* was found in 100% of the counties in Iowa, 29% of the counties in Illinois, 40% of the counties in Indiana, 50% of the counties in NC, and 100% of the

counties in both Ohio and Wisconsin. Virulence to *Htn1* was found in 50% of the counties in Iowa, 41% of the counties in Illinois, 20% of the counties in Indiana, one location in North Carolina, 100% of the counties in Ohio, and 50% of the counties in Wisconsin. In counties where samples were collected over multiple years, virulence profiles were not consistent across years.

Virulence to multiple resistance genes was common among the isolates, which led to the high number of observed races (Table 2.2). Ten races were observed in Iowa, 18 races in Illinois, 6 races in Indiana, 1 race in Minnesota, 4 races in North Carolina, 9 races in Ohio, and 3 races in Wisconsin. Race 1, race 0, and race 1mn were the most commonly observed races across all 156 isolates, which made up 27%, 21%, and 13% of the total isolates tested, respectively (Table 2.2). Race 1 was the predominant race in Iowa and Ohio, while race 0 was predominant in Illinois, Indiana, and Minnesota (though, only one isolate was tested from Minnesota). Isolates were evenly distributed among races in North Carolina, and 1mn was the predominant race in Wisconsin. Generally, states with a greater number of isolates collected and tested resulted in more races observed.

The frequency of virulence to the specific *Ht* genes also was examined across states (Table 2.3). In Illinois, Indiana, Iowa, Ohio, and Wisconsin, virulence to *Ht1* was present in greater than 50% of the collected isolates. In Minnesota and North Carolina isolates, which were all collected prior to 1986, no virulence to *Ht1* was observed. Virulence to *Ht2* and *Ht3* were observed together except in Wisconsin and Illinois. *Htn1* virulence was in all states, except Minnesota, and varied in its frequency. *Htm1* virulence was present in all states, except Minnesota, at levels similar to or greater than *Ht2*, *Ht3* and *Htn1*. Isolates avirulent to all *Ht* genes (race 0) were present in all states but often at a low frequency. Of the total 156 isolates

tested, 21% were avirulent to all *Ht* genes, 64% were virulent to *Ht1*, 20% were virulent to both *Ht2* and *Ht*, 30% were virulent to *Htn1*, and 30% were virulent to *Htm1*.

Similarly, when examining temporal race distribution, the number of races identified within years tended to increase with the number of isolates tested (Table 2.4). Five races were observed for isolates collected between 1979 and 1985, two races were observed in 2007, nine races in 2009, 16 races in 2010, 11 races in 2011, seven in 2012, one race in 2013, and four races in 2014. Race 0 was the most prevalent among isolates collected between 1979 and 1985, as well as isolates collected in 2014. Race 1mn was observed in greater frequency than any other race in 2009 and 2010. In 2011, 2012, and 2013 (only one isolate tested in 2013), race 1 was present in the highest number of isolates.

A shift in virulence to *Ht* genes was observed among years (Table 2.5). Greater than half of the isolates collected between 1979 and 1985 were race 0, inducing resistance responses on hosts containing any of the *Ht* genes. No virulence to *Ht1* was found in isolates collected prior to 2009; however, *Ht1* virulence was observed in the majority of isolates collected in 2009 and subsequent years. After 2010, the frequency of *Ht2* and *Ht3* virulence decreased compared to isolates collected previously. A similar reduction was observed in *Htn1* and *Htm1* virulence after 2010.

Discussion

In this race survey, a much greater number of races were observed than previously reported in the U.S. In previous reports, the commonly reported races found in U.S. fields were 0, 1, 23, 2n, and 23n, with race 0 and 1 being the most frequent (Fallah and Pataky, 1994; Simcox et al.,

1993; Windes and Pedersen, 1991). In the isolates tested here, 20 races were observed at fairly equal frequencies, with the exception of races 0, 1, and 1mn, which were more common. The increased number of races identified resembles recent results from other corn-producing regions, such as Europe, Africa, and China, where races are more diverse (Dong et al., 2008; Muiru et al., 2010). It is possible that more races have developed over time in the U.S., since previous studies focused on isolates collected prior to 1995 (Ferguson and Carson, 2004; Ferguson and Carson, 2007; Thakur et al., 1989; Windes and Pedersen, 1991). Another reason more races were identified in this study is the inclusion of lines containing the *Htm1* resistance gene in the differential test, which was not commonly used in earlier race typing studies (Ferguson and Carson, 2004; Thakur et al., 1989; Windes and Pedersen, 1991). The inclusion of *Htm1* allowed another level of race separation, increasing the number of races observed.

There was a high diversity of races observed within regions. Multiple races could be found within counties and even fields. Some inconsistency among years could be the result of isolates collected from different fields within counties and the relatively limited sample size. The diversity of races is somewhat unexpected in a population considered highly clonal in the North Central U.S. and under limited selection pressure, with minimal utilization of *Ht* genes in commercial breeding programs (Ferguson and Carson, 2004; Welz and Geiger, 2000; White, 1999). Ferguson and Carson (2004) did report high genetic diversity with approximately equal gametic phase equilibrium and equal proportions of mating types in Indiana, Kentucky, North Carolina and Ohio, which suggested that sexual recombination was occurring in those regions. Furthermore, they concluded that *E. turcicum* inoculum may travel long distances, which could cause new race introductions and race diversity in fields. The diversity of races in this collection does suggest a genetically diverse population, possibly due to sexual recombination or mutation,

and the presence of some selection pressure or limited fitness penalty associated with physiological races.

Races 0 and 1 remained the most frequently observed and were widely distributed across states, similar to earlier studies (Ferguson and Carson, 2004; Jordan et al., 1983). Races 1m, 1mn, and 1n were the second most frequently observed. The wide distribution of race 0 could be the result of reduced use of *Ht* resistance genes in breeding programs, which shifted to the use of more prolific quantitative resistance to control NLB after virulence to *Ht* genes were observed in the 1970s and 1980s (Welz and Geiger, 2000). The *Ht1* gene, and to a lesser extent the *Htn1* gene, were commonly incorporated in commercial lines until virulence was readily observed (Leonard, 1993; Raymundo and Hooker, 1982; Turner and Johnson, 1980). It is not surprising that populations with races virulent to *Ht1* and *Htn1* would be present in regions traditionally used for corn production due to selection pressure.

Races with virulence to *Ht1* were prevalent across major corn producing regions where isolates were collected after 2007. It is likely, if more isolates were available to test prior to 2009 virulence to *Ht1* would have been observed. Isolates from 1979 to 1985 were arbitrarily selected from part of a collection used for a genetic diversity study by Ferguson and Carson (2004; 2007) in which they observed virulence to *Ht1* across the eastern U.S. at lower frequencies than found in the more recent isolates tested here. This suggests the frequency of virulence to *Ht1* has increased in *E. turcicum* populations; however, a previous study conducted on isolates collected between 1979 and 1981 determined that 72 out of 89 isolates collected in 1981 were virulent to *Ht1* (Jordan et al., 1983). The variation among these previous studies makes it hard to determine if a population shift in virulence has occurred over time or if small sample size is confounding conclusions. Whether the population has shifted or remained

consistent, virulence to *Ht1* is present at high frequencies in *E. turcicum* populations across the north central U.S., which limits the usefulness of the *Ht1* gene in breeding for NLB control.

Races virulent to *Htn1*, *Htm1*, or both were fairly prevalent in all states, except Minnesota. Virulence to *Htn1* was expected given the *Htn1* gene may have been previously utilized in breeding programs and virulence had been reported in U.S. *E. turcicum* populations (Ferguson and Carson, 2004; Raymundo and Hooker, 1982; Thakur et al., 1989a). The high frequency of virulence to *Htm1* found in the collection was surprising since there is little evidence available to suggest that the gene has been deployed in field corn breeding programs, and virulence has not been previously reported. Virulence to *Htm1* was found in the isolates collected from North Carolina in 1985; however, the *Htm1* gene was not identified until 1993 (Robbins and Warren, 1993). The *Htm1* resistance response is characterized as reduced disease severity or no disease response, without the telltale chlorosis of *Ht1*, *Ht2*, and *Ht3*. It is possible the *Htm1* gene was unknowingly incorporated into quantitative resistance breeding programs, prior to its identification. *Htm1* virulence is common in this *E. turcicum* isolate collection, but whether that is a response to selection pressure or if it naturally occurs is unclear. The frequency of *Htm1* virulence may have been over-represented in this study. Part of the *Htm1* source seed for this study was provided by the U.S. National Plant Germplasm System (NPGS, http://www.ars-grin.gov/npgs) and problems with contamination and misclassification of *Ht* lines have been encountered previously (Simcox and Bennetzen, 1993).

Races with virulence to *Ht2*, *Ht3*, or both were the least frequently observed of the isolates tested. Virulence to these *Ht* genes was observed in all years, except 2013 when only one isolate was tested. Despite the presence of virulence across years and in most states, virulent isolates were the least distributed within states. These results are similar to other studies which

found virulence to *Ht2* and *Ht3* to be present at low frequency across the U.S. (Fallah and Pataky, 1994; Ferguson and Carson, 2004; Jordan et al., 1983; Lipps and Hite, 1982). The presence of virulence to one of these genes without the presence of the other is confusing since virulence appears to be conferred by the same single gene (Welz, 1998). In spite of this, multiple studies have reported races virulent to one gene without virulence to the other gene (Dong et al., 2008; Fallah and Pataky, 1994; Muiru et al., 2010; Ogliari et al., 2005). It is unclear what causes the variation in virulent races.

Since nearly all of the commercial corn production in the U.S. is with hybrids developed by private companies, the degree of *Ht* gene deployment is publicly unknown, which makes interpretation of the results more difficult. Most reports have determined use of qualitative resistance for NLB control in the U.S. is limited due to the observation of virulence races, though it has not been confirmed (Ferguson and Carson, 2004; Schechert et al., 1999; Welz and Geiger, 2000). Ferguson and Carson (2007) indicated that virulence to *Ht1* may reduce fitness of *E. turcicum*, as the mutation was not observed prior to widespread use of the *Ht1* gene. The high frequency of virulence to the *Ht1* gene observed in the isolates screened here would suggest either the *Ht1* gene is still present in corn lines or there is no fitness penalty associated with *Ht1* virulence. Furthermore, it is difficult to determine if the increase in races and the high frequency of virulence to *Ht* genes were due to current selection pressure, sexual recombination, or random mutation without knowing if *Ht* genes are present. Knowing the distribution of *Ht* genes in the states where isolates were collected would have complimented the race screening data.

NLB is increasing in prevalence across the U.S. Corn Belt due to changes in cultural practices and reductions in effective partial host resistance in commercially available corn hybrids (Munkvold et al., 2008; Wise and Mueller, 2011). Qualitative *Ht* resistance genes could

be useful in commercial breeding programs. In a study conducted by Lipps et al. (1997), the progeny of susceptible lines with the *Ht1* gene crossed with a line containing strong quantitative resistance demonstrated reduced disease severity when inoculated with *E. turcicum* race 0 than the progeny of the same cross with the isogenic susceptible lines without the *Ht1* genes. From the results observed in this isolate collection, virulence to *Ht1*, *Htn1*, and *Htm1* was frequent and widespread. The *Ht2* gene and *Ht3* gene would offer the greatest resistance to NLB in most regions. Ferguson and Carson (2007) concluded that virulence to *Ht2*, *Ht3*, and *Htn1* are not often expressed under field conditions, so hybrids containing these resistance genes may offer control even when virulent populations are present. Virulence to *Htm1* was more widespread than expected and may not be as effective; however, little has been reported about virulence under field conditions. Breeding multiple *Ht* resistance genes into lines may not offer as much protection as may have been thought based on previous race screening studies. Races conferring virulence to multiple *Ht* genes were diverse and widespread, and several isolates were virulent to all *Ht* genes. More *Ht* genes have been discovered than were included in this study and more are likely to be found (Ogliari et al., 2005; Welz and Geiger, 2000). Breeding programs could possibly benefit from using other *Ht* genes as well. Whether or not *Ht* genes could help improve control of NLB long term is hard to predict, but they could offer a level of disease control while quantitative resistance is improved and introgressed into popular corn lines.

Table 2.1. Origin by state, county, and year of *Exserohilum turcicum* collection, number of isolates, and race determined by differential screening lines containing *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htn1*.

State	County	Year	No. of isolates	Isolate races
Iowa	Story	2009	4	1(1)[a], 12(1), 1mn(1), m(1)
		2010	8	123(2), 123mn(1), 1m(1), 1mn(3), m(1)
	Washington	2011	21	0(3), 1(13), 123(1), 13(1), 123m(1), 1m(2)
Illinois	Champaign	2007	2	23(1), 23m(1)
		2009	1	0(1)
		2010	4	1m(2), 2(1), 23(1)
		2011	1	1mn(1)
		2012	2	1(1), 1n(1)
		2014	1	1(1)
	DeKalb	2010	12	1(3), 123(2), 123mn(1), 1mn(4), 23mn(2)
		2012	5	1(2), 123m(1), 13(1), 1n(1)
		2014	1	1(1)
	Ford	2009	3	1(1), 123m(1), 1n(1)
		2011	3	1(1), 1mn(2)
	Gallatin	2014	1	0(1)
	Iroquois	1979	2	0(2)
	Johnson	2010	1	0(1)
	Kane	2010	2	12mn(1), 23n(1)
	Mclean	2011	1	0(1)
	Piatt	2009	3	12(2), 2mn(1)
		2011	3	0(2), n(1)
		2012	3	0(1), 1m(1), 1mn(1)
	Pike	2010	1	0(1)
	Pope	2014	1	0(1)
	Saline	2014	1	0(1)
	Sangamon	2010	5	0(2), 1(2), 1n(1)
		2014	1	0(1)
	St. Clair	2009	3	23(3)
	Vermilion	1981	2	n(2)
	Warren	2012	2	1(2)
	Woodford	1981	1	0(1)
Indiana	Clark	1980	2	0(1), 23(1)
	Henry	2014	3	1(2), 1m(1)
	Knox	2014	1	0(1)
	North IN[b]	2012	1	1(1)
	Tippecanoe	2011	3	0(2), 1n(1)
		2013	1	1(1)
	Wabash	2014	2	0(1), 123m(1)

Table 2.1. (cont.)

Minnesota	Swift	1981	1	0(1)
North Carolina	Wilkes	1985	3	0(1), 23m(1), 23mn(1)
	NA[C]	1985	1	23(1)
Ohio	Clark	2009	4	1(1), 1mn(1), 1n(2)
		2010	9	0(2), 1m(1), 1mn(3), mn(2), n(1)
	Wayne	1980	1	0(1)
		2011	18	0(3), 1(9), 123mn(1), 1m(1), 1mn(2), 1n(1), m(1)
Wisconsin	Grant	2009	3	1mn(3)
		2010	2	13(1), 1m(1)

[a] The number of isolates belonging to a race are in parenthesis following the race nomenclature.
[b] The county where the isolate collected was not recorded but the general location was northern Indiana.
[c] The location where the isolate was collected is not available (NA).

Table 2.2. The number of *Exserohilum turcicum* isolates of each race found in states.

	E. turcicum race distribution by state							Number of isolates per race
Race	Iowa	Illinois	Indiana	Minnesota	North Carolina	Ohio	Wisconsin	
0	3	16	5	1	1	6		32
1	14	14	4			10		42
12	1	2						3
13	1	1					1	3
123	3	2						5
123m	1	2	1					4
12mn		1						1
123mn	1	1				1		3
1m	3	3	1			2	1	10
1mn	4	8				6	3	21
1n		4	1			3		8
2		1						1
23		5	1		1			7
23m		1			1			2
2mn		1						1
23mn		2			1			3
23n		1						1
m	2					1		3
mn						2		2
n		3				1		4
Total Isolates[a]	33	68	13	1	4	32	5	156[b]

[a] The total number of isolates evaluated for race from each state.

[b] The total number of isolates evaluated for race.

Table 2.3. The frequency of *Exserohilum turcicum* isolates virulent to the *Ht* resistance genes within states.

	Number of virulent isolates for each *Ht* gene						Number of isolates per state
State	No *Ht*	*Ht1*	*Ht2*	*Ht3*	*Htm1*	*Htn1*	
Iowa	3[a]	28	6	6	11	5	33
Illinois	16	38	19	15	19	21	68
Indiana	5	7	2	2	2	1	13
Minnesota	1	0	0	0	0	0	1
North Carolina	1	0	3	3	2	1	4
Ohio	6	22	1	1	12	13	32
Wisconsin	0	5	0	1	4	3	5
Total Isolates	32[a]	100	31	28	50	44	156[b]

[a] The total number of virulent isolates for each *Ht* resistance gene.
[b] The total number of isolates evaluated for virulence to *Ht* resistance genes.

Table 2.4. The number of *Exserohilum turcicum* isolates of each race found in years.

	E. turcicum race distribution by year								
Race	1979-1985	2007	2009	2010	2011	2012	2013	2014	Number of isolates per race
0	7		1	6	11	1		6	32
1			3	5	23	6	1	4	42
12			3						3
13				1	1	1			3
123				4	1				5
123m			1		1	1			3
12mn				1					1
123mn				2	1			1	4
1m				5	3	1		1	10
1mn			5	10	5	1			21
1n			3	1	2	2			8
2				1					1
23	2	1	3	1					7
23m	1	1							2
2mn			1						1
23mn	1			2					3
23n				1					1
m			1	1	1				3
mn				2					2
n	2			1	1				4
Total isolates	13[a]	2	21	44	50	13	1	12	156[b]

[a] The total number of isolates evaluated for race from each year.
[b] The total number of isolates evaluated for race.

Table 2.5. The frequency of *Exserohilum turcicum* isolates virulent to the *Ht* resistance genes within years.

	Number of virulent isolates for each *Ht* gene						Number of isolates per year
Race	No *Ht*	*Ht1*	*Ht2*	*Ht3*	*Htm1*	*Htn1*	
1979-85	7	0	4	4	2	2	13
2007	0	0	2	2	1	0	2
2009	1	15	8	4	8	9	21
2010	6	29	12	11	23	20	44
2011	11	37	3	4	11	9	50
2012	1	12	1	2	3	3	13
2013	0	1	0	0	0	0	1
2014	6	6	1	1	2	0	12
Total Isolates	32[a]	100	31	28	50	43	156[b]

[a] The total number of virulent isolates for each *Ht* resistance gene.
[b] The total number of isolates evaluated for virulence to *Ht* resistance genes.

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CHAPTER 3: SENSITIVITY OF *EXSEROHILUM TURCICUM* TO DEMETHYLATION INHIBITOR FUNGICIDES

Abstract

Exserohilum turcicum, the causal agent of northern leaf blight (NLB) of corn, can cause significant yield reductions in Illinois. Foliar fungicides are often used for NLB control in corn seed and grain fields. Metconazole, propiconazole, and prothioconazole are demethylation inhibitor (DMI) fungicides applied as solo active ingredients or premixed with other fungicide groups to control NLB. Monitoring for shifts in DMI sensitivity in *E. turcicum* populations is important for making management decisions and maintaining fungicide efficacy. Sensitivity to metconazole, propiconazole, and prothioconazole was determined for *E. turcicum* isolates collected prior to DMI use on corn (baseline group) and *E. turcicum* isolates collected in 2009, 2010, 2011, 2012, and 2014. An in vitro mycelial growth assay was used to determine the effective fungicide concentration at which 50% of the fungal growth was inhibited (EC_{50}) for each isolate-fungicide combination. Baseline EC_{50} value lsmeans for metconazole, propiconazole, and prothioconazole were 0.032 µg/ml, 0.060 µg/ml, and 0.254 µg/ml, respectively. When lsmeans of EC_{50} values for 2009, 2010, 2011, 2012, 2013, and 2014 *E. turcicum* isolates were compared to the lsmean of the baseline *E. turcicum* EC_{50} values, no significant ($P \leq 0.05$) shift towards reduced sensitivity was observed in metconazole, propiconazole, or prothioconazole. Three isolates had EC_{50} values significantly higher ($P \leq 0.05$) than the least sensitive baseline isolate for metconazole, and one isolate had an EC_{50} value significantly higher ($P \leq 0.05$) than the least sensitive baseline isolate for propiconazole. These isolates will require further evaluation to determine if they demonstrate reduced field sensitivity. Small but statistically significant ($P \leq 0.05$) positive correlations were found between

metconazole and propiconazole (r = 0..3269), as well as metconazole and prothioconazole (r = 0.0.0295) but not between propiconazole and prothioconazole. Positive correlations between metconazole and the other fungicides suggest the potential for cross-resistance between these DMI fungicides. To date, no loss of NLB control has been observed with the use of metconazole, propiconazole, and prothioconazole in the field.

Introduction

Northern leaf blight (NLB), caused by *Exserohilum turcicum* (Luttrell) Leonard and Suggs, is a fungal foliar disease that can reduce grain yields of maize (Bowen and Pedersen, 1988; Fisher et al., 1976; Raymundo and Hooker, 1981; Ullstrup and Miles, 1957). Leaf symptoms appear as long elliptical lesions that are initially pale green and turn necrotic over time (Carson, 1999; Ullstrup, 1966; Ullstrup and Miles, 1957). Under cool, moist conditions conidia will form on the lesion surface and rain will spread the conidia causing secondary infections up the canopy (Carson, 1999; Ullstrup, 1966; Ullstrup and Miles, 1957). Severe disease causes reduction in plant photosynthetic potential and increases the incidence of stalk rots and lodging (Dodd, 1980a; Dodd, 1980b; Fisher et al., 1976; Fajemisin and Hooker, 1974; Pedersen and Oldham, 1992; Raymundo and Hooker, 1981). Grain yields can be reduced by greater than 50 percent if infection occurs early in the growing season and conditions are favorable for disease development (Fisher et al., 1976; Raymundo and Hooker, 1981). NLB is found in maize producing regions around the world, and recently, it has increased in incidence across the United States (Carson, 1999; Wise and Mueller, 2011).

Several management strategies can be used to control NLB. Tillage and rotation are effective at limiting initial inoculum present on residue (Pedersen and Oldman, 1992; Wise and Mueller, 2011). Unfortunately, increased adoption of reduced tillage practices and continuous corn rotations has led to increased disease, especially in the northcentral regions of the country Pedersen and Oldman, 1992; Wise and Mueller, 2011). Resistant hybrids are available with both single-gene and multi-gene resistance (Ferguson and Carson, 2004; Schechert et al., 1999; Welz

and Geiger, 2000). Although host resistance can provide some protection, virulent races of the pathogen are present that can overcome the single-gene forms of resistance, and polygenic resistance does not offer complete control (Ferguson and Carson, 2004; Lipps et al., 1997; Welz and Geiger, 2000). Fungicides have become an economically viable option for control of maize foliar diseases in the last decade (Mallowa et al., 2015; Wise and Mueller, 2011). Producers often use foliar applied fungicides alone or in conjunction with other management practices to control NLB (Mallowa et al., 2015; Munkvold et al., 2008).

Corn producers are limited to a few fungicide groups for foliar application, one of which is the demethylation inhibitors (DMIs) (Mallowa et al., 2015; Munkvold et al., 2008). The DMIs are a group of fungicides in the sterol biosynthesis inhibitor class often used for NLB control in the field (FRAC, 2015). Three triazole chemicals make up the majority of the DMIs fungicides applied to production corn fields: metconazole (Caramba, BASF Corporation, Research Triangle Park, NC), propiconazole (Tilt, Syngenta Crop Protection, Greensboro, NC), and prothioconazole (Proline, Bayer CropScience, Research Triangle Park, NC). These chemicals are applied alone or in combination with a quinone outside inhibitor (QoI) fungicide. DMIs inhibit the C14 demethylation step in fungal sterol production limiting ergosterol, which is necessary for cell membrane construction (FRAC, 2015; Yoshida, 1993). The Fungicide Resistance Action Committee (FRAC) considers DMIs to be at medium risk of developing pathogen resistance, which appears as a stepwise decrease in sensitivity due to prolonged exposure to the fungicide (Brent and Hollomon, 2007b; Klix et al., 2007). Multiple independent mutations can be accumulated within the pathogen resulting in quantitative resistance; however, there is often a fitness penalty associated with resistance and a partial recovery of sensitivity may occur in the absence of fungicide selection pressure (Brent and Hollomon, 2007b). Cross-

resistance to fungicides in the DMI group has been observed in other pathogens, which can limit the fungicide options (Brent and Hollomon, 2007b).

Pathogen resistance to DMI fungicides is a concern due to their repeated use by producers across the U.S. Corn Belt placing selection pressure on a large *E. turcicum* population. The first objective of this study was to establish the in vitro *E. turcicum* baseline sensitivity to metconazole, propiconazole, and prothioconazole. The second objective was to monitor isolate sensitivity from 2009 to 2014 and compare to the baseline sensitivities.

Materials and Methods

Isolate collection. The *E. turcicum* collection consisted of 170 isolates obtained from corn: 168 isolates were tested for metconazole and prothioconazole sensitivity and 155 isolates were tested for propiconazole sensitivity. Isolates collected prior to 1992 were considered baseline isolates, because their collection predates metconazole, propiconazole, and prothioconazole being labeled for use on corn, thus, they were likely never exposed to DMI fungicides. Baseline isolates were provided from a collection compiled by Dr. Martin Carson and curated by Dr. Peter Balint-Kurti (United States Department of Agriculture, North Carolina State University, Raleigh, NC) from 8 states (Table 3.1). The baseline isolates were previously used for research including quantification of *E. turcicum* genetic variation, mating-type identification, and race identification and population distribution (Ferguson and Carson, 2004; Ferguson and Carson, 2007). Isolates were collected between 2009 and 2014 from random seed production fields and research fields across Illinois, except for two Indiana isolates collected in 2014 provided by Dr. Kiersten Wise (Purdue University, West Lafayette, IN) (Table 3.1). Many of the isolates from 2009 to 2014

were collected from fields known to have been treated with metconazole, propiconazole, or prothioconazole alone or in combination with a quinone outside inhibitor (QoI) fungicide in the year of collection: 7 isolates in 2009, 9 isolates in 2010, 21 isolates in 2011, and 15 isolates in 2012. The remaining isolates collected from 2009 to 2014 were from fields treated with a QoI fungicide alone, that did not receive a fungicide application, or in which it was unknown if fungicide had been applied.

Baseline *E. turcicum* isolates and the two isolates from Indiana were received as cultures grown on potato dextrose agar (PDA) (Fisher Scientific, Pittsburgh, PA), while all remaining isolates were isolated from NLB symptomatic corn leaf tissue samples. Leaf samples were placed in humidity chambers (sealed plastic bags containing a damp paper towel) to induce sporulation. After approximately four days, conidia were rinsed off the leaf surface with 200 to 500 µl of water, and the conidial suspension was spread on Petri plates containing PDA amended with 25 mg/L of rifampicin (Fisher Scientific, Pittsburgh, PA). The following day, single germinated *E. turcicum* conidia were collected and transferred to new Petri plates with PDA + rifampicin. Cultures were grown for 7 to 14 days at 20° to 25°C with 12 hour light/dark cycles until a thick mycelial mat formed. Mycelia samples were cut from isolate cultures and placed in individual 1.5 ml tubes containing 850 µl of 15% glycerol solution. Tubes were stored in a -80°C freezer until isolated were screened for fungicide sensitivity.

Fungicide sensitivity testing. Technical-grade formulations of metconazole (97% a. i.; BASF Corporation, Research Triangle Park, NC), propiconazole (95% a. i.; Syngenta Crop Protection, Greensboro, NC), and prothioconazole (97.7% a. i.; Bayer CropScience, Research Triangle Park, NC) were used to prepare stock solutions at concentrations of 100 mg/ml in acetone. The stock solution of each fungicide was used to make serial dilutions in acetone. PDA was autoclaved

and allowed to cool to approximately 55°C before it was amended with each fungicide dilution. The fungicide-amended PDA was poured in 65 x 15 mm Petri plates (Fisher Scientific, Pittsburgh, PA) at concentrations of 0, 0.001, 0.01, 0.1, 1, 10, and 100 µg/ml for the three fungicides. To measure fungicide sensitivity, mycelial growth of each isolate was assessed on PDA amended with the different concentrations of each fungicide. This method of sensitivity evaluation is commonly used for DMI fungicides (Brent and Hollomon, 2007b; Demirci et al, 2003; Miller et al., 2002; Russell, 2004; Wise et al, 2011).

In preparation for fungicide sensitivity testing, *E. turcicum* isolates were removed from long term storage and cultured on lactose casein agar (LCA) to promote conidia formation (Tuite, 1969). Isolates were grown on LCA for 7 to 14 days at 20° to 25°C with 12 hour light/dark cycles. Pieces (approximately 5 mm^2) of the sporulating cultures were placed in 1.5 ml tubes containing 500 to 800 µl of water and vortexed to dislodge the conidia. The conidia suspensions were evenly spread on PDA + rifampicin and allowed to grow for 5 to 10 days under temperature and light conditions previously described, until a dense, evenly distributed mycelial mat was present. Mycelial plugs (5 mm diameter) were cut from the PDA plates and placed mycelia side down in the center of the fungicide-amended PDA plates containing the different concentrations. Plates were placed in a growth chamber and incubated at 23°C in the dark for 4 days. Plates were then digitally scanned with a flatbed scanner (Epson Expression 10000XL, Epson American, Inc., Long Beach, CA) and the surface area (mm^2) of mycelia growth was quantified using specialized software (Assess 2.0: Image Analysis Software for Plant Disease Quantification, American Phytopathological Society, St. Paul, MN). The surface area of the plug was subtracted from the total surface area measured on each fungicide concentration and the percent inhibition was calculated as: 100 - [(mycelia surface area of the fungicide amended

media/mycelia surface area of the non-amended media) × 100]. From this, a linear interpolation method (Pasche et al., 2004; Wise et al., 2009; Zhang et al., 2012) was used to calculate the effective concentration where mycelia growth was reduced by 50% (EC_{50}) for each isolate.

Due to space limitations, *E. turcicum* isolates were tested in groups of approximately 10. An internal positive control isolate was included in each group to ensure assay reproducibility (Wong and Wilcox, 2000). An isolate from 2011 was selected as the internal control isolate and tested in five separate trials for each fungicide. The mean, standard error, and 95% confidence intervals were calculated based on the resulting EC_{50} values. If the internal control isolate EC_{50} values were within the 95% confidence intervals previously determined, trials were combined for statistical analysis; however, groups of isolates were retested if the internal control isolate EC_{50} values did not satisfy the reproducibility assay (Wong and Wilcox, 2000).

Each isolate was tested in two trials repeated over time with two replications per trial, using a completely randomized design (CRD). Isolates collected prior to 1992 were grouped together as baseline isolates for statistical analysis, while the non-baseline isolates were analyzed by the year they were collected. Isolate EC_{50} values for each fungicide were analyzed separately using the mixed models procedure (PROC MIXED) in SAS (version 9.4, SAS institute, Inc., Cary, NC). Residuals were examined with the Shapiro-Wilk test for normality (alpha = 0.01) using the univariate procedure (PROC UNIVARIATE) and Brown-Forsythe test for homogeneity of variance (alpha = 0.05) with the general linear model procedure (PROC GLM). Isolate EC_{50} values (calculated as μg/ml) were multiplied by 10^3 and natural log transformed to meet assumptions of normality and homogeneity for all analyses. For the analysis of years, the baseline and years were considered fixed effects, while trials, isolates nested in years, and interactions with trial and isolates were considered random effects. *Exserohilum turcicum*

baseline and year EC_{50} value means were compared and the 'PDMIX800' macro was used to format the pairwise differences from the PDIFF option in the LSMEANS statement in PROC MIXED, creating groups of similar means for fixed effects, designated by letters (Saxton, 1998). Boxplots of *E. turcicum* isolates base-10 log transformed EC_{50} values grouped by years were made using the boxplot procedure (PROC BOXPLOT). A separate analysis was performed to identify significant differences between isolate EC_{50} values, specifically, non-baseline isolates with EC_{50} values significantly greater than that of the least sensitive baseline isolate. For this analysis, isolates were treated as fixed effects while trials and interactions with trials were treated as random effects in PROC MIXED. Again, the 'PDMIX800' macro was used to group isolates with similar means. Correlation analysis was performed on EC_{50} values for metconazole, propiconazole, and prothioconazole using Pearson's correlation procedure (PROC CORR). Terms and estimates were considered significant when $P \leq 0.05$.

Results

The range of EC_{50} values determined for *E. turcicum* baseline isolates (collected prior to 1992) exposed to metconazole was 0.001-0.180 µg/ml (Table 3.1) with 89% of the isolate EC_{50} values below 0.1 µg/ml (Figure 3.1). The least squares mean (lsmean) of the baseline was 0.032 µg/ml (Figure 3.2). The ranges of the isolate EC_{50} values from 2009, 2010, 2011, 2012, and 2014 exposed to metconazole were 0.002-0.853, 0.009-3.898, 0.015-0.032, 0.012-0.072, and 0.025-0.191 µg/ml, respectively (Table 3.1). No Significant differences ($P = 0.0625$) between the metconazole EC_{50} value lsmeans of *E. turcicum* baseline and years were observed. The metconazole EC_{50} value lsmeans for the 2009, 2010, 2011, 2012, and 2014 were 0.030, 0.057,

0.047, 0.032, and 0.036 µg/ml, respectively (Figure 3.2). Significant differences ($P < 0.0001$) in metconazole sensitivity were found among isolates. Three non-baseline isolates were significantly greater than the baseline isolate with the greatest EC_{50} value. Two isolates from 2010 and one isolate from 2009 had EC_{50} values of 3.898, 0.921, and 0.860, respectively. The greatest EC_{50} value measured in the baseline isolate was 0.180 µg/ml.

The range of EC_{50} values determined for *E. turcicum* baseline isolates exposed to propiconazole was 0.007-0.670 µg/ml (Table 3.1), with 84% of isolate EC_{50} values below 0.13 µg/ml (Figure 3.3). The lsmean of the baseline was 0.060 µg/ml (Figure 3.4). The ranges of the isolate EC_{50} values from 2009, 2010, 2011, 2012 and 2014 were 0.005-0.977, 0.011-3.318, 0.006-0.446, 0.029-0.555, and 0.042-0.300 µg/ml, respectively (Table 3.1). No significant differences ($P = 0.3189$) between the propiconazole EC_{50} value lsmeans of *E. turcicum* baseline and years were observed. The propiconazole EC_{50} value lsmeans for 2009, 2010, 2011, 2012, and 2014 were 0.107, 0.117, 0.072, 0.093, and 0.071 µg/ml, respectively (Figure 3.4). Significant differences ($P < 0.0001$) in propiconazole sensitivity were found among isolates. One 2010 isolate EC50 value was significantly greater than the baseline isolate with the greatest EC_{50} value. The 2010 isolate EC50 value was 3.318 µg/ml. The greatest EC_{50} value measured in the baseline isolate was 0.670 µg/ml.

The range of EC50 values determined for *E. turcicum* baseline isolates exposed to prothioconazole were 0.007-2.143 µg/ml (Table 3.1), with 86% under 0.9 µg/ml (Figure 3.5). The lsmean of the baseline was 0.254 µg/ml (Figure 3.6). The ranges of the isolate EC_{50} values from 2009, 2010, 2011, 2012, and 2014 were 0.017-1.261, 0.018-0.963, 0.007-1.909, 0.013-0.924, and 0.179-4.170 µg/ml, respectively (Table 3.1). No significant differences ($P = 0.2631$)

between the prothioconazole EC_{50} value lsmeans of *E. turcicum* baseline and years were observed (Figure 3.6). The prothioconazole EC_{50} value lsmeans for 2009, 2010, 2011, 2012, and 2014 were 0.254, 0.196, 0.237, 0.381, 0.147, and 0.544 μg/ml, respectively (Figure 3.6). A significant difference ($P < 0.0001$) in prothioconazole sensitivity was found among isolates. A 2014 isolate with an EC_{50} value of 4.170 μg/ml was measured, but it was not significantly different than the least sensitive baseline isolate with an EC_{50} value of 2.1427 μg/ml.

Correlation analysis was conducted between log EC_{50} values for each fungicide to assess cross-resistance. A significant correlation was found between metconazole EC_{50} values and propiconazole EC_{50} values ($r = 0.3269$, $P < 0.0001$, $n = 153$) (Figure 3.7). A significant positive correlation was found between metconazole EC_{50} values and prothioconazole EC_{50} values ($r = 0.1690$, $P = 0.0295$, $n = 166$) (Figure 3.8). No significant positive correlation was also found between propiconazole EC_{50} values and prothioconazole EC_{50} values ($r = 0.1411$, $P = 0.0820$, $n = 153$) (Figure 3.9).

Discussion

There are some concerns that must be considered when establishing baseline fungicide sensitivity and making non-baseline comparisons including: dose rates, sample size, region of isolate collection, and methods for measuring fungicide sensitivity (Russell, 2004). The dose rates used for this study encompassed all EC_{50} values measured, included enough rates for accurate EC_{50} measurement, and offered 100% control at the higher concentrations for the baseline and non-baseline isolates. There is no set number of isolates required to establish a baseline; however, the histograms for all three fungicide baseline EC_{50} values demonstrated

fairly normal distributions and 100% control was achieved for isolates suggesting an adequate number of isolates were used. Similar results were found for the non-baseline isolates but not included. In the study, the baseline included isolates from multiple states across years, while the non-baseline focused on Illinois isolates; however, it was reasonable to make comparisons between the non-baseline isolate EC_{50} values from Illinois and the baseline EC_{50} values since baseline isolates from Illinois had EC_{50} values similar to those measured across other states. Finally, methods for sensitivity evaluation were consistent between baseline and non-baseline isolates, following procedures commonly used for in vitro DMI fungicide evaluations (Demirci et al., 2003; Miller et al., 2002; Russell, 2004; Wise et al., 2011).

Intrinsic differences in efficacy of the three fungicides were observed based on the baseline EC_{50} value lsmeans. Metconazole had the greatest activity, followed by propiconazole, followed by prothioconazole. Harlapur et al. (2007) found similar intrinsic differences when testing multiple DMI fungicides for efficacy against *E. turcicum*. The percent mycelial growth inhibited by five DMI fungicides, including propiconazole, tested in vitro at 0.1% concentrations ranged from 69 to 99% inhibition (Harlapur et al., 2007). Inherent differences in the efficacy of fungicides within the DMI chemical group also have been reported in other fungal species (Hsiang et al., 1997; Karaoglanidis et al., 2000; Sombardier et al., 2009).

The *E. turcicum* baseline isolates used for this study had varying ranges in sensitivity to metconazole, propiconazole and prothioconazole, approximately: 0.179 µg/ml, 0.663 µg/ml, and 2.136 µg/ml between the most and least sensitive isolates, respectively. The ranges in the *E. turcicum* EC_{50} values for the metconazole and propiconazole are similar to baseline ranges established for other species (Burlakoti et al., 2010; Forster et al., 2011; Wong and Midland, 2007). Similar EC_{50} ranges were reported in metconazole baseline sensitivity tests performed on

Fusarium spp. (0.0058 to 0.084 µg/ml) and *Alternaria* spp. (0.014 to 0.224 µg/ml) (Burlakoti et al., 2010; Forster et al., 2011). Furthermore, the metconazole baseline EC_{50} range found in this study was also very similar to the baseline EC_{50} value range found in baseline *E. turcicum* isolates previously tested from Georgia and Florida (0.008 to 0.155 µg/ml) (Arcibal, 2013). A similar EC_{50} range in propiconazole baseline sensitivity has been reported for *Colletotrichum cereale* (0.025 to 0.35 µg/ml) (Wong and Midland, 2007). The fold-differences for the prothioconazole baseline EC_{50} values were greater than those found for *Ascochyta rabiei* (0.0526 to 0.2958 µg/ml) and *Rhizoctonia solani* (0.20 to 5.58 µg/ml) (Ajayi and Bradley, 2014; Wise et al., 2011). The wide fold differences between the most sensitive and least sensitive baseline *E. turcicum* isolates suggest an increased potential for resistance development; however, none of the baseline lsmeans for metconcazole, propiconazole, and prothioconazole were skewed towards the less sensitive end of the distributions, suggesting development of resistance is less likely (Russell, 2004).

No significant reductions in sensitivity for the years of non-baseline isolates from Illinois were observed when compared to the baseline isolates for metconazole, propiconazole, or prothioconazole. It is likely some of the non-baseline isolates tested may not have been previously exposed to DMI fungicides. Unknown isolate histories, sprayer error, and migration of *E. turcicum* conidia from non-treated to fungicide-treated fields increase the level of variability of fungicide exposer. In years when the majority of isolates tested were from fields where DMI fungicides were applied, the level of exposure provided insufficient selection pressure to cause a shift in fungicide sensitivity. Many of the isolates were collected from seed production fields where fungicides were applied annually; however, the repeated selection pressure was too limited to cause shifts in sensitivity. In a study testing *Monilinia fructicola*

sensitivity to propiconazole in peach orchards, there was no significant reduction in sensitivity between the baseline isolate population mean (never exposed to propiconazole) and the population mean of isolates collected from the same orchard after 23 applications of propiconazole (Zehr et al., 1999). Multiple studies have reported significant shifts in DMI sensitivity in populations only after prolonged and repeated exposure to fungicides (Keller et al., 1997; Wong et al., 1997; Zehr et al., 1999). These results are not surprising considering DMI fungicide resistance is quantitative, requiring repeated DMI exposure to select for populations containing multiple mutations to reduce fungicide sensitivity (Brent and Hollomon, 2007b; Luke, 2014).

The absence of significant population shifts in sensitivity to the DMI fungicides could be partially explained by the biology of *E. turcicum* and the DMI resistance mechanisms. *Exserohilum turcicum* primarily reproduces asexually, forming conidia as both the primary and secondary inoculum (Carson, 1999). While NLB is a polycyclic disease which favors fungicide resistance development, asexual reproduction limits the amount of genetic recombination and variation necessary for rapid spread and development of fungicide resistance (McDonald and Linde, 2002). The Fungicide Resistance Action Committee (FRAC) has labeled *E. turcicum* as medium risk of developing fungicide resistance (FRAC, 2013). As stated previously, multiple mutations are necessary to reduce DMI sensitivity. The three primary mechanisms are: i) mutations in the target-encoding CYP51 gene causing decreased affinity of the protein for inhibitors, ii) over-expression of the CYP51 gene caused by insertions in the predicted promoter regions, and iii) increased efflux caused by over-expression of genes encoding membrane transporters (Cools et al., 2013; Ma and Michailides, 2005). Mutations in the target-encoding CYP51 gene tend to be fungicide specific, while over-expression of the CYP51 gene and

increase fungicide efflux confer reduced sensitivity to multiple fungicides. Enhanced efflux has only been shown to affect fungicide performance in *Botrytis cinerea* in the field, thus far (Cools et al., 2013). None of the mutations appear to confer complete resistance and there seems to be a fitness penalty associated with the over-expression of CYP51 (Cools et al., 2013).

Though no shifts in *E. turcicum* population sensitivity were observed between the baseline and years, several isolate EC_{50} value lsmeans were significantly higher than the least sensitive baseline isolates for metconazole and propiconazole. These isolates could be the result of exposure to DMI fungicides resulting in reduced sensitivity; however, they also may be the result of experimental error due to sample size. In vivo fungicide testing will be required in the future to establish if these isolates confer resistance, as fungicide sensitivity in vitro is generally higher than in the field and does not always correlate to the field performance (Karaoglanidis et al., 2000; Reis et al., 2015; Russell, 2004).

Significant cross-sensitivity was observed between metconazole and propiconazole, metconazole and prothioconazole, but not propiconazole and prothioconazole. Though statistically significant, correlations between fungicide sensitivities varied and were low. Variations in the levels of cross-sensitivity are common in the DMI fungicides (Hildebrand et al., 1988; Karaoglanidis et al., 2000; Klix et al., 2006). Variations in the cross-sensitivity between the three fungicides could be related to the differences in their intrinsic activity, differences in their mode of interference with the demethylation process, and the multigene mutations necessary for resistance development (Hildebrand et al., 1988; Karaoglanidis et al., 2000; Klix et al., 2006). Due to the relatively low level of correlation among the fungicides, monitoring for shifts in *E. turcicum* population sensitivity should be carried out for each fungicide.

This study established *E. turcicum* baseline sensitivity for metconazole, propiconazole, and prothioconazole which can be utilized to monitor for resistance. The study also determined that no significant shift in population sensitivity has occurred in Illinois between 2009 and 2014. Fungicides currently are applied to less than 20% of corn production fields in the corn belt, leaving the remaining corn fields to act as 'refuge' to reduce selection pressure and mitigate wide spread population shifts in sensitivity (Mallowa et al., 2015; Munkvold et al., 2008; Wise and Mueller, 2011). Multiple DMI fungicides are available for rotation to producers with low levels of cross-sensitivity (as concluded in this research) and can be applied in combination with other fungicides to further reduce the potential for resistance development while offering NLB control (Da Costa and Boller, 2008). These management practices plus the limitations imposed by the asexual lifecycle of *E. turcicum*, the multigene mutations required for DMI resistance, and the potential fitness penalties associated with the mutations slow the development of DMI resistance. Increased DMI foliar fungicide applications and repeated use of the same fungicide could cause reduced sensitivity to DMI fungicides (Brent and Hollomon, 2007a). Resistance to other fungicide groups has been observed in closely related species. The causal agent of sugarcane leaf spot, *Helminthosporium halodes*, developed in vitro resistance to mancozeb (Reddy, 1989). Polyoxin-resistant mutants of *Cochliobolus heterostrophus*, the causal agent of southern leaf blight of corn, were produced in the laboratory (Gafur et al., 1998). Field resistance to thiabendazole and thiophanate-methyl also has been reported in *Helminthosporium solani*, the causal agent of silver scurf on potato (Geary et al., 2007). Monitoring DMI resistance progression in *E. turcicum* can aid in making management decisions and maintain fungicide efficacy (Brent and Hollomon, 2007b).

Table 3.1. Collection information and results of in vitro assays for isolates of *Exserohilum turcicum* for metconazole, propiconazole, and prothioconazole baseline sensitivity and non-baseline sensitivity.

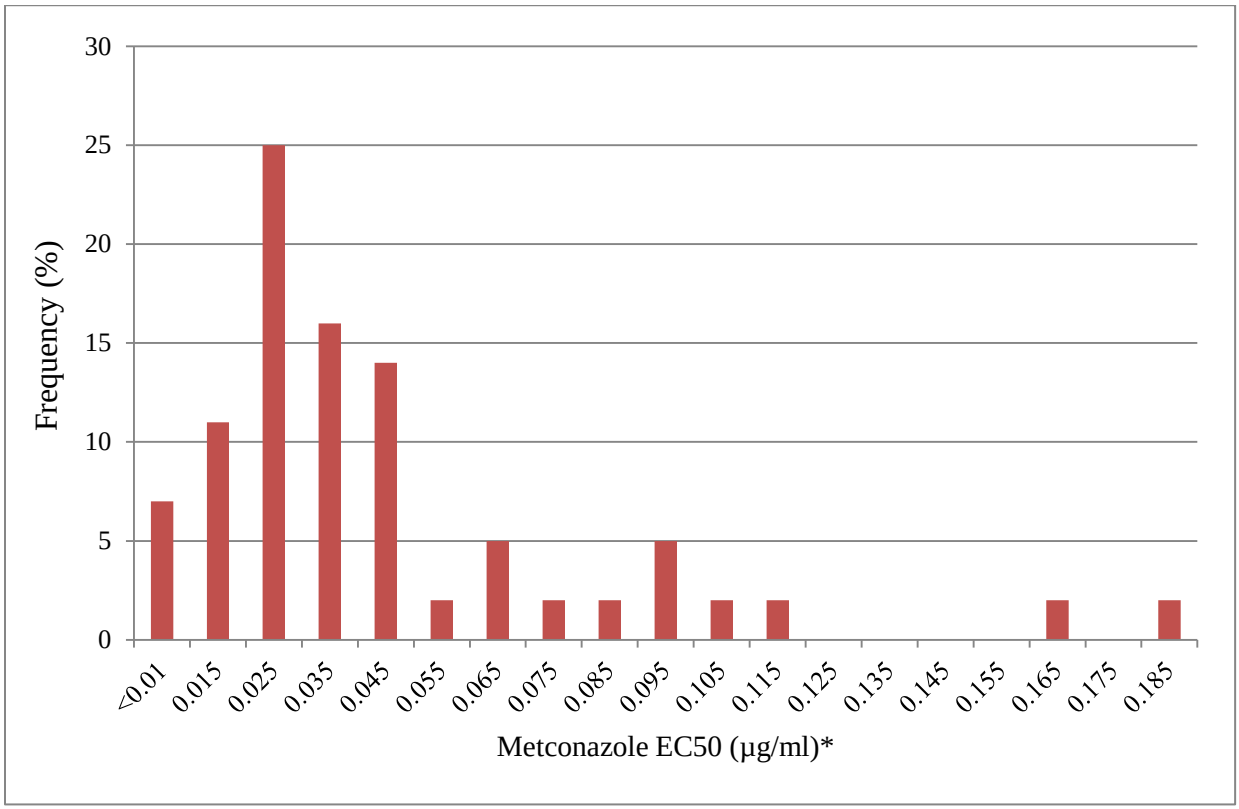
				Metconazole			Propiconazole			Prothioconazole		
Year	State	County	Number of isolates/location	N	Mean EC50 (µl/ml)	EC50 range	N	Mean EC50 (µl/ml)	EC50 range	N	Mean EC50 (µl/ml)	EC50 range
1975	GA	Lee	1	1	0.012		1	0.035		1	0.475	
1977	MN	Redwood	1	1	0.104		1	0.049		1	0.175	
1978	IA	Webster	1	1	0.037		1	0.047		1	0.750	
1979	IL	Iroquis	4	4	0.046	0.020-0.089	4	0.230	0.007-0.670	4	0.207	0.298-0.074
1980	IA	Webster	1	1	0.005					1	0.432	
	IN	Clark	1	1	0.030		1	0.121		1	0.219	
	OH	Wayne	3	3	0.060	0.029-0.095	3	0.055	0.038-0.064	3	1.100	0.061-2.143
	VA	Fauquier	1	1	0.022		1	0.060		1	2.242	
1981	IL	Henry	2	2	0.025	0.006-0.043	2	0.261	0.094-0.428	2	0.348	0.215-0.482
		Iroquis	1	1	0.113		1	0.123		1	0.593	
		Stark	3	3	0.041	0.012-0.064	3	0.082	0.054-0.123	3	0.127	0.007-0.306
		Vermilion	2	2	0.105	0.029-0.180	2	0.222	0.212-0.232	2	0.258	0.141-0.375
		Whiteside	1	1	0.024		1	0.021				
		Woodford	1	1	0.032		1	0.051		1	0.638	
	MN	Swift	1	1	0.032		1	0.044		1	0.060	
1985	NC	Wilkes	7	7	0.032	0.005-0.075	7	0.054	0.026-0.082	7	0.475	0.144-1.033
	NC	NA	4	4	0.040	0.013-0.093	4	0.052	0.037-0.075	4	0.446	0.032-0.671
1990	IA	Clay	2	2	0.029	0.018-0.041	2	0.058	0.031-0.084	2	0.219	0.100-0.337
	IL	Champaign	1	1	0.164		1	0.196		1	0.711	
	IN	Montgomery	2	2	0.034	0.022-0.046	2	0.136	0.021-0.250	2	0.139	0.087-0.191
1991	MN	Steele	4	4	0.029	0.001-0.060	4	0.033	0.001-0.072	4	0.150	0.028-0.324
2009	IL	Champaign	11	10	0.123	0.002-0.853	9	0.157	0.061-0.303	11	0.177	0.017-0.423
		Logan	2	2	0.046	0.037-0.055	2	0.533	0.089-0.977	2	0.458	0.293-0.624
		Piatt	15	15	0.040	0.002-0.127	14	0.172	0.005-0.682	15	0.428	0.018-1.261
		Pope	2	2	0.027	0.027-0.028	2	0.114	0.079-0.150	2	0.443	0.362-0.523
		St. Clair	1	1	0.038		1	0.027		1	0.510	

Table 3.1. (cont.)

2010	IL	Champaign	15	14	0.398	0.009-3.898	11	0.477	0.054-3.318	14	0.383	0.018-0.963
		DeKalb	2	2	0.079	0.042-0.115	2	0.121	0.027-0.214	2	0.145	0.136-0.154
		Kane	1	1	0.063		1	0.039		1	0.340	
		McLean	2	2	0.059	0.051-0.066	2	0.038	0.031-0.044	2	0.358	0.333-0.383
		Pike	1	1	0.025		1	0.124		1	0.222	
		Pope	3	3	0.081	0.019-0.129	3	0.315	0.123-0.490	3	0.390	0.042-0.644
		Sangamon	5	5	0.033	0.014-0.047	5	0.071	0.011-0.135	5	0.306	0.095-0.499
		Warren	4	4	0.072	0.014-0.135				4	0.132	0.025-0.286
2011	IL	Champaign	8	8	0.057	0.019-0.202	7	0.135	0.043-0.273	8	0.492	0.007-1.621
		Dewitt	5	5	0.115	0.025-0.194	5	0.165	0.013-0.446	5	0.864	0.236-1.699
		Ford	3	3	0.200	0.024-0.491	2	0.054	0.051-0.058	3	0.446	0.018-0.719
		McLean	8	8	0.059	0.021-0.188	8	0.142	0.006-0.417	8	0.524	0.284-0.946
		Piatt	6	6	0.022	0.015-0.032	6	0.076	0.044-0.109	6	0.650	0.111-1.909
2012	IL	Champaign	7	7	0.040	0.020-0.072	7	0.148	0.047-0.336	7	0.469	0.013-0.924
		DeKalb	4	4	0.025	0.012-0.047	4	0.058	0.035-0.099	4	0.084	0.041-0.116
		Piatt	3	3	0.035	0.018-0.062	3	0.044	0.029-0.061	3	0.108	0.019-0.232
		Warren	2	2	0.052	0.048-0.055	2	0.360	0.166-0.555	2	0.232	0.025-0.439
		NA	1	1	0.036		1	0.422		1	0.310	
2014	IL	Champaign	2	2	0.035	0.031-0.040	2	0.056	0.044-0.069	2	0.509	0.179-0.840
		Gallatin	4	4	0.068	0.025-0.191	4	0.110	0.042-0.300	4	1.467	0.474-4.170
		Johnson	4	4	0.049	0.030-0.097	3	0.051	0.050-0.053	4	0.803	0.553-1.413
		Saline	2	2	0.031	0.028-0.033	2	0.049	0.046-0.052	2	0.440	0.348-0.533
		Sangamon	1	1	0.033		1	0.105		1	0.840	
	IN	Rush	1	1	0.013		1	0.041		1	0.517	
		North IN	1	1	0.017		1	0.057		1	0.659	
Total isolates			170	168	0.038	0.001-3.898	155	0.082	0.005-3.318	168	0.270	0.007-4.170

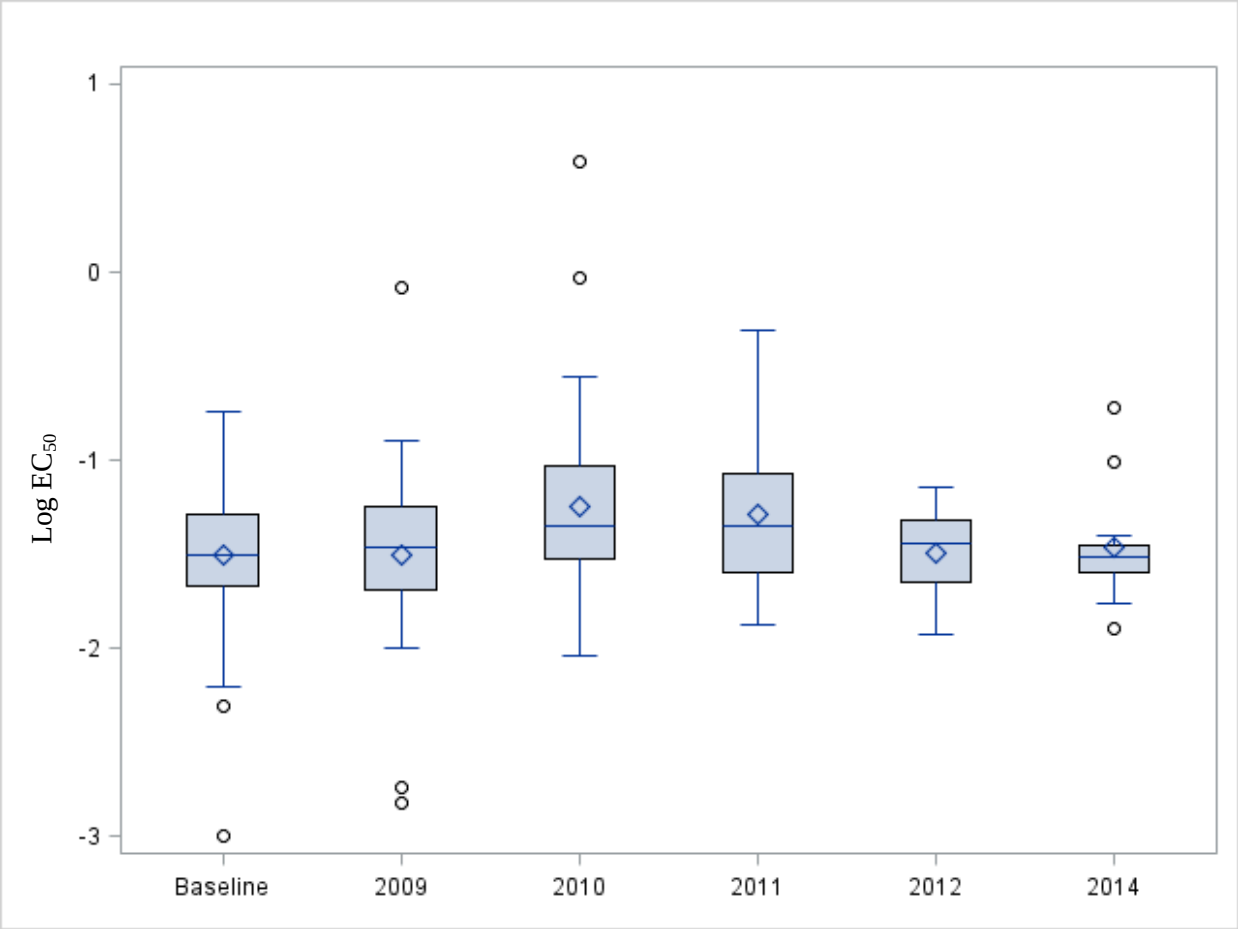
Fungicide sensitivity was determined by calculating the mean effective fungicide concentration that inhibited mycelia growth by 50% of the non-treated control (EC50 value; μg/ml).

Figure 3.1. Frequency distributions of effective metconcazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline *Exserohilum turcicum* isolates.



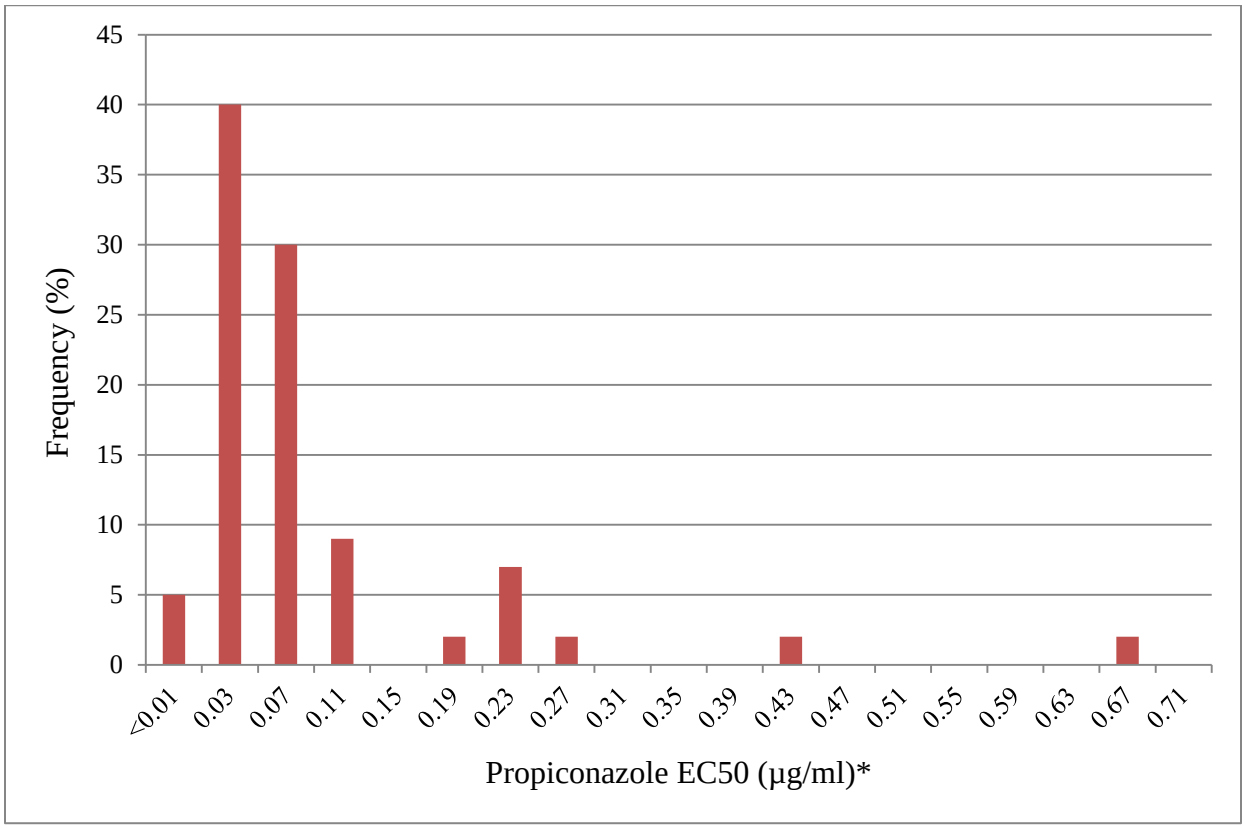
*Individual isolates are grouped in class intervals of 0.010 µg/ml; values on the *X*-axis indicate the midpoint of the interval.

Figure 3.2. Boxplots of effective metconcazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline, 2009, 2010, 2011, 2012, and 2014 *Exserohilum turcicum* isolates.



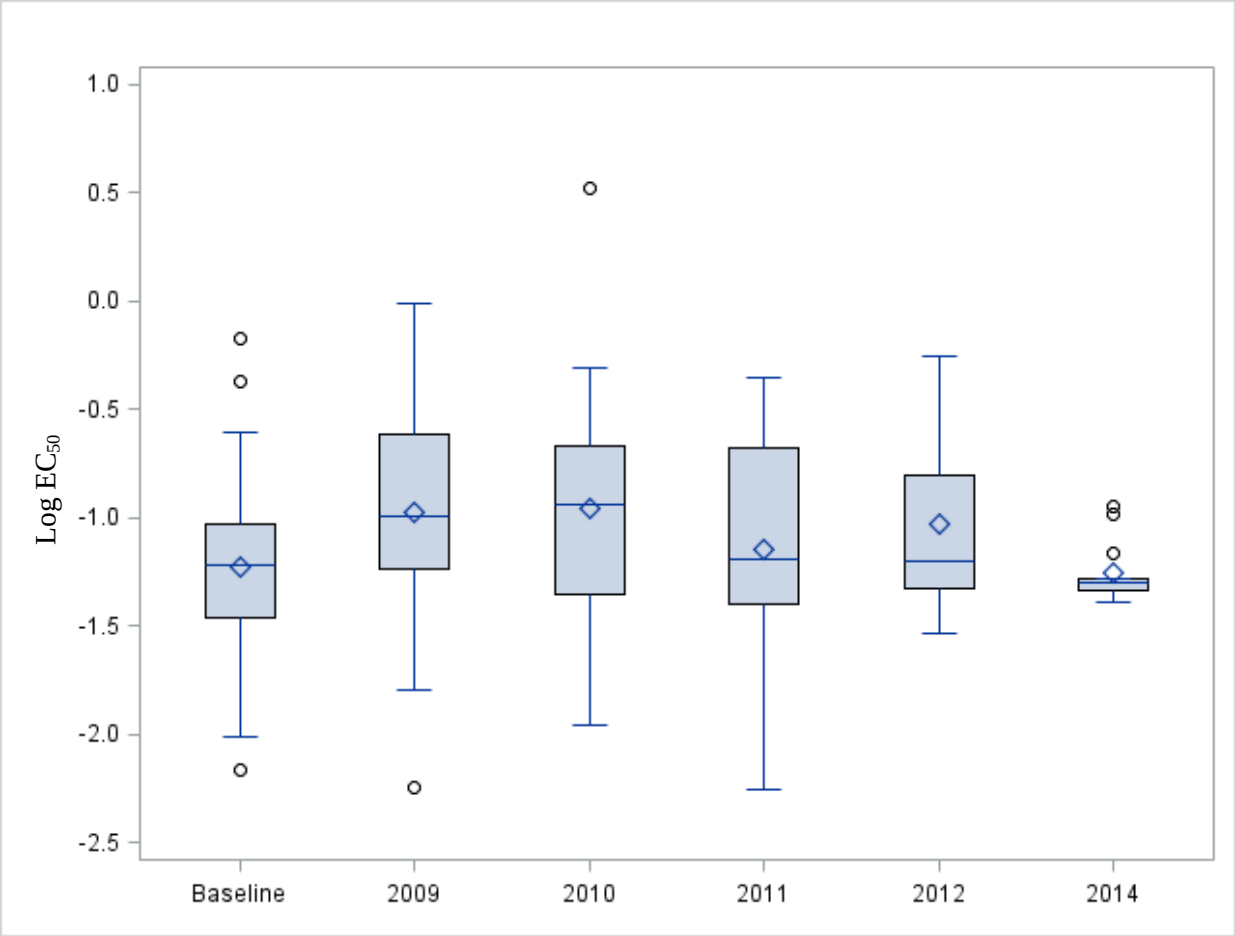
For each box, the diamond represents the mean, the solid line in the box represents the median, the top of each box represents the 75th percentile, the bottom of each box the 25th percentile of EC50 values. Whiskers represent the maximum observation below the upper fence (top) and the minimum observation below the lower fence (bottom) of EC50 values. Circles represent outliers.

Figure 3.3. Frequency distributions of effective propiconazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline *Exserohilum turcicum* isolates.



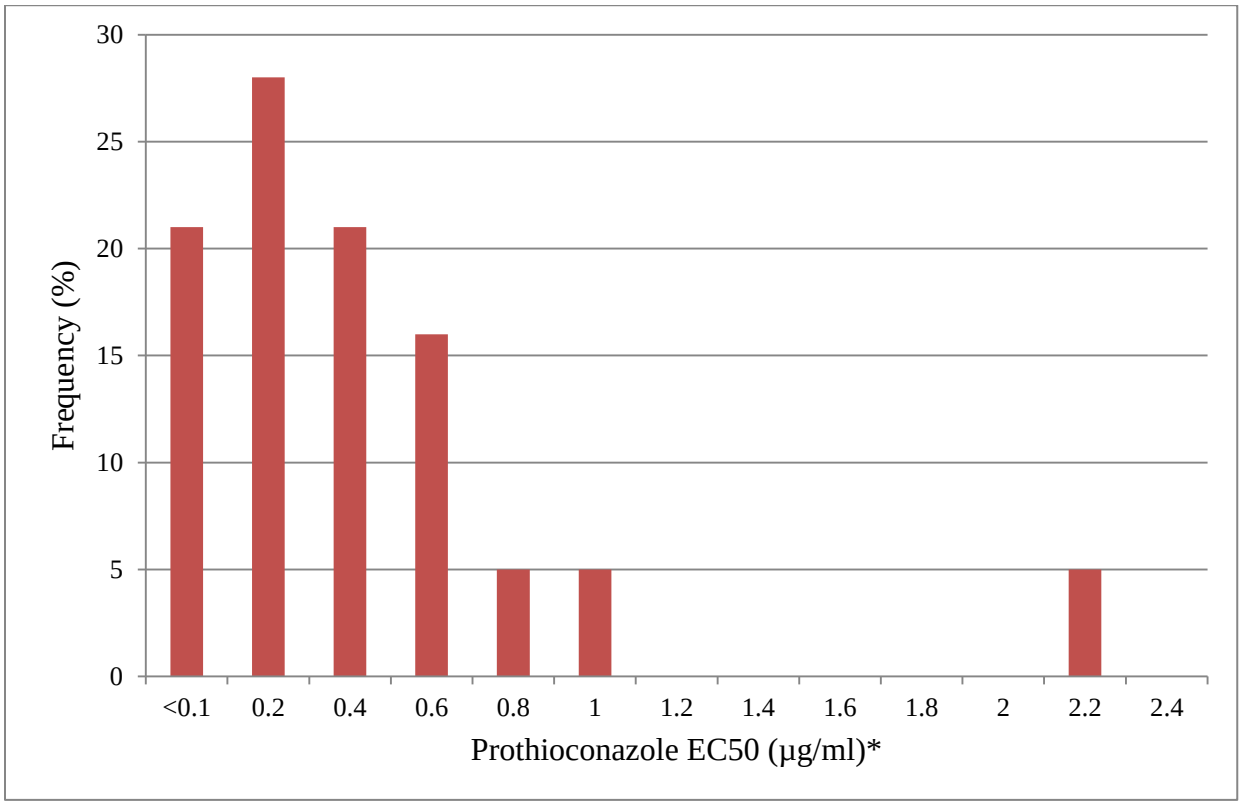
*Individual isolates are grouped in class intervals of 0.040 µg/ml; values on the *X*-axis indicate the midpoint of the interval.

Figure 3.4. Boxplots of effective propiconazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline, 2009, 2010, 2011, 2012, and 2014 *Exserohilum turcicum* isolates.



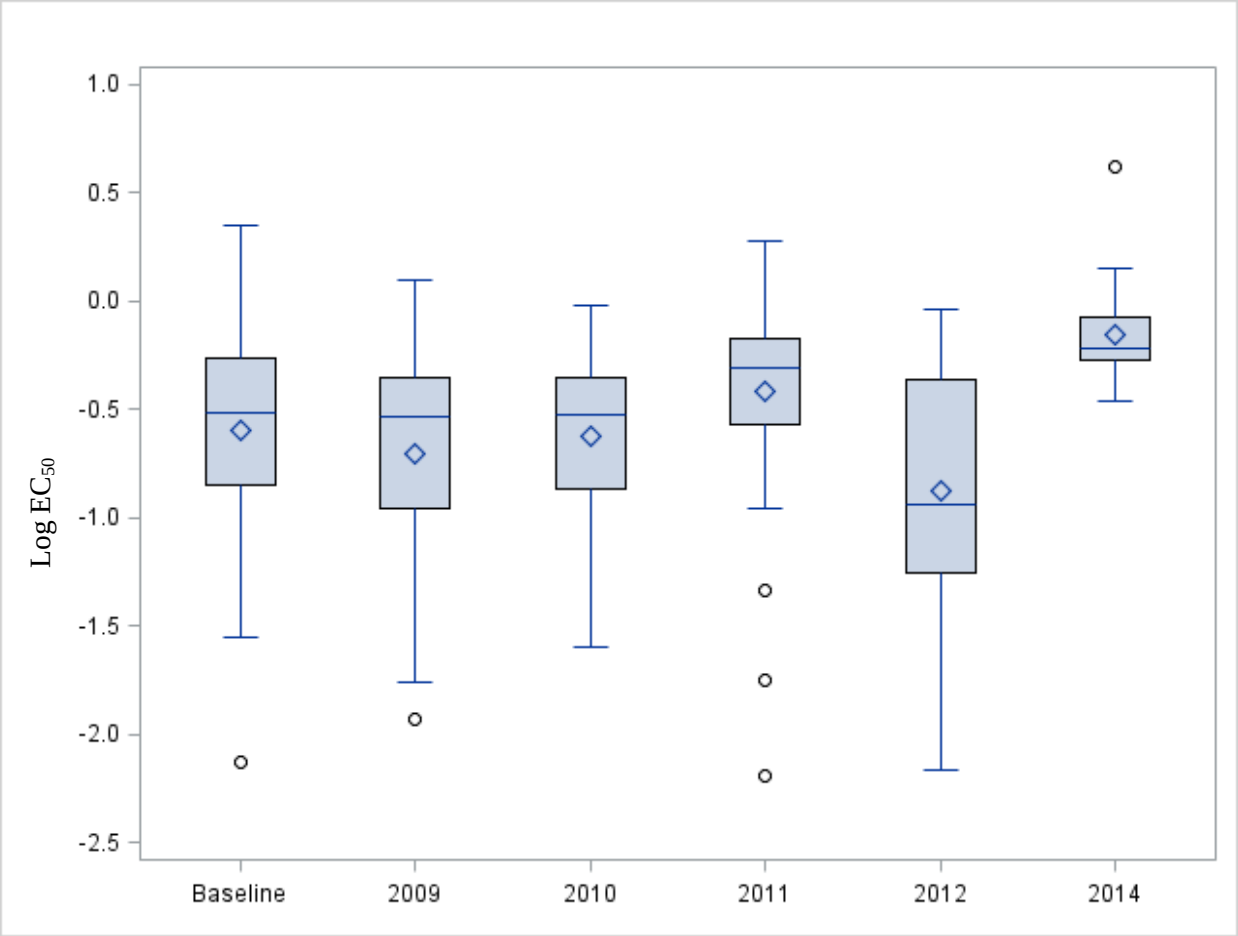
For each box, the diamond represents the mean, the solid line in the box represents the median, and the top of each box represents the 75th percentile, the bottom of each box the 25th percentile of EC50 values. Whiskers represent the maximum observation below the upper fence (top) and the minimum observation below the lower fence (bottom) of EC50 values. Circles represent outliers.

Figure 3.5. Frequency distributions of effective prothioconazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline *Exserohilum turcicum* isolates.



*Individual isolates are grouped in class intervals of 0.200 µg/ml; values on the *X*-axis indicate the midpoint of the interval.

Figure 3.6. Boxplots of effective prothioconazole concentrations that inhibited mycelia growth by 50% (EC_{50} value; µg/ml) for baseline, 2009, 2010, 2011, 2012, and 2014 *Exserohilum turcicum* isolates.



For each box, the diamond represents the mean, the solid line in the box represents the median, and the top of each box represents the 75th percentile, the bottom of each box the 25th percentile of EC50 values. Whiskers represent the maximum observation below the upper fence (top) and the minimum observation below the lower fence (bottom) of EC50 values. Circles represent outliers.

Figure 3.7. Relationship between in vitro mean effective fungicide concentration that inhibited mycelia growth by 50% (EC_{50}; µg/ml) for metconazole and propiconazole of *Exserohilum turcicum* isolates.

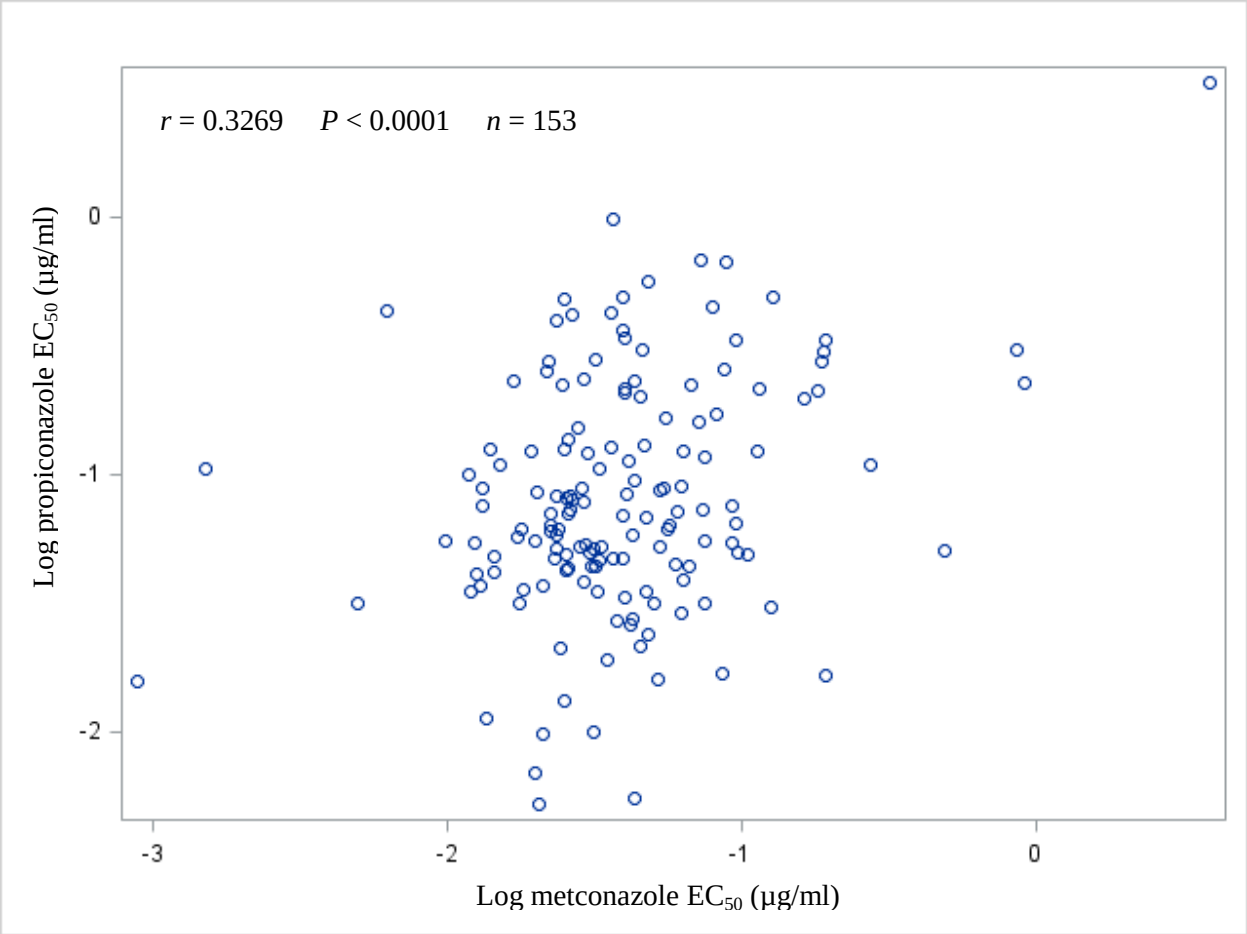


Figure 3.8. Relationship between in vitro mean effective fungicide concentration that inhibited mycelia growth by 50% (EC_{50}; µg/ml) for metconazole and prothioconazole of *Exserohilum turcicum* isolates.

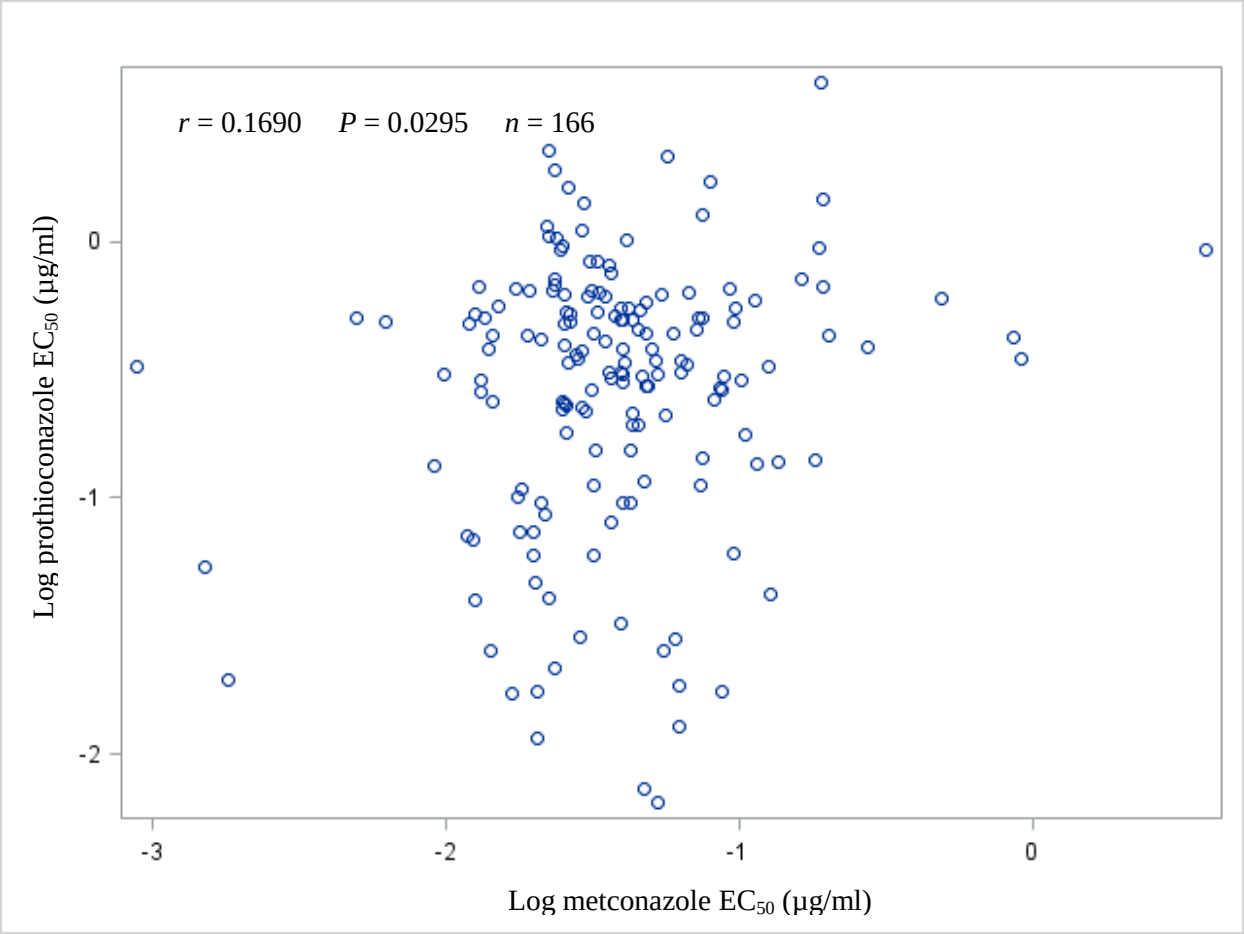
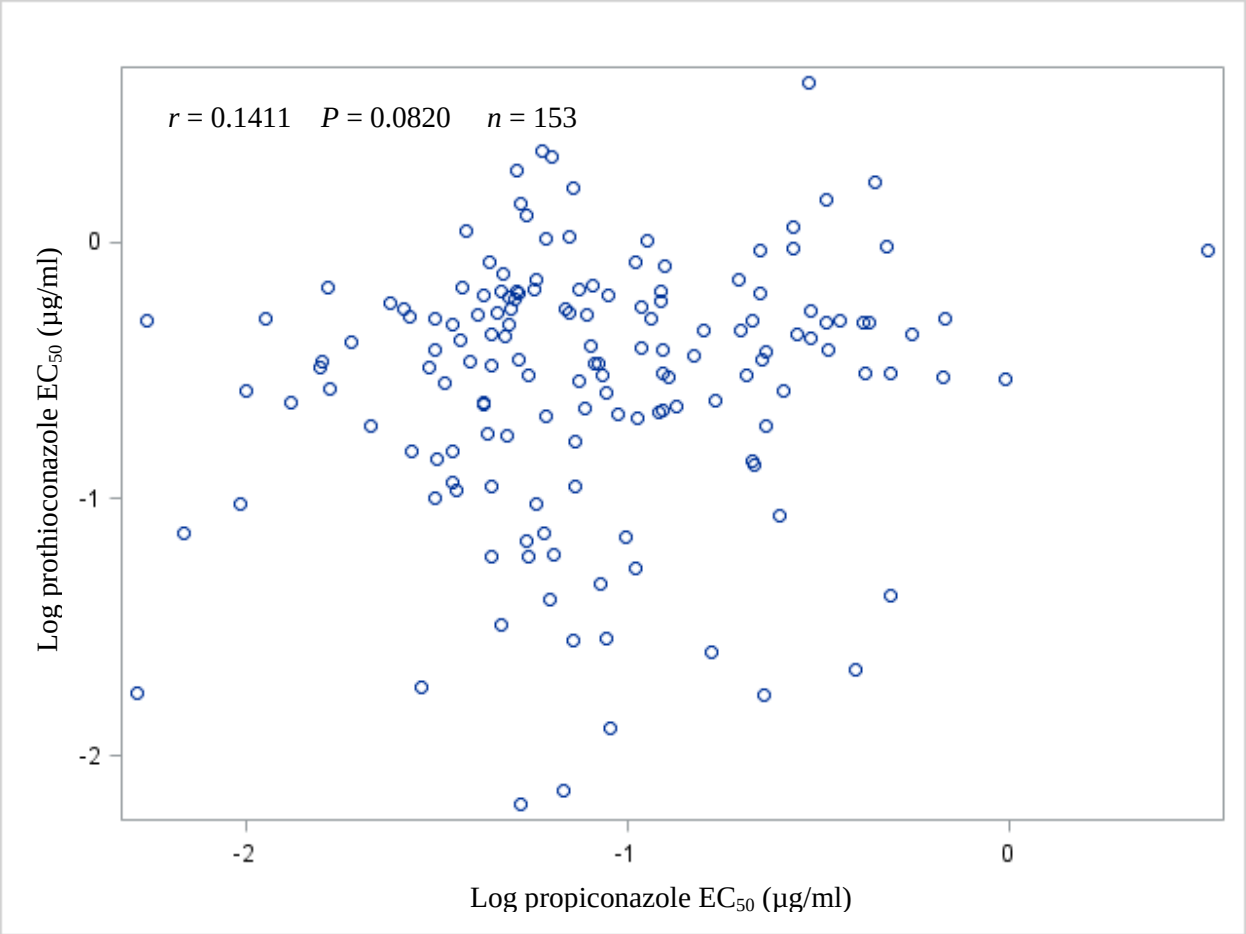


Figure 3.9. Relationship between in vitro mean effective fungicide concentration that inhibited mycelia growth by 50% (EC_{50}; µg/ml) for propiconazole and prothioconazole of *Exserohilum turcicum* isolates.



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CHAPTER 4: FIELD AND GREENHOUSE EVALUATION OF QUINONE OUTSIDE INHIBITOR AND DEMETHYLATION INHIBITOR FUNGICIDES FOR CONTROL OF NORTHERN LEAF BLIGHT OF CORN, CAUSED BY *EXSEROHILUM TURCICUM*

Abstract

Northern leaf blight (NLB), caused by *Exserohilum turcicum*, is a yield reducing foliar disease commonly found in field and sweet corn production. Fungicides containing quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) active ingredients alone or in combination are frequently applied to control NLB. Field trials were conducted in Illinois at DeKalb, Monmouth, and Urbana in 2012 and Dixon Springs and Urbana in 2013 to evaluate NLB control of DMI, QoI, and QoI + DMI fungicides applied at the solo label rates and the reduced rates present in QoI + DMI premixed fungicides. A moderately susceptible field corn hybrid (Pioneer 33W84) was planted at all site locations across years. Trials were inoculated at the 4-leaf growth stage and fungicides were applied at silk emergence. The mean NLB percent leaf infection for the ear leaf, leaf above the ear, and below the ear and the plot NLB percent severity were evaluated at corn reproductive stages R1, R2, R3, R4 and R5. Stalk rot severity, plant maturity, and yield data were collected. Sweet corn trials were conducted in Urbana in 2012 and 2013 using the same methods. NLB leaf and plot severities were evaluated at reproductive stages R1, R2, and R3 and mean ear weight was calculated at harvest. In the greenhouse, trials were conducted to evaluate NLB percent leaf severity on plants inoculated with *E. turcicum* up to seven days before and after fungicide application with azoxystrobin, propiconazole, prothioconazole, or pyraclostobin. In field trials with low disease severity, no significant differences in treatments were observed for NLB severity ratings, stalk rot severity, plant maturity, or yield. In field corn trials with moderate disease severity, label rates of metconazole and azoxystrobin +

propiconazole significantly ($P \leq 0.05$) reduced NLB leaf and plot disease severity compared to the non-treated control across reproductive stages. DMI fungicides at high rates and QoI + DMI premixes offered greater NLB control than other treatments. Fungicide treatments did not significantly affect stalk rot, plant maturity, or yield in field trials with moderate disease severity. In sweet corn trials, metconazole, propiconazole, and azoxystrobin + propiconazole significantly ($P \leq 0.05$) reduced plot disease severity compared to the non-treated control at R2 and R3. DMI fungicides controlled NLB better than other treatments when compared by fungicide chemical group and rates. Ear weight was not significantly affected by treatments in sweet corn trials. In greenhouse trials, all fungicides significantly ($P \leq 0.05$) reduced disease severity when applied 3 days, 1 day, and 3 hours before inoculation and 3 days and 7 days after inoculation. QoI and DMI fungicides can control NLB when applied prior and post infection; however, products containing DMI fungicides offered better NLB control in the field.

Introduction

Northern leaf blight (NLB) of corn, caused by *Exserohilum turcicum*, is a common foliar fungal disease in corn-producing areas in the U.S. (Carson, 1999). Symptoms initially present as long, pale green-gray elliptical lesions on leaves, which expand and turn necrotic over time (Carson, 1999; Ullstrup and Miles, 1957). NLB is a polycyclic disease able to spread up the canopy in favorable cool, wet field conditions (Carson, 1999). Significant grain yield reductions have been reported but vary based on disease severity, timing, and host susceptibility (Bowen and Pedersen, 1988; Fisher et al., 1976; Raymundo and Hooker, 1981; Ullstrup and Miles, 1957). Fisher et al. (1976) reported 0.2% yield reduction for each 1% increase in NLB severity measured 3 to 4 weeks after corn silk emergence; although, disease early in the season (near pollination) has a greater effect on yield than late season infections (Ullstrup and Miles, 1957). Yield losses greater than 50% have been reported (Bowen and Pedersen, 1988; Perkins and Pedersen, 1987; Raymundo and Hooker, 1981; Ullstrup and Miles, 1957). Plants stressed by NLB have an increased potential for root and stalk lodging due to translocation of carbohydrates and increased susceptibility to stalk rot diseases, which can further reduce yields and harvestablity (Dodd, 1980a; Dodd, 1980b; Fisher et al., 1976; Pedersen and Oldham, 1992; Raymundo and Hooker, 1981).

Exserohilum turcicum overwinters as mycelia, conidia, and chlamydospores contained in conidia on corn residue (Boosalis et al., 1967; Carson, 1999; Levy, 1984). Conidia serve as primary inocula and are wind- and rain-dispersed to the host (Carson, 1999; Ferguson and Carson, 2004). Conidial germination and direct infection can occur in as little as five hours in the presence of free water on the host leaf and a dark environment. Conidia, formed on NLB necrotic lesions under cool and wet conditions, act as secondary inocula and are spread up the

canopy by wind and rain. Latent and incubation periods can vary based on environmental conditions and host susceptibility (Carson, 1995; Thakur et al., 1989; Welz and Geiger, 2000).

Several methods of NLB control are available to producers. Rotations with non-host species and tillage practices, which reduce surface corn residue, can limit initial *E. turcicum* inoculum resulting in reduced disease (Boosalis et al., 1967; Summer et al., 1981; Lipps, 1983; de Nazareno et al., 1993). Single-gene and multi-gene resistance is available and can offer various levels of disease control, though neither form of resistance appears to offer complete control of NLB (Welz and Geiger, 2000). Foliar-applied fungicides also can provide NLB control, and their use has increased in the past decade due to higher corn prices and greater chemical availability (Bradley and Ames, 2010; Munkvold et al., 2008; Wise and Mueller, 2011). Thus far, the majority of foliar-applied fungicides used on corn contain active ingredients from the demethylation inhibitor (DMI, a.k.a. triazole) and quinone outside inhibitor (QoI, a.k.a. strobilurin) chemical groups, either alone or in combination with each other.

DMI fungicides were first introduced in the late 1960s and 1970s (Schwabe et al., 1984). DMI fungicides bind to the cytochrome P450 lanosterol 14α-demethylase (*CYP51*) enzyme, which interferes with ergosterol production in fungi (Köller, 1992; Yoshida, 1993). Fungi cannot develop functional cell membranes without ergosterol leading to reduced mycelial growth and eventual death (Köller, 1992; Ma and Michailides, 2005; Yoshida, 1993). Mycelial growth inhibition leading to death is considered the main mode of action for DMI fungicides; however, metconazole, prothioconazole, and tebuconazole (DMI fungicides) have been shown to inhibit spore germination, as well (Klix et al., 2007). DMI fungicides are systemic within plants and demonstrate translaminar movement and movement through the xylem from the point of application up the plant from the base to the tips of leaves and from the stalk to new growth

(Edgington, 1981). They are unable to move through the phloem and down into older growth, stalk, and roots (Edgington, 1981).

QoI fungicides were first introduced in 1996 (Bartlett et al., 2002). QoI fungicides target the cytochrome *bc1* enzyme complex (complex III) at the quinol outer binding site in the mitochondrial respiration pathway of fungi, disrupting electron transport (Bartlett et al., 2002; Gisi et al., 2002). This halts the energy cycle and interferes with spore germination, specifically, but can also inhibit mycelia growth (Bartlett et al., 2002; Gisi et al., 2002). QoI fungicides vary in their level of systemic mobility within the host (Bartlett et al., 2002). Some QoI fungicides, such as azoxystrobin and picoxystrobin, have translaminar movement and are xylem-systemic, similar to the DMIs (Bartlett et al., 2002; Godwin et al., 1999), while fungicides, such as kresoxim-methyl, trifloxystrobin, and pyraclostrobin, demonstrate translaminar movement only (Ammermann et al., 2000; Ammermann et al., 1992; Bartlett et al., 2002; Margot et al., 1998; Reuvini, 2001).

NLB, caused by *E. turcicum*, has become more prevalent in the U.S. due to increased continuous corn production, reduced-tillage practices, and possible reduction in NLB resistance in production corn hybrids (Mallowa et al., 2015; Wise and Mueller, 2011). DMIs, QoIs, and QoI + DMI fungicide mixtures are labeled for management of NLB on corn. The QoI + DMI formulations often have reduced concentrations of the active ingredients (especially the DMI active ingredients) (Da Costa and Boller, 2008). The objectives of this research were to: i) evaluate NLB control with DMI, QoI, and DMI + QoI fungicides at labeled rates, as well as, DMI and QoI fungicides at the rates contained in the DMI + QoI formulations in the field, and ii) evaluate DMI and QoI fungicide efficacies based on application timing relative to inoculation with *E. turcicum* in the greenhouse.

Materials and Methods

Field experiment. Field trials were conducted at University of Illinois research farms located near DeKalb, Monmouth, and Urbana, IL in 2012, and Dixon Springs and Urbana, IL in 2013. Field corn trials were carried out at all locations using a NLB moderately susceptible hybrid ('Pioneer 33W84', Pioneer Hi-Bred International, Inc., Johnston, IA). At Urbana in 2012 and 2013, the trials also were repeated on a NLB susceptible sweet corn hybrid ('Merit', Seminis Vegetable Seeds, St. Louis, MO). Field corn trials were planted at a population of 84,014 seeds/ha, while sweet corn trials were planted at 64,246 seeds/ha. Field corn and sweet corn trials in Urbana received natural rainfall and were irrigated both years, while the other trials only received natural rainfall. In Urbana trials, starting at approximately the 10 leaf vegetative growth stage and ending at maturity, overhead impact irrigation was applied for 15 minutes every 90 minutes between 10am and 7pm each day to maintain free water on the leaves to promote disease development. Impact sprinklers (model 14VH, Rain Bird Corporation, Azusa, CA) with code 04 nozzle tips (1.59 mm orifice diameter) applied 3.3 L of water per minute at 4.1 bars of pressure. The sprinklers were on 3 m risers spaced on 9.1 m centers and had a spray radius of 9.8 m. Plots were 4 rows wide (76 cm row spacing) and 7.6 meters long. In all trials, all plots were arranged in a randomized complete block design (RCBD) with four replications.

Plants in all trials were inoculated with a *E. turcicum* mycelia and conidia suspension between the 4-leaf and 6-leaf growth stage. Inoculum was prepared following methods described by Pataky et al. (1988), and inoculations were carried out according to Pataky (1992). Isolates of *E. turcicum* race 0 and 1 (provided by Dr. Jerald Pataky, previously at the University of Illinois) were grown on lactose-casein hydrolyslate agar (LCA) at 20° to 25°C with 12 hour

light/dark cycles for 2 to 3 weeks (Tuite, 1969). *Exserohilum turcicum* cultures were then stored at 4°C until plants were at the proper growth stage. Immediately prior to inoculation, *E. turcicum* race 0 and 1 cultures were ground at a 1:1 ratio in a blender with water and filtered through a 125 micron paint strainer to remove large particles of agar and mycelia which could plug the sprayer. Conidia suspensions were prepared separately for each trial when plants were the proper growth stage and ranged in concentration between 1.0×10^4 to 2.0×10^4 conidia per ml water containing Tween 20 (10 µl/L) (Fisher Scientific, Pittsburgh, PA). Approximately 15 mls of conidia suspension was sprayed directly in the whorls of all plants in the trials using a hand pump backpack sprayer. Each trial was inoculated twice within a week to increase the likelihood of infection.

All fungicide treatments were applied at corn silk emergence (reproductive growth stage R1). Fungicide applications were made using a CO_2-pressurized backpack sprayer and boom set at 2.8 bars with 4 TJ-60 8002VS twinjet nozzles (Teejet Technologies, Urbandale, IA) on 50.8 cm centers. The spray volume applied was 281 L/ha to the center 2 rows of each plot. A total of 17 fungicide treatments were tested and a non-treated control was included. Fungicide treatments were comprised of eight active ingredients (a.i.), including four DMI and four QoI fungicides. Products containing both DMI and a QoI active ingredients were applied at labeled rates and included: fluoxastrobin + tebuconazole (Evito T, Arysta LifeScience North America, LLC, Cary, NC); pyraclostrobin + metconazole (Headline AMP, BASF Corp., Research Triangle Park, NC); azoxystrobin + propiconazole (Quilt Xcel, Syngenta Crop Protection, Greenboro, NC); and trifloxystrobin + prothioconazole (Stratego YLD, Bayer CropScience, Research Triangle Park, NC). Products with formulations containing each individual a.i. also were applied at the companies' suggested rates and the a.i. rates present in the DMI + QoI. DMI products

were: metconazole (Caramba, BASF Corp.); tebuconazole (Folicur, Bayer CropScience); propiconazole (Tilt, Syngenta Crop Protection); and prothioconazole (Proline, Bayer CropScience). The QoI fungicides were: fluoxastrobin (Evito, Arysta LifeScience North America, LLC, Cary, NC); trifloxystrobin (Gem, Bayer CropScience); pyraclostrobin (Headline, BASF Corp.); and azoxystrobin (Quadris, Syngenta Crop Protection). The products and application rates are listed in Table 4.1.

NLB disease severity was assessed for the effects of the fungicide treatments and the non-treated control. NLB disease severity was initially scored at the time of fungicide application. Five plants from the center 2 rows were selected arbitrarily and tagged for repeated disease evaluation. NLB severity in field corn trials was rated 5 times after fungicide application, while sweet corn trials were rated only 3 times before harvest. Disease was rated at late R1, R2, R3, R4 and R5 corn reproductive stages for field corn and R1, R2, and R3 corn reproductive stages for sweet corn. In each plot, 5 plants were evaluated by quantifying disease severity (percent leaf area covered with lesions) on the ear leaf, one leaf above the ear leaf, and one leaf below the ear leaf. The mean of the percent leaf lesion area was calculated for each plot (Mallowa et al., 2015). The center 2 rows of plots also were given a percent disease severity score based on total leaf lesion area of all plants in their entirety. Disease ratings were not collected at the R1 growth stage at Dixon Springs in 2013 and the R5 growth stage from DeKalb in 2012.

Corn stalk rot severity, plant maturity, and yield were measured for plots in the field corn trials at all locations across years. To evaluate stalk rot severity, 3 consecutive plants were selected for 1 of the center 2 rows of each plot at growth stage R6 (physiological maturity). The stalk of each plant was split in half from the ear node to the soil surface to assess node and

internode stalk rot disease infection. Stalk rot was scored on a 0 to 5 rating scale, where: 0 = no visible discoloration of the internal stalk nodes or pith; 1 = internal discoloration at the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot (Hines, 2007). QoI fungicides have been shown to preserve green tissue and delay senescence (Byamukama et al., 2013; Wu and Von Tiedemann, 2001). To identify any delays in maturity, plots were rated at approximately R5 to R6 for 'stay green effect' which was scored on a 0 to 9 scale based on visual assessment of the center 2 rows: 0 = a completely mature plant and 9 = a plant with completely green leaves and stalk. Each increasing integer between 0 and 9 represented an approximate 10% increase in green tissue. Sweet corn harvest is prior to plant maturity, therefore, stalk rot and plant maturity were not evaluated for sweet corn trials. The center 2 rows of field corn plots were harvested at maturity using a small plot combine (Kincaid 8-XP, Kincaid Equipment Manufacturing, Haven, KS) and grain yields were standardized to 13% seed moisture. To measure yield for sweet corn, ears were harvested by hand from 10 consecutive plants in one of the center 2 rows of each plot and then weighed to determine the mean ear weight.

Data were analyzed using the mixed models procedure (PROC MIXED) in SAS v. 9.4 (SAS Institute, Cary, NC) to examine the effect of fungicide treatments on disease severity, stalk rot, plant stay green (maturity), and yield. Fungicide was considered a fixed effect, while environment (trial location within year), replications, and the interaction between environment and fungicide were considered random effects. Preliminary analysis of field corn trials indicated variations in disease severity; therefore, environments were separated by NLB disease severity of the non-treated control. Environments where the non-treated control plots had NLB leaf and plot

disease severity means less than 10% and 15%, respectively, at the R5 growth stage were considered to have low disease severity. Environments with greater non-treated control NLB leaf and plot disease severity means were considered to have moderate disease severity. Environments with low disease severity (Dixon Springs, 2013; Monmouth, 2012; and Urbana, 2012) were analyzed separately from environments with moderate disease severity (DeKalb, 2012 and Urbana, 2013). The 2012 and 2013 sweet corn trials were combined but analyzed separately from field corn trials. NLB severity leaf ratings in trials with low disease severity contained zeros in the data set. To meet assumptions of normality and homogeneity of variance, the transformation ($x = \sqrt{(\%\ severity + 0.1)}$) was used for leaf disease ratings collected between R1 and R5 growth stages and square root transformations were used for plot disease ratings taken at R2 and R5 growth stages. Square root transformations were used to meet assumptions of normality and homogeneity of variance for R3 leaf and plot disease ratings, R4 leaf disease ratings, and R5 leaf disease ratings in the analysis of trials with moderate disease severity. For the sweet corn trials, square root transformation was used for leaf disease ratings at the R3 rating time. The test of -2log likelihood was used to compare models to determine if the environment by fungicide interaction could be removed from the model for each dependent variable. The interaction effect of environment and fungicide was removed from the model for all dependent variables except the R5 leaf disease ratings in trials with low disease severity. Fungicide main effect was considered statistically significant at $\alpha = 0.05$. Fungicide treatments were compared and the 'PDMIX800' macro was used to format the pairwise differences from the PDIFF option in the LSMEANS statement in PROC MIXED, creating groups of similar means for fixed effects, designated by letters (Saxton, 1998). Estimate statements were used to group treatments to determine least-squared means (lsmeans) and make comparisons between the non-

treated control, DMI fungicides at high rates, DMI fungicides at low rates, QoI fungicides at high rates, QoI fungicides at low rates, and QoI + DMI fungicides. Groups of similar means for estimates were designated by letters. Terms and estimates were considered significant when $P \leq 0.05$.

Greenhouse experiment. The greenhouse trials were carried out on the same susceptible sweet corn hybrid that was used for the field trial ('Merit', Seminis Vegetable Seeds). Seeds were planted in 3.8-liter plastic pots (Classic 300, Nursery Supplies Inc., Chambersburg, PA) with a steam-pasteurized soil mix (1:1:1 soil:peat:perlite). Plants were irrigated as needed and fertilized with Osmocote Classic 13-13-13 (N-P-K) controlled release fertilizer (The Scott's Company, Marysville, OH) to maintain plant health and growth. The greenhouse was maintained at 21°C ± 3° daytime and 18°C ± 3° nighttime temperatures. Natural light and supplemental lighting, set at 25 to 50 klux (325 to 650 μE m^{-2} s^{-1}), was used with 15 hour daytime and 9 hour nighttime. Each individually potted plant represented a plot.

Exserohilum turcicum race 0 conidia suspensions were prepared immediately prior to plant inoculations, following the method previously described for the field trials. The final concentrations of all conidial suspensions were calculated using a hemocytometer and diluted to 1,000 conidia/ml in water containing Tween 20 (10 μl/L). Prior to inoculation, all plant leaves were marked with a permanent felt tip pen near the base to identify where the conidial suspension was applied. Entire plants were sprayed until runoff using a hand pump sprayer. Following inoculation, plants were placed in a humidity chamber for 24 hours to promote infection. The humidity chamber, constructed in the greenhouse, consisted of an opaque plastic tent containing 2 humidifiers which maintained relative humidity at 100%. Sets of plants (three replicates) for each fungicide tested were inoculated at 3 h, 1, 3, and 7 days prior to fungicide

application and 3 h, 1, 3, and 7 days after fungicide application. Sets of non-treated control plants were inoculated at each timing, as well. The earliest inoculation timing commenced at the corn 4-leaf growth stage (V4).

Fungicide applications were made at the 5-leaf to 6-leaf growth stage (V5 to V6), 7 days after the earliest inoculation timing. Applications were performed using the methods described for the field trials. The base of plant leaves were marked with a permanent felt tip pen prior to fungicide application to identify fungicide treated tissue, similar to when inoculated. Fungicides were applied at labeled rates and included azoxystrobin at 112 g a.i./ha, pyraclostrobin at 112 g a.i./ha, propiconazole at 123 g a.i./ha, and prothioconazole at 202 g a.i./ha.

NLB severity was evaluated 21 days after inoculation. The percent infected leaf area was assessed for each leaf where fungicide was applied. Due to infections being fairly localized to leaves still in the whorl at the time of inoculation, the mean was calculated for the percent infected leaf area of the 2 leaves with the greatest disease severity of each plant.

Trials were pooled for analysis. Data were analyzed using the generalized linear mixed models procedure (PROC GLIMMIX) in SAS v. 9.4 (SAS Institute) to examine the effect of fungicide treatments on percent disease control. Data for each fungicide timing interval were analyzed independently. Fungicide was considered a fixed effect, while replications were consider random. Terms were considered statistically significant when $P \leq 0.05$.

Results

Field Experiment. Table 4.2 summarizes the probability values ($P > F$) of the fixed effect of fungicide in field corn trials with low disease severity, field corn trials with moderate disease severity, and sweet corn trials with moderate disease severity. For trials with low disease severity, fungicide had no significant effect on leaf or plot disease severity, stalk rot, corn stay green (maturity), or yield. For trials with moderate disease severity, fungicide had a significant effect on R2, R3, R4, and R5 leaf and plot disease ratings. For sweet corn trials, fungicide had a significant effect on R2 plot disease severity and R3 plot disease severity. Only significant terms will be discussed.

Trials with moderate disease severity. Significant differences among fungicide treatments were observed in leaf disease severity during the corn R2, R3, R4, and R5 growth stages (Table 4.3). At the R2 rating time, treatments of metconazole at 67 g a.i./ha and prothioconazole at 202 g a.i./ha significantly reduced leaf disease severity compared to the non-treated control. At the R3 rating time, significant treatment differences were observed, but no fungicide treatment was significantly different than non-treated control. At R4 and R5 rating times, treatments with azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha and metconazole at 67 g a.i./ha had significantly lower leaf disease than the non-treated control.

Estimates statements with treatments grouped by fungicide and rate identified significant differences in leaf disease severity at R2, R3, R4, and R5 rating times (Table 4.4). High rate DMIs had significantly lower leaf disease severity than all other treatments except the non-treated control at the R2 rating time. At the R3 rating time, no fungicide group significantly varied from the control; however, high rate DMIs had significantly lower disease than the low

rate QoIs. At the R4 rating time, leaf disease severity for the QoI + DMI fungicides was significantly lower than disease severities from high rate QoI and low rate QoI treatments. At the R5 rating time, disease severities in high rate DMI treatments and QoI + DMI treatments were significantly lower than disease severities in low rate QoI treatments.

Significant differences among fungicide treatments were observed in plot disease severity during the corn R2, R3, R4, and R5 growth stage (Table 4.3). At the R2 rating time, azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha and metconazole at 45 g a.i./ha treatments significantly reduced total plot disease severity compared to the non-treated control. At the R3 rating time, plots treated with azoxystrobin at 112 g a.i./ha, azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha, metconazole at 67 g a.i./ha, propiconazole at 90 g a.i./ha, and propiconazole at 123 g a.i./ha showed significantly less disease severity than the non-treated control. At R4 and R5 rating times, treatments with azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha and metconazole at 67 g a.i./ha had significantly lower leaf disease than the non-treated control, similar to observed leaf disease severity at the same rating times.

Estimates statements for grouped treatments identified significant differences in leaf disease severity at R3, R4, and R5 rating times (Table 4.4). At the R3 rating time, high rate DMI treatments had significantly lower plot disease severity than the non-treated and low rate QoI treatments. At the R4 rating time, high rate DMI treatments had significantly lower plot disease than high rate QoI and low rate QoI treatments. At the R5 rating time, leaf disease severities in the high rate DMI, QoI + DMI, and low rate QoI treatments were significantly lower than severities in the low rate DMI treatments.

Sweet corn trials. Significant differences among fungicide treatments were observed in plot disease severity during the corn R2 and R3 growth stages (Table 4.5). At the R2 rating time, azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha, metconazole at 45 g a.i./ha, metconazole at 67 g a.i./ha, propiconazole at 90 g a.i./ha, propiconazole at 123 g a.i./ha, pyraclostrobin at 112 g a.i./ha, and pyraclostrobin at 112 g a.i./ha + metconazole at 45 g a.i./ha treatments had significantly lower plot disease severity than the non-treated control. At the R3 rating time, treatments of azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha, metconazole at 45 g a.i./ha, metconazole at 67 g a.i./ha, and propiconazole at 123 g a.i./ha showed significantly lower plot disease severity than the non-treated control.

Estimate statements for groups identified significant differences in the plot disease severity at growth stages R2 and R3 in the sweet corn trial (Table 4.6). When plot disease was rated at R2, the high rate DMI treatments, low rate DMI treatments, and QoIs + DMI treatments had significantly less disease than the high rate QoI treatments and non-treated control. The R3 ratings for plot disease severity for high rate DMI treatments and low rate DMI treatments were significantly lower than the high rate QoI treatments, non-treated control, and QoI + DMI treatments.

Greenhouse Experiment. Significant differences among treatments were observed when fungicides were applied 3 days, 1 day and 3 hours prior to inoculations with *E. turcicum*, as well as, 3 days and 7 days after inoculations (Table 4.7). At inoculation times where significant differences were observed, all fungicides had significantly less disease than the non-treated control.

Differences also were observed among fungicides (Table 4.7). When fungicides were applied 3 days prior to inoculation, pyraclostrobin significantly reduced leaf disease compared to prothioconazole. When fungicides were applied 3 hours prior to inoculation, prothioconazole significantly reduced disease compared to propiconazole. When fungicides were applied 7 days after inoculation, propiconazole significantly reduced disease compared to azoxystrobin and prothioconazole.

Discussion

Despite two inoculations with *E. turcicum* at the V4 to V6 growth stage to promote disease development, three of the field corn trials developed low levels of disease, while the other two field corn trials and the sweet corn trials had moderate levels of disease. Similar inconsistencies in NLB disease development following inoculation have been previously reported (Mallowa et al., 2015) and, likely, are the result of differences in environmental conditions.

Fungicides varied in NLB control depending on the level of disease present. In environments with low disease severity, no significant differences in leaf and plot severity were observed between fungicides and the non-treated control. In environments with moderate disease severity and sweet corn trials, significant fungicide effects were observed starting at the R2 growth stage and continuing through the rating period. Variations in fungicide control at different disease severity have been observed. Mallowa et al. (2015) were able to identified reductions in NLB disease severity in fields with low and moderate disease severity when fungicides were applied; however, control was not consistent between fields. Similar results were found in a meta-analysis conducted by Paul et al. (2011).

Azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha and metconazole at 67 g a.i./ha provided significant disease control in field trials with moderate NLB severity and azoxystrobin at 112 g a.i./ha + propiconazole at 90 g a.i./ha, metconazole at 45 g a.i./ha, metconazole at 67 g a.i./ha, and propiconazole at 123 g a.i./ha provided significant control in sweet corn trials. Though not all treatments were significantly different than the non-treated control, DMI fungicides at higher rates (solo application label rates) and QoI + DMI fungicides provided greater NLB control than other treatments, generally. *Exserohilum turcicum* forms large lesions, capable of infecting much of the leaf area with relatively few lesions. It is possible the DMI mode of action is more effective against *E. turcicum* than QoI fungicides, since it primarily limits mycelial growth, which may slow lesion expansion and limit sporulation. In a field corn study, Bowen and Pedersen (1988) found that propiconazole controlled NLB more effectively than mancozeb, which only inhibited spore germination.

Though several fungicides reduced foliar disease significantly, those symptom reductions were insufficient to significantly affect stalk rot, plant maturity, or yield. In this study, stalk rot severity tended to be greater in trials where NLB was more severe. High foliar disease has been associated with increased potential for stalk rot (Dodd, 1980a); however, Mallowa et al. (2015) also observed reductions in foliar disease using fungicides without reducing stalk rot, similar to results observed in this study. QoI fungicide effects on plant maturity appear to be inconsistent, and reports of this phenomenon vary among studies (Blandino et al., 2012; Byamukama et al., 2013). While no significant differences in yield were found in this study, fungicide-treated plots tended to yield more than the non-treated control. Research has proven yield responses to fungicide to be fairly variable, even when disease is reduced (Mallowa et al., 2015; Munkvold and Gorman, 2012; Paul et al., 2011, Shah and Dillard, 2010).

In the greenhouse experiment, fungicides reduced disease when inoculated before and after application. This is similar to results reported by Mueller et al. (2004) who tested fungicide application relative to inoculation of ornamental plant species with rust fungi (*Puccinia* spp.). While inoculation times were not compared, there was some variation in the disease severity on the non-treated plants between inoculation times. No fungicide proved to be consistently more effective than the others at controlling disease across all application times. Mueller et al. (2004) observed reduced disease control with increased time between fungicide application and inoculation; however, that study included longer time intervals between fungicide application and inoculation than used in this experiment. It may have been informative to include inoculation timings greater than 7 days; however, most *E. turcicum* infection took place on new growth near the whorl. Inoculation dates would have been on new tissue where fungicides were not applied. DMI and some QoI fungicides are capable of xylem movement toward leaf tips, while other QoI fungicides only have translaminar movement (Bartlett et al., 2002; Edgington, 1981; Godwin et al., 1999; Vincelli, 2002). Research has been conducted to test if fungicide applications made during early vegetative stages, like those tested in the greenhouse trial, is effective at controlling disease and increasing yield. Blandino et al. (2012) evaluated azoxystrobin + propiconazole applied at several growth stages and did not observe significant difference in disease control between the V4 application and the non-treated control; however, they did report a small but significant yield increase.

Products containing DMI fungicides were more effective in managing NLB than QoI fungicides and could be a better option for producers when disease is moderately severe. DMI and QoI fungicides were able to reduce disease when applied before and after inoculation with *E. turcicum*. Reduction in disease due to fungicides did not result in improved yields. Other

studies also have observed inconsistencies between disease control and yield; however, fungicide applications are more likely to be profitable when foliar disease severity is moderate to high (Mallowa et al., 2015; Paul et al. 2011). Fungicides are a valuable tool in IPM and can be used for control of NLB in corn.

Table 4.1. The fungicide group, commercial product name, active ingredient, and application rates for all field trials.

Fungicide group[a]	Product name	Active ingredient (a.i.)	Product use rate (ml/ha)	Fungicide use rate (g a.i./ha)
DMI	Caramba	metconazole	497	45
DMI	Caramba	metconazole	731	67
DMI	Tilt	propiconazole	205	90
DMI	Tilt	propiconazole	292	123
DMI	Proline	prothioconazole	95	45
DMI	Proline	prothioconazole	417	202
DMI	Folicur	tebuconazole	292	123
QoI	Quadris	azoxystrobin	438	112
QoI	Evito	fluoxastrobin	146	67
QoI	Headline	pyraclostrobin	438	112
QoI	Headline	pyraclostrobin	446	112
QoI	Gem	trifloxystrobin	256	123
QoI	Gem	trifloxystrobin	270	134
QoI + DMI	Quilt Xcel	azoxystrobin + propiconazole	767	112 + 90
QoI + DMI	Evito T	fluoxastrobin + tebuconazole	292	56 + 78
QoI + DMI	Headline AMP	pyraclostrobin + metconazole	731	112 + 45
QoI + DMI	Stratego YLD	trifloxystrobin + prothioconazole	365	134 + 45

[a] Fungicide groups included the demethylation inhibitors (DMI) and the quinone outside inhibitors (QoI).

Table 4.2. Probability values ($P > F$) associated with the main effect of fungicide on northern leaf blight severity at corn reproductive growth stages (R1-R5), stalk rot, plant maturity, and yield for low disease severity, moderate disease severity, and sweet corn trials.

	Northern leaf blight severity (%)												
	Leaf[b]					Plot[c]					Stalk	Plant	
Trial[a]	R1[d]	R2	R3	R4	R5	R1	R2	R3	R4	R5	rot[e]	maturity[f]	Yield[g]
low disease severity	0.3905[hi]	0.4359[h]	0.6470[h]	0.0770[h]	0.2660[h]	0.3950[j]	0.6569[ij]	0.4060[j]	0.4067[j]	0.1499[j]	0.6178	0.1771	0.5165
moderate disease severity	0.1320[k]	0.0472	0.0092[j]	0.0234[j]	0.0260[j]	0.1320[k]	0.0378	0.0106	0.0118	0.0113	0.4357	0.4094	0.2460
sweet corn	0.3074	0.0909	0.1091[j]			0.3074	0.0111	<0.0001					0.5000

[a] Trials grouped for analysis as low disease severity field corn trials (Dixon Springs, 2013; Monmouth, 2012; Urbana 2012), high disease severity field corn trials (DeKalb, 2012; Urbana, 2013), and 2012 and 2013 sweet corn trials for analysis.

[b] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[c] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[d] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

[e] Stalk rot severity assessed on a 0 to 5 scale at plant maturity: 1 = internal discoloration a the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot.

[f] Plant maturity ('stay green') rated on 0 to 9 scale: 0 = completely mature plant and 9 = a plant with completely green leaves and stalk.

[g] Grain yield was calculated as kg/ha at 13% moisture for field corn trials. Mean ear weight (grams) was calculated for ten ears collected from consecutive plants.

[h] Data were transformed to meet assumptions of variance using the formula: $x = \sqrt{(\%\ severity + 0.1)}$.

[i] Data did not include 2013 Dixon Springs disease ratings.

[j] Data were square root transformed to meet assumptions of variance.

[k] Data did not include 2012 DeKalb disease ratings.

Table 4.3. Effects of fungicides on northern leaf blight leaf and plot disease severity observed at the corn growth stages R1 to R5 in field trials with moderate disease severity.

		Northern leaf blight severity (%)									
		Leaf[a]					Plot[b]				
Fungicide	Rate (g a.i./ha)	R1[c]	R2	R3[d]	R4[d]	R5[de]	R1	R2	R3[d]	R4	R5[e]
non-treated		3.6 a[f]	6.5 ab	9.1 abcde	17.7 abcd	28.5 abc	8.0 a	16.8 ab	16.9 a	25.0 ab	42.5 ab
azoxystrobin	112	4.0 a	6 abcd	8.9 bcde	14.7 bcdef	23.5 bcde	9.1 a	13.4 bc	12.4 bcdef	24.8 abc	40.0 abc
fluoxastrobin	67	4.8 a	7.2 a	11.7 ab	19.4 ab	34.3 a	7.9 a	16.6 ab	15.9 abc	27.5 a	46.3 a
metconazole	45	4.6 a	6.8 ab	10.3 abc	17.5 abcd	32.7 ab	8.8 a	11.9 c	14.5 abcde	25.6 ab	42.5 ab
metconazole	67	3.0 a	4.5 cd	6.3 e	12.1 f	17.5 e	7.6 a	14.4 abc	9.9 f	18.1 d	31.3 d
propiconazole	90	4.2 a	5.9 abcd	8.3 cde	16.6 abcde	26.9 abcd	9.3 a	16 ab	11.9 def	23.1 abcd	43.8 ab
propiconazole	123	3.2 a	4.9 bcd	7.3 de	14.3 cdef	22.7 cde	7.9 a	15.6 ab	12.0 cdef	21.3 bcd	40.0 abc
prothioconazole	45	3.2 a	5.1 bcd	9.0 bcde	17.6 abcd	28.9 abc	8.9 a	16.4 ab	15.7 abcd	26.3 ab	45.0 a
prothioconazole	202	2.3 a	4.1 d	7.8 cde	14.4 cdef	28.5 abc	8.3 a	16.4 ab	13.6 abcdef	25.6 ab	42.5 ab
pyraclostrobin	112	3.8 a	6.4 ab	9.4 abcd	15.8 abcdef	25.9 abcd	8.8 a	17.6 a	15.5 abcd	26.3 ab	37.5 bcd
pyraclostrobin	112	3.4 a	5.8 abcd	9.1 abcd	15.4 bcdef	26.6 abcd	8.5 a	15.8 ab	16.0 abc	25.6 ab	37.5 bcd
tebuconazole	123	4.0 a	6.6 ab	10.8 abc	20.8 a	29.7 abc	9.1 a	16.0 ab	15.6 abcd	27.5 a	41.3 abc
trifloxystrobin	123	4.5 a	7.4 a	12.3 a	17.8 abc	29.9 abc	8.9 a	16.9 ab	16.0 ab	28.1 a	41.3 abc
trifloxystrobin	134	3.5 a	6.6 ab	10.5 abc	16.4 abcdef	31.1 abc	7.6 a	16.6 ab	15.0 abcd	25.6 ab	43.8 ab
azoxystrobin + propiconazole	112 + 90	3.7 a	5.7 abcd	6.8 de	12.6 ef	19.1 de	6.6 a	11.8 c	10.8 ef	19.4 cd	35.0 cd
fluoxastrobin + tebuconazole	56 + 78	3.4 a	5.6 abcd	9.6 abcd	15.9 abcdef	27.4 abcd	9.0 a	16.9 ab	15.4 abcd	28.1 a	40.0 abc
pyraclostrobin + metconazole	112 + 45	4.0 a	6.0 abc	9.4 abcd	15 bcdef	24.8 bcde	9.3 a	15.8 ab	14.0 abcde	23.8 abc	41.3 abc
trifloxystrobin + prothioconazole	134 + 45	3.5 a	5.5 abcd	8.7 bcde	13.2 def	23.1 cde	7.9 a	17.0 a	15.4 abcd	24.4 abc	40.0 abc

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

[d] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

Table 4.3 (cont.)

[e] Data did not include 2012 DeKalb disease ratings.

Table 4.3. (cont.)

[f] Values followed by the same letter within a column are not significantly different ($\alpha = 0.05$).

Table 4.4. Least-squared means and comparisons, calculated using estimate statements, of northern leaf blight leaf and plot disease severity for fungicide groups at corn growth stages R1 to R5 in field trials with moderate disease severity.

Treatment groups	Northern leaf blight severity (%)									
	Leaf[a]					Plot[b]				
	R1[c]	R2	R3[d]	R4[d]	R5[de]	R1	R2	R3[d]	R4	R5[e]
non-treated	3.6 a[f]	6.5 ab	9.1 abc	17.7 ab	28.5 ab	8.0 a	16.8 a	16.9 a	25.0 ab	42.5 ab
high rate DMIs[g]	3.1 a	5.0 b	8.0 c	15.3 ab	24.3 b	8.2 a	15.6 a	12.7 b	23.1 b	38.8 b
high rate QoIs[h]	4.0 a	6.6 a	10.1 ab	16.5 a	28.5 ab	8.4 a	16.1 a	14.7 ab	26.0 a	41.9 ab
low rate DMIs[i]	4.0 a	5.9 a	9.2 abc	17.2 a	29.5 a	9.0 a	14.8 a	14.0 ab	25.0 ab	43.8 a
low rate QoIs[j]	3.9 a	6.6 a	10.7 a	16.6 ab	28.2 ab	8.7 a	16.3 a	16.0 a	26.9 a	39.4 b
QoI + DMIs[k]	3.6 a	5.7 a	8.6 bc	14.1 ab	23.5 b	8.2 a	15.3 a	13.8 ab	23.9 ab	39.1b

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

[d] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

[e] Data did not include 2012 DeKalb disease ratings.

[f] Values followed by the same letter within a column are not significantly different ($\alpha = 0.05$).

[g] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[h] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[i] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[j] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[k] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.

Table 4.5. Effects of fungicides on northern leaf blight leaf and plot disease severity observed at different corn growth stages (R1 to R3) in 2012 and 2013 sweet corn field trials conducted at Urbana, IL.

		Northern leaf blight severity (%)					
		Leaf[a]			Plot[b]		
Fungicide	Rate (g a.i./ha)	R1[c]	R2	R3[d]	R1	R2	R3
non-treated		5.4 a[f]	11.6 a	21.0 a	18.3 a	28.1 a	41.3 abcd
azoxystrobin	112	4.1 a	9.3 a	16.2 a	12.5 a	24.6 abcdefg	36.3 cdef
fluoxastrobin	67	6.0 a	10.6 a	17.6 a	14.3 a	26.3 abcde	44.4 a
metconazole	45	5.1 a	8.9 a	13.6 a	17.0 a	21.5 efg	35.0 ef
metconazole	67	5.5 a	8.6 a	13.4 a	16.1 a	23.0 bcdefg	34.4 ef
propiconazole	90	5.7 a	9.5 a	15.4 a	16.8 a	23.0 bcdefg	35.6 def
propiconazole	123	3.8 a	7.3 a	13.0 a	18.0 a	20.1 fg	31.9 f
prothioconazole	45	4.6 a	9.5 a	17.4 a	14.9 a	24.8 abcdefg	36.3 cdef
prothioconazole	202	5.5 a	9.3 a	14.9 a	18.8 a	23.9 abcdefg	36.3 cdef
pyraclostrobin	112	4.7 a	10.9 a	16.6 a	17.6 a	25.9 abcde	38.1 bcde
pyraclostrobin	112	4.0 a	8.7 a	14.4 a	15.4 a	21.8 defg	36.3 cdef
tebuconazole	123	6.2 a	11.3 a	19.4 a	15.1 a	26.8 abcd	41.9 abc
trifloxystrobin	123	6.4 a	12.3 a	19.0 a	14.4 a	28.0 ab	41.3 abcd
trifloxystrobin	134	6.0 a	11.5 a	18.4 a	13.9 a	27.5 abc	42.5 ab
azoxystrobin + propiconazole	112 + 90	4.7 a	8.9 a	13.8 a	16.3 a	19.8 g	31.9 f
fluoxastrobin + tebuconazole	56 + 78	5.8 a	9.6 a	16.0 a	14.9 a	26.4 abcde	42.5 ab
pyraclostrobin + metconazole	112 + 45	5.8 a	10.8 a	17.9 a	15.8 a	22.9 cdefg	39.4 abcde
trifloxystrobin + prothioconazole	134 + 45	5.3 a	10.4 a	16.2 a	15.5 a	25.1 abcdef	42.5 ab

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

[d] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

[e] Data did not include 2012 DeKalb disease ratings.

[f] Values followed by the same letter within a column are not significantly different ($\alpha = 0.05$).

Table 4.6. Least-squared means and comparisons, calculated using estimate statements, of northern leaf bight leaf and plot disease severity for fungicide groups at corn R1 to R3 growth stages, in 2012 and 2013 sweet corn trials.

	Northern leaf blight severity (%)					
	Leaf[a]			Plot[b]		
Treatment group	R1[c]	R2	R3[d]	R1	R2	R3
non-treated	5.4 a[e]	11.6 a	21.0 a	18.3 a	28.1 a	41.3 a
high rate DMIs[f]	5.2 a	9.1 a	15.1 a	16.4 a	23.4 b	36.1 b
high rate QoIs[g]	5.2 a	10.6 a	17.2 a	15.6 a	26.1 a	40.3 a
low rate DMIs[h]	5.1 a	9.3 a	15.4 a	15.4 a	23.1 b	35.6 b
low rate QoIs[i]	5.2 a	10.5 a	16.6 a	16.3 a	24.9 ab	38.8 ab
QoI + DMIs[j]	5.4 a	9.9 a	15.9 a	15.0 a	23.5 b	39.1 a

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, and R3 = kernel milk.

[d] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

[e] Values followed by the same letter within a column are not significantly different from each other ($\alpha = 0.05$).

[f] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[g] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[h] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[i] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[j] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.

Table 4.7. Effect of fungicides applied at different times, relative to *E. turcicum* inoculation, on northern leaf blight severity scored 21 days after inoculation in greenhouse trials.

		Northern leaf blight severity (%)[a]							
	Rate	Fungicide applied before inoculation				Fungicide applied after inoculation			
Fungicide	(g a.i. /ha)	7days	3 days	1 days	3 hours	3 hours	1 day	3 days	7 days
non-treated		14.6 a[b]	24.1 a	13.4 a	12.1 a	14.8 a	20.5 a	27.3 a	28.9 a
azoxystrobin	112	3.8 a	7.9 cb	2.9 b	3.1 cb	2.2 a	6.9 a	12.5 b	13.5 b
propiconazole	123	5.8 a	10.6 cb	3.4 b	5.1 b	2.8 a	5.2 a	5.9 b	8.8 c
prothioconazole	202	5.6 a	11.7 b	5.6 b	1.4 c	4.4 a	7.8 a	9.0 b	13.7 b
pyraclostrobin	112	6.0 a	7.0 c	2.7 b	1.9 cb	1.4 a	7.0 a	8.1 b	11.9 cb
P value[c]		0.1261	<0.0001	0.0016	<0.0001	0.1343	0.0608	0.0009	<0.0001

[a] Northern leaf blight disease severity was the mean of the % infected leaf area of the two most diseased leaves.

[b] Values followed by the same letter within a column are not significantly different from each other at alpha = 0.05.

[c] Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

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APPENDIX A

Table A.1. Effects of fungicides on northern leaf blight leaf and plot disease severity observed at the corn R1 to R5 growth stages in trials with low disease severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

		Northern leaf blight severity (%)									
		Leaf[a]					Plot[b]				
Fungicide	Rate (g a.i./ha)	R1[cef]	R2[e]	R3[e]	R4[e]	R5[e]	R1[g]	R2[g]	R3[g]	R4[g]	R5[g]
non-treated		0.2	0.3	0.4	1.0	2.5	3.8	5.0	5.3	7	6.1
azoxystrobin	112	0.3	0.5	0.7	1.4	3.3	3.3	4.8	5.3	7.2	4.5
fluoxastrobin	67	0.0	0.2	0.3	0.9	2.4	3.6	4.9	4.8	7.3	5.3
metconazole	45	0.1	0.3	0.5	1.0	1.6	3.4	4.2	4.6	6.6	4.7
metconazole	67	0.1	0.2	0.3	0.6	1.5	3.1	3.6	4.5	5.6	4.0
propiconazole	90	0.1	0.2	0.3	0.6	1.3	2.9	3.6	4.6	6.3	4.8
propiconazole	123	0.2	0.4	0.6	1.1	1.7	3.5	4.7	4.9	6.7	4.7
prothioconazole	45	0.1	0.3	0.5	1.2	2.6	3.3	4.0	4.1	7.6	5.0
prothioconazole	202	0.1	0.2	0.3	0.8	1.8	3.1	4.2	4.2	7.0	4.2
pyraclostrobin	112	0.1	0.3	0.5	1.1	2.4	3.1	4.4	4.6	6.7	4.5
pyraclostrobin	112	0.2	0.2	0.4	1.0	1.8	3.9	4.6	5.1	6.8	5.4
tebuconazole	123	0.1	0.2	0.4	1.0	2.2	3.8	4.5	4.4	7.4	5.9
trifloxystrobin	123	0.2	0.4	0.7	1.6	2.7	3.3	5.2	6.2	7.9	5.6
trifloxystrobin	134	0.1	0.3	0.6	1.4	2.9	3.1	4.3	5.8	7.3	6.1
azoxystrobin + propiconazole	112 + 90	0.1	0.2	0.3	0.7	1.3	2.6	4.9	4.9	6.6	4.7
fluoxastrobin + tebuconazole	56 + 78	0.1	0.2	0.4	0.9	2.4	3.1	4.2	4.5	6.5	4.4
pyraclostrobin + metconazole	112 + 45	0.2	0.2	0.4	1.0	1.9	3.4	4.6	4.7	7.8	5.5
trifloxystrobin + prothioconazole	134 + 45	0.0	0.2	0.2	0.7	1.8	3.3	4.1	4.0	6.1	4.2

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

Table A.1 (cont.)

[e] Data were transformed to meet assumptions of variance using the formula: $x = \sqrt{(\%\ severity + 0.1)}$. Data shown are the back transformed least-squared means.

[f] Data did not include 2013 Dixon Springs disease ratings.

[g] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

Table A.2. Least-squared means and comparisons, calculated using estimate statements, of northern leaf blight leaf and plot disease severity for fungicide groups at corn growth stages R2 to R5 in field trials with low northern leaf blight severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

	Northern leaf blight severity (%)									
	Leaf[a]					Plot[b]				
Treatment groups	R1[cde]	R2[d]	R3[d]	R4[d]	R5[d]	R1[f]	R2[f]	R3[f]	R4[f]	R5[f]
non-treated	0.2	0.3	0.4	1.0	2.5	3.8	5.0	5.3	7.0	6.1
high rate DMIs[g]	0.1	0.2	0.4	0.9	1.8	3.4	4.2	4.5	6.7	4.7
high rate QoIs[h]	0.1	0.3	0.5	1.2	2.8	3.3	4.6	5.1	7.1	5.1
low rate DMIs[i]	0.1	0.3	0.4	0.9	1.8	3.2	3.9	4.4	6.8	4.8
low rate QoIs[j]	0.2	0.3	0.5	1.3	2.2	3.6	4.9	5.6	7.3	5.5
QoI + DMIs[k]	0.1	0.2	0.4	0.8	1.8	3.1	4.5	4.5	6.8	4.7

[a] Mean percent infected leaf area of the ear leaf, leaf below the ear, and leaf above the ear estimated for 5 plants per plot.

[b] Percent of total plant leaf area infected estimated for all plants in the center two rows of plots.

[c] Approximate reproductive growth stage at which disease severity was assessed: R1 = silk emergence, R2 = kernel blister, R3 = kernel milk, R4 = kernel dough, and R5 = kernel.

[d] Data were transformed to meet assumptions of variance using the formula: $x = \sqrt{(\%\ severity + 0.1)}$. Data shown are the back transformed least-squared means.

[e] Data did not include 2013 Dixon Springs disease ratings.

[f] Data were square root transformed to meet assumptions of variance. Data shown are the back transformed least-squared means.

[g] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[h] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[i] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[j] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[k] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.

Table A.3. Effects of fungicides on stalk rot severity, plant maturity, and yield in trials with low disease severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Fungicide	Rate (g a.i./ha)	Stalk rot[a]	Plant maturity[b]	Yield (kg/ha)[c]
non-treated		0.5	5.3	11,557
azoxystrobin	112	0.3	5	11,909
fluoxastrobin	67	0.5	5.6	12,245
metconazole	45	0.6	5.3	12,115
metconazole	67	0.3	5.3	11,915
propiconazole	90	0.6	5.8	11,813
propiconazole	123	0.5	5.6	12,044
prothioconazole	45	0.6	5.3	11,843
prothioconazole	202	0.5	5.3	12,326
pyraclostrobin	112	0.5	6.1	12,488
pyraclostrobin	112	0.2	6.1	12,316
tebuconazole	123	0.2	5.4	12,178
trifloxystrobin	123	0.3	5.3	12,154
trifloxystrobin	134	0.7	5.3	11,846
azoxystrobin + propiconazole	112 + 90	0.3	5.8	11,630
fluoxastrobin + tebuconazole	56 + 78	0.3	5.3	12,124
pyraclostrobin + metconazole	112 + 45	0.6	5.5	12,124
trifloxystrobin + prothioconazole	134 + 45	0.6	5.3	11,999

[a] Stalk rot severity assessed on a 0 to 5 scale at plant maturity: 1 = internal discoloration at the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot.

[b] Plant maturity ('stay green') rated on 0 to 9 scale: 0 = completely mature plant and 9 = a plant with completely green leaves and stalk.

[c] Grain yield was calculated at 13% moisture.

Table A.4. Least-squared means and comparisons, calculated using estimate statements, of stalk rot, plant maturity, and grain yield in trials with low disease severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Treatment groups	Stalk rot[a]	Plant maturity[b]	Yield (kg/ha)[c]
non-treated	0.5	5.3	11,557
high rate DMIs[d]	0.4	5.4	12,115
high rate QoIs[e]	0.5	5.5	12,122
low rate DMIs[f]	0.6	5.4	11,924
low rate QoIs[g]	0.2	5.7	12,235
QoI + DMIs[h]	0.4	5.5	11,969

[a] Stalk rot severity assessed on a 0 to 5 scale at plant maturity: 1 = internal discoloration at the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot.

[b] Plant maturity ('stay green') rated on 0 to 9 scale: 0 = completely mature plant and 9 = a plant with completely green leaves and stalk.

[c] Grain yield was calculated at 13% moisture.

[d] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[e] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[f] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[g] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[h] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.

Table A.5. Effects of fungicides on stalk rot severity, plant maturity, and grain yield in trials with moderate northern leaf blight severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Fungicide	Rate (g a.i./ha)	Stalk rot[a]	Plant maturity[b]	Yield (kg/ha)[c]
non-treated		2.0	5.9	9,750
azoxystrobin	112	2.1	5.5	10,551
Fluoxastrobin	67	2.0	5.6	10,410
Metconazole	45	2.4	5.1	9,907
Metconazole	67	2.2	5.1	10,800
propiconazole	90	1.8	5.6	10,396
propiconazole	123	2.0	5.6	10,440
prothioconazole	45	2.2	5.3	10,024
prothioconazole	202	2.0	5.8	9,869
pyraclostrobin	112	0.9	6.1	10,336
pyraclostrobin	112	2.0	5.9	10,806
tebuconazole	123	2.3	5.0	10,461
trifloxystrobin	123	1.7	5.5	10,367
trifloxystrobin	134	2.2	4.6	9,885
azoxystrobin + propiconazole	112 + 90	1.6	5.8	11,149
fluoxastrobin + tebuconazole	56 + 78	1.9	5.1	10,178
pyraclostrobin + metconazole	112 + 45	2.3	6.4	10,885
trifloxystrobin + prothioconazole	134 + 45	2.2	6.1	11,065

[a] Stalk rot severity assessed on a 0 to 5 scale at plant maturity: 1 = internal discoloration at the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot.

[b] Plant maturity ('stay green') rated on 0 to 9 scale: 0 = completely mature plant and 9 = a plant with completely green leaves and stalk.

[c] Grain yield was calculated at 13% moisture.

Table A.6. Least-squared means and comparisons, calculated using estimate statements, of stalk rot, plant maturity and grain yield in field trials with moderate northern leaf blight severity. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Treatment groups	Stalk rot[a]	Plant maturity[b]	Yield (kg/ha)[c]
non-treated	2.0	5.9	9,750
high rate DMIs[d]	2.1	5.4	10,392
high rate QoIs[e]	1.8	5.5	10,296
low rate DMIs[f]	2.1	5.3	10,109
low rate QoIs[g]	1.9	5.7	10,587
QoI + DMIs[h]	2.0	5.8	10,819

[a] Stalk rot severity assessed on a 0 to 5 scale at plant maturity: 1 = internal discoloration at the stalk nodes; 2 = internal discoloration at the stalk nodes and in the pith; 3 = pith separation from the rind; 4 = complete discoloration and decay of the pith between 2 or more nodes, but not lodged; and 5 = stalk is lodged due to stalk rot.

[b] Plant maturity ('stay green') rated on 0 to 9 scale: 0 = completely mature plant and 9 = a plant with completely green leaves and stalk.

[c] Grain yield was calculated at 13% moisture.

[d] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[e] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[f] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[g] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[h] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.

Table A.7. Effects of fungicides on sweet corn ear weight in 2012 and 2013 field trials conducted in Urbana, IL. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Fungicide	Rate (g a.i./ha)	Ear weight (g)[a]
non-treated		206
azoxystrobin	112	222
Fluoxastrobin	67	227
Metconazole	45	236
Metconazole	67	225
propiconazole	90	208
propiconazole	123	218
prothioconazole	45	213
prothioconazole	202	221
pyraclostrobin	112	208
pyraclostrobin	112	230
tebuconazole	123	198
trifloxystrobin	123	206
trifloxystrobin	134	216
azoxystrobin + propiconazole	112 + 90	210
fluoxastrobin + tebuconazole	56 + 78	210
pyraclostrobin + metconazole	112 + 45	200
trifloxystrobin + prothioconazole	134 + 45	237

[a] Mean ear weight was calculated for ten ears collected from consecutive plants in one of the center two rows of each plot.

Table A.8. Least-squared means and comparisons, calculated using estimate statements, of corn ear weight in 2012 and 2013 sweet corn trials. Treatment effects were not significant ($P \leq 0.05$) according to the F-test.

Treatment groups	Ear weight (g)[a]
non-treated	206
high rate DMIs[b]	211
high rate QoIs[c]	222
low rate DMIs[d]	221
low rate QoIs[e]	204
QoI + DMIs[f]	220

[a] Mean ear weight was calculated for ten ears collected from consecutive plants.

[b] Treatments included in the group are metconazole at 67 g a.i./ha, propiconazole at 123 g a.i./ha, prothioconazole at 202 g a.i./ha, and tebuconazole at 123 g a.i./ha.

[c] Treatments included in the group are azoxystrobin at 112 g a.i./ha, fluoxastrobin at 67 g a.i./ha, pyraclostrobin at 112 g a.i./ha., and trifloxystrobin at 134 g a.i./ha.

[d] Treatments included in the group are metconazole at 45 g a.i./ha, propiconazole at 90 g a.i./ha, and prothioconazole at 45 g a.i./ha.

[e] Treatments included in the group are pyraclostrobin at 112 g a.i./ha and trifloxystrobin at 123 g a.i./ha.

[f] Treatments included in the group are azoxystrobin at 112 g a.i./ha + propiconazole 90 g a.i./ha, trifloxystrobin 134 g a.i./ha + prothioconazole 45 g a.i./ha, pyraclostrobin 112 g a.i./ha + metconazole 45 g a.i./ha, fluoxastrobin 56 g a.i./ha + tebuconazole 78 g a.i./ha.