

# INTEGRATED MANAGEMENT OF SOUTHERN CORN RUST AND NORTHERN CORN LEAF BLIGHT USING HYBRIDS AND FUNGICIDES

by

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(Under the Direction of Robert C. Kemmerait, Jr.)

## ABSTRACT

Southern corn rust (SCR) caused by *Puccinia polysora* and northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* are important foliar diseases of corn in the southern United States. Field experiments were conducted to determine the effect of hybrid, fungicide and timing of fungicide application on NCLB and SCR epidemics and corn yield. The *Rpp9*-virulent and *Rpp9*-avirulent races of *P. polysora* were characterized in the field. Onset of SCR in Pioneer 33M52 was delayed in early-planted trials but not in later-planted trials. Area under the disease progress curves (AUDPC) for SCR were lower and yields were higher in Pioneer 33M52 than in Pioneer 33M57 when this disease was severe. Fungicides were usually most effective when applied near disease onset. When both diseases were severe, multiple fungicide applications improved disease management and yield. In vitro sensitivity assays indicated a range of EC<sub>50</sub> values from 0.008 to 0.155 µg/ml. These results can be used to further develop management guidelines for SCR and NCLB.

INDEX WORDS: Southern corn rust, *Puccinia polysora*, *Rpp9*-virulent race, northern corn leaf blight, *Exserohilum turcicum*, pyraclostrobin, metconazole, fluxapyroxad, fungicide timing, area under the disease progress curve, severity, incidence, necrosis, yield, fungicide sensitivity

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## DEDICATION

To my parents, Leopoldo and Rosalia, I cannot thank you enough for your selfless love.

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## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

#### **Purpose and Significance of the study**

Southern corn rust (SCR) caused by *Puccinia polysora* and northern leaf blight (NCLB) caused by *Exserohilum turcicum* are foliar diseases that can potentially cause severe yield losses for growers. In Georgia, SCR is a significant problem to growers in some years. Also, a new *Rpp9*-virulent race of *P. polysora* was confirmed in 2008 (14). Field characterization of the new *Rpp9*-virulent race of *P. polysora* in the field is crucial to determine if previously-resistant hybrids can still provide protection to SCR. Epidemics of NCLB have become severe in the years since 2008 (31).

Management of SCR and NCLB requires integrated use of resistant hybrids, tillage practices, crop rotation with a non-host and chemical control (14). The use of fungicides may be needed when SCR, NCLB or southern corn leaf blight (SCLB) is present in the field. Fungicides were rarely used in the past to control corn foliar diseases but have become popular because of high market demands for corn and because of damage losses incurred by diseases (6). A number of quinone outside inhibitor (QoI), demethylation inhibitor (DMI) and QOI-DMI premix fungicides are registered to manage foliar diseases of corn. Additionally, a new product which is a pre-mix of a QoI and a succinate dehydrogenase inhibitor (SDHI) was recently labeled for use.

Efficacy of fungicides for managing diseases depends on timing of application, host growth stage, level of host resistance, and environmental conditions. Yield benefits can be achieved when registered fungicides are judiciously applied at the time of disease onset. Multiple

applications and combination of different chemistries may be necessary when the environment is conducive for disease development. Initiation of a fungicide program typically has been recommended between early tassel (VT) and blister (R2) growth stages when many foliar diseases seem to develop (37). Fungicide application at the vegetative stages should be evaluated to determine the impact of such treatments to disease epidemics and corn yield. Due to large-scale use of fungicides, it is also important to monitor fungicide resistance of *E. turcicum*.

The overall objectives of this study were to characterize the *Rpp9*-avirulent and *Rpp9*-virulent races of *P. polysora* in the field and to assess the impact of integration of corn hybrids and fungicide programs on the management of SCR and NCLB.

## **Literature review**

Corn (*Zea mays* L.) is one of the most domesticated of all field crops and is considered as one of the three most important grain crops in the world along with rice and wheat. Corn is an important source for food, feed, fiber and by-products such as oil, syrup, biofuel and alcohol (35). In 2011 and 2012, the United States produced a total of 314,162 million kilograms and contributed 44.4% of the world corn exports (38). Since the 1990's, at least 141,640 hectares has been planted to corn in Georgia annually. In 2011, the Georgia Farm Gate Value for corn was \$319,538,540 contributing 2.47% to the total agricultural commodity value for the state (2).

Numerous plant diseases can affect all plant parts and at different growth stages of corn resulting in poor quality and yield loss. Optimal yield is not always possible to attain because several corn diseases may occur every field season. Aside from diseases, abiotic factors and other plant pests such as insects and weeds also contribute to yield reduction. The reported annual disease loss for corn in the United States is approximately 2 to 15 % (62). Among foliar



diseases of corn in Georgia, SCR and NCLB are the most destructive. In 2010, SCR and NCLB caused a combined damage and cost of control of 8.3 and 2.3 million dollars, respectively (64).

#### *Southern corn rust*

Southern corn rust (SCR) is a potentially devastating foliar disease caused by *Puccinia polysora* Underwood. The pathogen is an obligate, microcyclic biotroph where only the uredinial and telial spore states have been reported. Other spore states and alternate hosts are not known. Urediniospores are yellowish to golden and oblong to ellipsoid. Walls are thin and lightly echinulate with symmetrical four to five equatorial pores (8). Smaller flattened urediniospores with two pores on each side have been observed in Cuba, Jamaica, Philippines and Christmas Islands (7). Teliospores are chestnut brown, angular and ellipsoid or oblong, two celled and borne on short pedicels. Symptoms are bright orange, circular to oval pustules scattered largely on the upper leaf surface. At maturity, teliospores are produced in horseshoe or circular patterns around the pustules. Infected leaves of severely damaged plants turn prematurely yellow and dry. Husk leaves, ear shanks and stalks can also be infected. Disease severity gradually increases as the plant reaches maturity (30, 62).

Southern corn rust (SCR) predominates in tropical and subtropical regions but can spread to temperate regions under suitable environmental conditions (62). The disease is favored by high temperature, high relative humidity, and heavy rainfall. Disease development is optimum at 23° to 28°C. The urediniospores penetrate the leaf of the susceptible corn hybrids through the stomates. Symptoms are observed three to six days after infection and yellow spots can be seen on the upper surface of the leaf. The host epidermis may rupture and expose the mature urediniospores seven to 10 days after infection (44). Urediniospores serve as both the primary and secondary inoculum. During the growing season, urediniospores from previously infected

corn plants are carried by the wind and then infect newly-planted plants. These urediniospores are blown northward in the mid-summer. In the United States, SCR is reintroduced to the corn belt every season from Mexico, the Caribbean region and Central America (30, 62).

Yield losses are significant when the disease appears early in the growing season or if corn is planted late during the growing season. Critical yield losses have been observed when infection occurred by early grain fill stages (15, 57). Historic outbreaks of SCR occurred in Africa in 1949 and yield losses of approximately 50% were reported (7, 25, 51). Yield losses of 80 to 84% were observed on susceptible cultivars in the Philippines in 1953 (50) and substantial yield losses of 42 to 53% were first reported in northern China in 1998 (13). In the United States, the disease prevails in the southeastern states, lower Mississippi Valley and Texas but can occasionally spread up to the northwestern states (14). Severe epidemics in the 1970s were associated with a change in cropping practice. To meet the increasing market demands, growers in the lower Mississippi valley planted two crops during a growing season and used early maturing hybrids from the Corn Belt states. These highly susceptible hybrids produced inoculum that could spread and infect later planted corn (15). Severe losses of up to 50% were reported from all inoculated and naturally-infected field trials (36). Losses of up to 45% have been reported in near isogenic resistant and susceptible hybrids due to reduced size and number of kernels. The authors suggested that these hybrids would have been killed prior to grain fill stage had they been inoculated at the seven- to eight-leaf stages (V7-V8) (53). Losses to SCR were reported to be 18 and 39% in inoculated field trials conducted in Pennsylvania and Maryland, respectively (45). In Florida, yields of corn planted in a double-cropped field were reduced by up to 45% due to SCR (44).

The most efficient method to manage SCR is the use of resistant varieties (12). Eleven single, dominant, resistance genes (*Rpp* genes) have been designated. Host genotypes that contain *Rpp* genes express hypersensitive, chlorotic fleck reactions when infected by corresponding avirulent race types of *P. polysora* (26). Likewise, ten races of *P. polysora* have been identified according to their differential reactions to various corn lines. Three races (EA1, EA2 and EA3) were found in East Africa. Six races (PP.3, PP.4, PP.5, PP6, PP.7 and PP.8) were identified from 11 isolates differentiated on 11 corn lines that carried corresponding resistance genes *Rpp3* to *Rpp8* (52). The *Rpp1* gene was identified in corn line AFRO 29 ('Colombia 2') and *Rpp2* in AFRO 24 ('SLP 20-4A'). The *Rpp1* gene confers complete resistance to EA1 and *Rpp2* conditions intermediate reaction to EA1 and EA2 races. The *Rpp10* gene in AFRO 761 ('Andaqui') confers complete resistance to EA1 and EA3 races. The *Rpp11* gene confers partial resistance to EA1 and EA3 races of *P. polysora* (55, 57). Ullstrup (59) identified the *Rpp9* gene from a South African corn cultivar Boesman yellow flint (PI 186208) which conferred resistance to PP.9. The *Rpp9* gene also confers resistance to EA2 and PP.5 (36, 59). Unfortunately, the host differential set used for race identification was lost (14). Moreover, most of these *Rpp* genes were not available to corn hybrids for use in temperate regions (12).

*Puccinia polysora* is known to have multiple races that can overcome single, dominant resistance genes (30). However, there are few recent studies that focus on identifying phenotypic virulence and genetic diversity of *P. polysora*. Casela and Ferreira (10) assessed the virulence pattern of 60 *P. polysora* isolates collected from six locations in Brazil. Seventeen virulence patterns were identified by inoculating the isolates in test hybrids. Most of the virulence patterns identified were found in all locations suggesting that there was no geographical grouping among predominant *P. polysora* populations. Unartngam et al. (60) recently analyzed genetic diversity

of 38 isolates from eight provinces in Thailand using inter-simple sequence repeat (ISSR) markers. Populations were distributed into 13 groups that were not geographically differentiated, presumably due to migration of urediniospores.

Utilization of the *Rpp9* gene and other sources of Rpp resistance have been successful for management of SCR in the United States for more than two decades (41). However, an *Rpp9*-virulent race of *P. polysora* was identified in Georgia in 2008. Dolezal et al. (14) determined infection types of *Rpp*-hybrids based on a modified cereal rust scale of 0 to 4 where 0 is highly resistant and 4 is susceptible. Reactions of corn lines Oh43 *Rpp9* and W64a *Rpp9* were scored resistant to moderately resistant while lines Pioneer 33M52 and Va59 were scored susceptible when inoculated with an isolate from Macon County, GA. Subsequently, Pataky et al. (41) evaluated the differential reactions of corn lines carrying the *Rpp9* gene. Seven lines (PI 186208, PI 186215, Green Giant 1, Green Giant 2, Pioneer 33M52, B1138T and NC 300) were susceptible and three lines (B37 *Rpp9*, Oh43 *Rpp9* and W64a *Rpp9*) were resistant to moderately resistant to the Georgia isolate. The authors suggested that resistance of some lines was due to the presence of more than one resistance gene or that *Rpp9* is located near the complex *Rp1* region. Several additional *Rpp* resistance sources were also mapped on the same chromosome or identified as allelic to *Rpp9* (13, 16, 26, 54). Most sources of resistance have been from sweet or dent corn genotypes since these types of corn have higher market values. Pioneer 33M52 is the only known cultivar of field corn carrying the *Rpp9* gene for SCR resistance.

Early detection of SCR is crucial for effective SCR management, especially when the *Rpp9*-virulent race is present because previously resistant hybrids may need to be protected with fungicides. An annual sentinel plot monitoring program was initiated in Georgia in 2009 to detect reintroduction of the pathogen and to enable growers to better time applications of protective

fungicides. Corn sentinel plots planted with Pioneer 33M52 and Pioneer 33M57 have been established annually since 2009 early in the growing season at 16 locations in Georgia. Each plot was monitored for development of SCR on a weekly basis from vegetative stage until late in the maturity of the corn crop. Data for sentinel plots were uploaded to the National Corn Rust Monitoring website (<http://scr.ipmpipe.org/cgi-bin/sbr/public.cgi>). Based upon the sentinel monitoring data, southern rust was much more widespread and the *Rpp9*-virulent race was also more abundant in 2010 than in years 2009 and 2011. Moreover, southern rust was detected earlier in 2010 than in either other year (36).

The use of fungicides to manage SCR in Georgia has been recommended since 2004 (31). This was in response to the serious damages inflicted by severe outbreaks of SCR in 2003 (63). Fungicide applications are thought to be effective until the crop reaches the dough stage (R4), at which time the crop is deemed safe from southern rust (31). In most cases, an application of fungicides at the tasselling (VT) stage was effective (31) to manage SCR and protect yields. Susceptible hybrids must be protected with fungicides, especially when the environment is favorable for disease development.

In field corn, several systemic fungicides have been tested that significantly reduced rust severity compared to non-fungicide treated controls. However, only pyraclostrobin (Headline, BASF Corporation, Research Triangle Park, NC), pyraclostrobin + metconazole (Headline AMP, BASF Corporation, Research Triangle Park, NC) and combined applications of azoxystrobin and azoxystrobin + propiconazole (Quilt and Quadris, respectively, Syngenta Crop Protection, Greensboro, NC) increased yields among all fungicides tested (1, 21, 32). The value of fungicide application to the previously rust-resistant hybrid P33M52 was also evaluated in Georgia and Alabama during the 2010 growing season. Overall rust severity was significantly reduced by

fungicide applications but yields were not significantly different from the non-fungicide treated control (3, 22-24).

#### *Northern corn leaf blight*

Northern corn leaf blight (NCLB) is caused by *Exserohilum turcicum* (Pass.) K.J. Leonard and Suggs. (syn. *Setosphaeria turcica*), formerly *Helminthosporium turcicum* (Luttr.). *Exserohilum turcicum* produces olive gray, spindle shaped conidia ( $20 \times 105 \mu\text{m}$ ) with three to eight septae and a distinct protruding hilum. The sexual structure, a pseudothecium, has been observed but only under laboratory conditions. Symptoms of the disease are long, grayish-green to tan, cigar-shaped lesions (3 to 15 cm long) on the foliage. Gray sporulation is produced on lesions, predominantly on the undersides of infected leaves (62).

The fungus overwinters as mycelia and conidia on crop residues. Conidia are carried by rain-splash or wind from crop debris onto new corn leaves. Optimum conditions for conidial germination are temperatures of 18 to 27°C and continuous leaf wetness for 6-18 hours. Under favorable conditions, secondary infection by conidia occurs in fields. Symptoms appear seven to 14 days after infection and spores can be produced in seven days. Lesions first appear on the lower leaves then disease progresses to the upper canopy. As the disease progresses, lesions become larger and eventually coalesce covering the entire leaf (62).

Northern corn leaf blight (NCLB) occurs in humid corn-growing areas of the world. In the United States, the disease can be destructive, especially in the midwestern states and Florida (62). In Georgia, the disease has become a serious problem for growers since 2008 (31). Yield losses can reach up to 70% when the disease becomes severe two to three weeks after pollination (58). Under favorable environmental conditions, yield losses increased as disease measured as the area under the disease progress curve (AUDPC) increased. Moreover, yield losses were

reported when disease symptoms were observed at the upper third of the canopy early during the grain-fill period or when disease severity exceeded 20% of the total leaf area. (40, 43, 48).

Resistant cultivars are primarily used to control NCLB. Single, dominant genes are designated as *Ht* (for *Helminthosporium turcicum*) genes (*Ht1*, *Ht2*, *Ht3* and *HtN*). Resistance conferred by *Ht1*, *Ht2* and *Ht3* is expressed as chlorotic lesions (19, 27-29). Meanwhile, *HtN* prolongs latent period and reduces sporulation of the fungus (47). However, virulent races of *E. turcicum* for each of the *Ht* genes have been identified and combinations of virulence have been observed (62). Names of the four races correspond to their virulence to *Ht* genes. Race 0 is avirulent on genotypes with all *Ht* genes while Race 1 is virulent on genotypes with *Ht1* gene. Race 23 is virulent on genotypes with both *Ht2* and *Ht3* while Race 23N is virulent to *Ht2*, *Ht3* and *HtN* (33, 34, 56). A single, recessive gene designated as *ht4* confers a chlorotic halo reaction (9). Both major genes and partial resistance can be combined for disease control, but identifying partial resistance has been prioritized due to practical limitations of *Ht* genes (34, 39, 40, 42). Quantitative trait loci (QTLs) for NCLB resistance have been mapped from both small and long arms of all ten chromosomes of *Z. mays* (61). However, high disease severities have been also observed in many partially resistant hybrids during extreme environmental conditions (45, 46). Thus, relying solely on host resistance to control of northern blight is challenging due to the presence of the four races of *E. turcicum* (40).

Application of fungicides is another way to manage NCLB. Broad-spectrum protectant fungicide with active ingredients such as chlorothalonil, maneb and mancozeb were commonly applied on or before disease onset for effective disease management. However, these fungicides had to be applied frequently and could be phytotoxic to corn. Systemic fungicides were necessary to prevent high levels of disease when conditions were favorable for disease and were

more cost-effective for growers. Propiconazole was reported to have longer effective period and significantly increased yields compared to mancozeb (5). Traditionally, only sweet corn has been frequently treated with fungicides due to high market value of this crop (5, 46). Fungicides were rarely applied to hybrid corn but have become more common due to several factors such as higher yield potentials (6). Fungicide applications are recommended when the disease affects corn early in the reproductive stage. The benefit of spraying fungicides during the vegetative growth stage and the need for multiple applications are still under investigation (31).

The repeated large scale use of fungicides with similar mode of action places selection pressure on the pathogen population that can lead to fungicide resistance (4, 20). With the increased fungicide use, sensitivity monitoring programs can be useful for detecting changes in the frequency of resistant isolates or reduced sensitivity before control failure occurs. Establishment of baseline sensitivity is the first step in the initiation of a fungicide sensitivity monitoring program. Bowen and Pedersen (5) observed that propiconazole inhibits mycelial growth of *E. turcicum* with mean EC<sub>50</sub> value of 0.01 µg/ml. Chapara et al. (11) recently established baseline sensitivities of *E. turcicum* to pyraclostrobin and observed normally distributed EC<sub>50</sub> values with a 10-fold sensitivity range.

Although not yet reported in *E. turcicum*, shifts in sensitivities have been observed in closely related species. *Helminthosporium halodes*, causal agent of sugarcane leaf spot, developed in vitro resistance to mancozeb (49). Resistant mutants of the southern corn leaf blight pathogen *Cochliobolus heterostrophus* were also produced in the laboratory (17). Recently, field resistance to thiabendazole and thiophanate-methyl by *Helminthosporium solani*, causal agent of potato silver scurf, were reported in the Columbia basin of Washington and Oregon (18). Resistance to QoIs and DMIs has not been reported in *E. turcicum* or closely related pathogens.



However, the long list of resistant pathogens strongly indicates that monitoring programs should be initiated to detect potential shifts in pathogen sensitivity.

## **Objectives**

Managing SCR and NCLB solely by use of resistant hybrids is difficult due to the presence of different pathogenic races that favor disease development. There is little information about the exact identity of the new, *Rpp9*-virulent race of *P. polysora* and hybrids containing the *Rpp9* gene may now be susceptible to SCR. The use of fungicides has become an important production practice in field corn. However there is limited research available that addresses management of these foliar diseases in field corn, especially for previously rust-resistant hybrids. Also, little research has been conducted with regards to the most effective timing and frequency of fungicide applications. Multiple applications on high-value corn have been the emphasis of most research; however this may not be applicable in all situations and may not be cost-effective for all producers. Additionally, fungicide resistance may develop due to extensive fungicide usage but shifts in sensitivities of foliar pathogens such as *E. turcicum* have not been carefully monitored. The objectives of this research were to characterize the new from the old race of *P. polysora* in the field, to better understand the epidemiology of *P. polysora* and *E. turcicum* and to assess management of these diseases with the effective use of hybrids and fungicides. Results from this research will be used to optimize fungicide programs and to provide appropriate recommendations to growers on how to effectively manage SCR and NCLB.

The specific objectives of this research were:

1. To characterize the new *Rpp9*-virulent race of *P. polysora* and evaluate SCR and NCLB epidemics in field corn based upon use of hybrids (Pioneer 33M52 and Pioneer 33M57),

fungicides (pyraclostrobin and pyraclostrobin+metconazole) and several timings of fungicide application.

2. To assess the efficacy of an established fungicides (pyraclostrobin) and a newly labeled mix of fungicides (pyraclostrobin + fluxapyroxad) combined with timing of application on disease epidemics and yield of a susceptible hybrid.
3. To determine in vitro sensitivities of *Exserohilum turcicum* isolates to metconazole.

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## CHAPTER 2

# INFLUENCE OF HYBRIDS AND FUNGICIDES ON EPIDEMICS OF SOUTHERN CORN RUST AND NORTHERN CORN LEAF BLIGHT

<sup>1</sup>Arcibal, S. S., Kemerait, R.C, Stevenson, K. L. and. L. J. Newsom. 2013. To be submitted to *Plant Disease*.

## **Abstract**

Southern corn rust (SCR), caused by *Puccinia polysora*, and northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, are important foliar diseases that can cause significant yield losses in the southern United States. The objectives of this study were to characterize the *Rpp9*-virulent race of *P. polysora* in the field and to determine the effect of hybrid, fungicide and fungicide timing on disease epidemics and corn yield. Field trials were conducted in Georgia (Tifton and Attapulgus) and Citra, Florida from 2011 to 2013 with the “rust-resistant” Pioneer 33M52 (P33M52) and rust-susceptible Pioneer 33M57 (P33M57). Treatments at each location included pyraclostrobin and pyraclostrobin + metconazole applied at seven different timings based upon corn growth stages. Onset of SCR was delayed in P33M52 as compared to P33M57 in early trials but not in later-planted trials. Area under the disease progress curves (AUDPC) for SCR severity and incidence were typically lower in P33M52 than in P33M57. Yields were usually greater in P33M52 than in P33M57 when SCR was severe. The efficacy of fungicide treatments was impacted by time of onset and intensity of SCR and NCLB. Fungicide applications at the early reproductive stages were effective but greatest yield improvement was attained with fungicides applied at the vegetative stages followed by a second application at the early reproductive stage. Yields from plots treated with fungicides were greater by up to 30% in Tifton, 6% in Attapulgus and 41% in Citra than were yields from untreated plots. Yield may have been impacted by southern corn leaf blight and ear rot at some locations.

## **Introduction**

Southern corn rust (SCR), caused by *Puccinia polysora* Underwood, and northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs., are the most destructive foliar diseases of corn in Georgia. The value of use of fungicides to manage southern



corn rust has been recognized Georgia since 2003 (30). *Puccinia polysora* does not overwinter on crop debris and must be reintroduced each growing season for much of the United States (14). Although SCR may not occur in the United States each year, yield losses of up to 50% have been reported in several field trials when the disease did occur (14). NCLB became widespread in Georgia in 2008 and was even more severe in 2009 (15). Unlike *P. polysora*, *E. turcicum* survives as mycelia or conidia on previously infected crop (16). Northern corn leaf blight has caused severe yield losses of up to 70% in susceptible lines (27). When both diseases are present in the field, it may be difficult to evaluate the inflicted damages separately.

Foliar diseases of corn are managed by adjusting planting date, planting resistant genotypes, tillage practices, crop rotation and use of fungicides (29). Host resistance is the best method to manage foliar diseases such as rusts and blights (14, 26). Management of SCR has heavily relied upon using hybrids containing the *Rpp9* resistance gene and other sources of Rpp resistance (3, 14, 27). *Rpp9*-virulent races had not been reported in the U.S. for almost 50 years until a second virulent race was identified in 2008 (4). Likewise, resistance to NCLB is conferred by four *Ht* genes but corresponding virulent *E. turcicum* races have also been reported (12, 13, 18, 20, 22). Fungicides were infrequently used in the past but may now be justified when foliar diseases become prevalent. Various fungicides are available in the market but only those within the quinone outside inhibitor (QoI), demethylation inhibitor (DMI) and succinate dehydrogenase inhibitor (SDHI) classes are recommended for use on corn in Georgia.

Timing of fungicide application is very important to effectively manage corn foliar diseases. Fungicide applications are usually recommended between vegetative tassel (VT) and blister (R2) stages as many foliar diseases begin to develop during this period (17). In most field trials in Georgia, fungicides sprayed at the VT stage have proved effective for managing SCR in

most field trials. Fungicide applications are recommended for controlling NCLB when disease symptoms appear in the early reproductive stages of the corn crop (15). Fungicide applications during the vegetative growth stages needs further investigation to determine the impact of such treatments on disease control and yield. Multiple applications of fungicides and combinations of fungicides from different classes may be appropriate when the environment is favorable for development of disease. However, this may also lead to excessive fungicide applications that incur additional costs to corn producers. Additionally, fungicide applications may only be cost-effective on susceptible hybrids (24, 25).

If fungicides are not properly used, yield improvement may not be sufficient to cover the cost of spraying. There has been little research focused on applying fungicides to control SCR and NCLB on hybrid corn (1, 6-9, 11, 21) as compared to sweet corn. Though fungicides were recommended for use on field corn in Georgia as early as 2004, use of fungicides on hybrid corn became popular nationally with the promotion that a VT application of a QoI fungicide could increase yield as a result of plant growth enhancement (17). The presence of the *Rpp9*-virulent *P. polysora* race means that the previously-resistant hybrids must now be monitored and the benefit of fungicide applications to these hybrids should be explored. Thus, knowledge on the interaction of hybrids and fungicides under different disease pressures is important to select appropriate management programs. Based on these considerations, the objectives of this study were to: (a) characterize the *Rpp9*-virulent and *Rpp9*-avirulent races of *P. polysora* in the field and (b) determine the effects of hybrids, fungicides and the timing of fungicide application on management of SCR and NCLB.

## Materials and Methods

*Locations and experimental design.* Field trials were conducted in Georgia (University of Georgia Coastal Plain Experiment Station in Tifton and Attapulgus Research and Education Center) and Florida (University of Florida Plant Science Research and Education Unit in Citra) from 2011 to 2013. Experiments were designed as a split-plot with four to six replications, where hybrid was the whole-plot factor and fungicide treatment was the sub-plot factor. Hybrids planted were Pioneer 33M52 (P33M52) and Pioneer (P33M57). The two hybrids are reported as nearly isogenic and differ in the presence (P33M52) or absence (P33M57) of the *Rpp9* gene conferring resistance to *P. polysora* race 9. Fungicides used were pyraclostrobin (PYR) (109 g a.i. ha<sup>-1</sup> Headline SC or 110 Headline EC; BASF Corporation, Research Triangle Park, NC) and pyraclostrobin + metconazole (PYR+MET) (107 + 40 g a.i. ha<sup>-1</sup>, Headline AMP; BASF Corporation, Research Triangle Park, NC) applied at different timings based upon crop growth stages. Fungicide timings are represented as follows: (i) VEG (single application between V5 and V7 stages), (ii) EREP (single application during early reproductive stages between VT and R1), (iii) MREP (single application during mid-reproductive stage between R2 and R3), (iv) VEG + EREP, (v) VEG + MREP, (vi) EREP + MREP and (vii) VEG + EREP + MREP. Planting dates, plot dimensions and fungicide application dates varied for each location (Table 2.1). Plots were maintained by using established management practices in each experimental station. All plots were treated with recommended rates of fertilizer, herbicide and insecticide.

*Fungicide applications.* Spray equipment and nozzle tips varied for each location. In Tifton, fungicides were applied using a Lee Spider Spray Trac (Lee Company, Idalou, TX) sprayer with four TeeJet 8003 (Spraying Systems Co., Wheaton, IL) flat fan nozzle tips in 2012 and CO<sub>2</sub> pressurized backpack sprayer with four TeeJet 8002 (Spraying Systems Co., Wheaton,

IL) flat fan nozzle tips in 2012 and 2013. In Attapulugus, Lee Spider Spray Trac and CO<sub>2</sub> backpack sprayers with four TeeJet 8002 flat fan nozzle tips were used in 2012 and 2013. In Citra, a CO<sub>2</sub> backpack sprayer with two TeeJet 8004E (2011) and four Teejet 8002 flat fan nozzle tips (2012 and 2013) was used. For all locations, all nozzle tips were spaced 0.46 m apart and the spray volume was 187 to 280 liters of water per hectare.

*Disease assessment.* Epidemics of SCR and NCLB were evaluated for all field trials. Southern corn leaf blight (SCLB) caused by *Cochliobolus heterostrophus* (syn. *Bipolaris maydis*) was also evaluated at Citra in 2012 and 2013 since it was considered as a predominant disease. Visual assessments were made by arbitrarily selecting 30 leaves from each plot on each sampling date. In 2012, sampling number from late planted trials in Citra and Attapulugus was reduced to 20 leaves per plot due to distance and time to assess trials with high disease severity. Disease severities were determined by visually estimating the percent leaf affected for each disease from each leaf. Those assisting with disease assessment were trained prior to assessing field trials. Disease incidence was calculated by dividing the number of infected leaves by the total number of sampled leaves. In 2012 and 2013, necrosis was also determined at the final assessment date by estimating the percentage of canopy affected by all foliar diseases. A scale of 0 to 100 % was used for all assessments. Seven trials were non-destructively assessed weekly for a total of five to six rating dates while two late-planted trials, Attapulugus in 2012 and Citra in 2013, were rated every two weeks for a total of three assessments. Initial observations of SCR and NCLB in each trial were recorded based on days after planting (DAP).

Area under the disease severity progress curves (AUDSPC) and area under the disease incidence progress curves (AUDIPC) were calculated from repeated severity and incidence assessments using the trapezoidal method. The formula used to calculate AUDSPC and AUDIPC

was as for area under the disease progress curve (AUDPC) where

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1} + 1}{2} \right) (t_{i+1} - t_i)$$

In this equation,  $t$  is the time in days after planting for each assessment,  $y$  is the reading of the variable that was assessed and  $n$  is the number of assessments. In 2011, final severity (%) and incidence (%) were used for SCR since disease symptoms were only observed on the last assessment date. Yields were obtained in pounds per plot and then converted to kilograms per hectare ( $\text{kg ha}^{-1}$ ). Yield data was not obtained from the late planted trial in Attapulugus in 2012 due to problems picking the corn with a new plot combine.

*Statistical analysis.* Data were analyzed separately by field trial due to differences in planting dates, disease pressures and environmental conditions for each year. The generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.3, SAS Institute, Cary, NC) was used to determine the effect of hybrid, fungicide treatment and interaction between the two factors on AUDSPC, AUDIPC, final disease severity, final disease incidence, total plot necrosis and yield. Data were pooled when there was no significant interaction between hybrids and fungicide treatment for each variable. Treatments were based on pairwise comparisons of least square means ( $P=0.05$ ).

## Results

*Disease detection and disease progress curves.* Foliar disease symptoms were observed sooner after planting in later-planted field trials than in the earliest planted trials (Table 2.1). Disease symptoms were first observed after early reproductive stages in early-planted trials but were first observed as early as the fifth-leaf vegetative stages in late planted-trials. Severity of NCLB tended to increase in later-planted trials as compared to the trials planted earlier in the season. Highest NCLB severities were observed in late-planted trials at Attapulugus and Citra in

2012 and 2013, respectively (Figs. 2.2 and 2.4). Severity of SCR was dramatically higher in trials planted in May than in those planted in April or in later-planted trials. Highest severities of SCR were observed in late-planted trials at Tifton and Attapulcus in 2012 and 2013, respectively. In 2012 and 2013, rust pustules of *P. polysora* were observed 7 to 14 days earlier in P33M57 than in P33M52 in early-planted trials but were concurrent in later-planted trials (Figs. 2.3 and 2.5). At Citra in 2011, rust symptoms were first observed on both hybrids at the same time but SCR was only detected on the final assessment date. Northern corn leaf blight was also first detected during the early reproductive growth stages but significantly increased later in the season (Fig. 2.1).

*Weather conditions.* Conditions varied over the three growing seasons (Table 2.2). The growing season in 2011 was characterized by prolonged periods of hot and dry weather followed by wet weather associated with tropical storm Irene in late August. The 2012 growing season was more favorable for disease development. Temperatures were warm to hot and rainfall was abundant. Ample rainfall in late May and late June was associated with tropical storms Beryl and Debby respectively. Heavy rainfall in August was associated with hurricane Isaac. The growing season in 2013 was initially cold and dry in March but dramatically shifted to warm and wet beginning in June. Abundant rainfall in June and July was caused by tropical storms Andrea and Dorian, respectively.

*2011 field trial.* In Citra, symptoms of NCLB were first observed 63 DAP and SCR was not observed until 107 DAP (Table 2.1). The effects of hybrids and fungicide treatments in this trial are presented in Table 2.3. Final SCR incidence was not significantly different between hybrids but final SCR severity was significantly higher in P33M52 than in P33M57. Values of AUDSPC and AUDIPC for NCLB were not different between hybrids. However, yields of

P33M57 were significantly higher than those of P33M52. Final SCR severity and final SCR incidence in fungicide treatments did not differ from the untreated control. For NCLB, AUDSPC in fungicide-treated plots was not significantly different from the control. However, three fungicide treatments had significantly higher AUDIPC for NCLB than the control. Nine fungicide treatments had yields significantly higher than that of the control.

*2012 field trials.* In the early-planted trial in Tifton, NCLB was first detected 61 DAP and SCR was not observed until 95 DAP (Table 2.1). The effects of hybrids and fungicide treatments in this trial are presented in Table 2.4. Yields were significantly greater in P33M57 than in P33M52 although AUDSPC and AUDIPC values for SCR were similar for both hybrids. Fungicide treatments had a significant effect for NCLB. Three fungicide applications resulted in significantly lower AUDSPC and AUDIPC values of NCLB as compared to the control.

In the early-planted trial in Attapulgus, NCLB and SCR were first observed at 64 DAP and 72 DAP, respectively (Table 2.1). The effects of hybrids and fungicide treatments in this trial are presented in Table 2.5. The AUDSPC values for SCR and NCLB, AUDIPC values for SCR and total plot necrosis were significantly greater in P33M57 than in P33M52. However, AUDIPC values for NCLB and yield were not significantly different between hybrids. Fungicide treatments that included applications at stage VEG + MREP applications significantly reduced AUDSPC and AUDIPC values for SCR compared to the control. Only the treatment of three applications of PYR+MET significantly reduced AUDSPC and AUDIPC values for NCLB compared to the control. Total plot necrosis ratings were significantly lower with multiple applications of PYR+MET and applications of PYR that included MREP. However, yields of fungicide-treated plots were not significantly different from the untreated plots.

In the late-planted trial in Tifton, NCLB and SCR were first observed at 32 DAP and 55 DAP, respectively (Table 2.1). The effects of hybrids and fungicide treatments in this trial are presented in Table 2.6. The AUDSPC and AUDIPC values for NCLB did not differ significantly between hybrids. However, AUDSPC and AUDIPC values for SCR and total plot necrosis were significantly higher in P33M57 than in P33M52. Yields were significantly higher in P33M52 than in P33M57. Fungicide treatments that included an application in the EREP stage resulted in significantly lower AUDSPC values for SCR than for the control. However, the AUDIPC values of all fungicide treatments were not significantly different from the AUDIPC of the control. Fungicide treatments that included a stage VEG application significantly reduced AUDSPC values for NCLB compared to the control. Treatments that included applications at the stage VEG + EREP stages resulted in significantly lower AUDIPC for NCLB compared to the control. Multiple fungicide applications resulted in significantly lower total plot necrosis ratings than the control. Fungicide had significant effects on yield of both hybrids. Significant yield improvement relative to the untreated control was most often observed for treatments with multiple applications of PYR+MET.

In Citra, NCLB and SCR were first observed at 36 DAP and 50 DAP, respectively (Table 2.1). Additionally, first detection of SCLB was concurrent with that of NCLB. The effects of hybrids and fungicide treatments in this trial are presented in Table 2.7. The AUDSPC and AUDIPC values for NCLB and SCLB were not significantly different between hybrids. However, AUDSPC and AUDIPC values for SCR and total plot necrosis were significantly higher in P33M57 than in P33M52. Yields of P33M52 were significantly higher than those of P33M57. Fungicide treatment had significant effect on all measures of disease development except for AUDSPC and AUDIPC for NCLB. For SCR, there was a significant hybrid by



fungicide interaction for AUDSPC ( $P \leq .0001$ ). In P33M52, AUDSPC of any given fungicide treatment did not differ from the control. In P33M57, all fungicide treatments resulted in a significantly lower AUDSPC value than the control, except for single applications at the MREP stage. The AUDIPC values for SCR in plots treated with multiple applications of PYR+MET or applications of PYR at the VEG + EREP stages were significantly lower than for those in the untreated plots. For SCLB, treatments that included an application of PYR+MET at the VEG stage or PYR at the VEG + MREP stages resulted in significantly lower AUDSPC and AUDIPC than the control. There was a significant hybrid by treatment interaction on total plot necrosis. In P33M57, treatments applied at VEG + EREP or EREP + MREP stages typically resulted in less necrosis than the control. In P33M52, treatments applied at the EREP or MREP stage tended to result in lower total plot necrosis. Yields of four fungicide treatments were significantly higher than that of the control. Treatments applied at the VEG + EREP stage significantly improved yield compared to the control. Among all treatments, single applications at stages VEG or MREP had the lowest yields.

In the late-planted trial in Attapulugus, NCLB and SCR were first observed at 33 DAP and 47 DAP, respectively (Table 2.1). Additionally, first detection of SCLB was concurrent with that of NCLB. The effects of hybrids and fungicide treatments on disease epidemics and corn yield in this trial are presented in Table 2.8. No significant differences between P33M57 and P33M52 were found for any measure of disease. However, significant differences among fungicide treatments were observed for all measures of disease. For NCLB, treatments that included an application at the VEG stage typically resulted in significantly lower AUDSPC and AUDIPC values than the control. Similar trends were observed for SCR and SCLB despite low disease pressures.

*2013 field trials.* In Tifton, NCLB and SCR were not detected until 85 DAP and 91 DAP, respectively (Table 2.1). The effects of hybrids and fungicide treatments on disease epidemics and corn yield in this trial are presented in Table 2.9. The AUDSPC and AUDIPC for SCR were significantly greater in P33M57 than in P33M52. For NCLB, AUDIPC was significantly higher in P33M57 than in P33M52 but AUDSPC values did not differ significantly between hybrids. No significant differences in disease development were found among fungicide treatments for either disease. Total plot necrosis ratings and yield were not significantly different between hybrids and among fungicide treatments.

In Attapulgus, NCLB and SCR were first observed at 55 DAP (Table 2.1). The effects of hybrids and fungicide treatments are presented in Table 2.10. The AUDSPC and AUDIPC for SCR and total plot necrosis ratings were significantly higher in P33M57 than in P33M52. The NCLB AUDPC values and yield were not significantly different between hybrids. Fungicide treatment had significant effect on AUDPC values except those for NCLB. For SCR AUDSPC values, there was a significant hybrid by fungicide treatment interaction ( $P=0.0003$ ). In P33M57, fungicides applied at the EREP + MREP stages tended to result in significantly reduced AUDPC values. In P33M52, AUDSPC values were not significantly different among treatments. Total plot necrosis ratings were significantly lower in treatments that included a stage MREP application compared to the control. Yields significantly differed among fungicide treatments; however, only PYR+MET applied at the EREP stage or EREP + MREP stages were significantly higher than the control. Only three fungicide treatments were not significantly different from the highest yielding treatment (PYR+MET at EREP). Yield may also have been impacted by ear rot present near the end of the growing season.

In Citra, NCLB, SCLB and SCR were first observed at 37 DAP (Table 2.1). The effects of hybrids and fungicide treatments on disease epidemics and corn yield in this trial are presented in Table 2.11. The AUDSPC and AUDIPC values for SCR and total plot necrosis ratings were significantly higher in P33M57 than in P33M52. However, AUDPC values for NCLB and SCLB, and yield were not significantly different between hybrids. For SCR, significantly lower AUDSPC values were typically observed with multiple fungicide applications compared to the control. Treatments applied at the VEG stage tended to result in lower AUDIPC values than the control. For NCLB, AUDSPC values tended to be lower in treatments applied at the VEG stage. Only multiple applications of PYR+MET that included VEG resulted in significantly lower AUDIPC values than the control. For SCLB, AUDSPC values were not significantly different from the control. Values of AUDIPC were lower relative to the control when PYR was applied at EREP or PYR+MET was applied at the VEG + EREP stages. Total plot necrosis ratings were significantly lower than the control following multiple applications of PYR+MET. Applications of PYR that included stages VEG or VEG + EREP stages also resulted in lower total plot necrosis than the control. Treatments that included at least one fungicide application at VEG significantly improved yields. Single applications at EREP and MREP stages resulted in yields that were not significantly different from the control.

## **Discussion**

In this study, P33M52 with and P33M57 without the *Rpp9* gene were evaluated to differentiate the new, *Rpp9*-virulent race from the old race of *P. polysora*. To our knowledge, this is the first study to report field characterization of the new race of *P. polysora* in the United States. The presence of the *Rpp9*-virulent race could only be confirmed when rust pustules were detected on P33M52. In nearly all early-planted trials, onset of SCR in P33M52 occurred 7 to 14

days later than in P33M57. In all late-planted trials, however, SCR occurrence was detected on both hybrids at the same date. The reason for the delay in detection of SCR on P33M52 may be due to a difference in infection efficiency and latent period of the *Rpp9*-virulent race in P33M52 than in P33M57. Another possible explanation is that the *Rpp9*-virulent race was introduced later than the *Rpp9*-avirulent race.

Since this study relied on natural inoculum, there was likely a mixture of both virulent and avirulent races throughout each trial. In this study, it was observed that uredinia on P33M57 were typically larger and denser in distribution than those on P33M52. The new race was visually distinguished from the old race in P33M52 where chlorotic and necrotic flecks resulting from the old race of *P. polysora* were observed along with sporulating uredinia from the new race. Mixture of both races was also observed on lines used in SCR trials at Waimanalo (19). However, it was not possible to determine the virulence of the new race and distinguish the population of the new from the old race on P33M57. According to Dolezal (5), the only information currently known about the new race is that it is virulent to the *Rpp9* gene. Known races of *P. polysora* were identified using the host differentials developed fifty years ago by Robert (23). However, all *P. polysora* isolates included in that study and five out of eleven corn lines used in that study are no longer available (4).

Hybrids with the *Rpp9* gene such as P33M52 will now be susceptible to SCR due to the presence of the virulent race in the United States. Susceptibility of P33M52 and five other lines carrying the *Rpp9* gene to the new race was previously reported (4, 19). In this study, P33M52 afforded protection to SCR compared to P33M57, despite the presence of the new virulent race. The AUDSPC values in P33M52 were significantly lower than for values in P33M57 except when this disease was very low in a given trial. The AUDIPC values in P33M52 were

significantly lower in all but one trial. The AUDIPC values did not differ between hybrids in the latest planted trial conducted at Attapulugus in 2012. This may be that the population of the new race possibly increased in magnitude during the earlier-planted trial. When SCR was predominant in a given trial, total plot necrosis was significantly lower in P33M52 than in P33M57. Under significant SCR pressure, P33M52 had significantly higher yields than P33M57. This was observed in two-late planted trials where SCR were severe. However, yield of P33M57 was greater than P33M52 in two early-planted trials where severity of SCR was very low. Higher yield in P33M57 was also observed in one early-planted trial conducted in 2011 (Appendix. A). Lower yields of P33M52 in these early planted trials may have occurred due to a fitness cost associated with the *Rpp9* gene; however such was not assessed in this study.

Unlike SCR, NCLB symptoms were detected on both P33M57 and P33M52 at the same assessment date regardless of planting date. The AUDSPC and AUDIPC for NCLB were generally not significantly different between hybrids. However, the values of AUDSPC and AUDIPC for NCLB were significantly different among hybrids at Attapulugus in 2012 and at Tifton 2013, respectively. Calculated AUDSPC or AUDIPC values of P33M57 were significantly higher than that of P33M52. However, NCLB was of minor importance in these trials and differences for calculated AUDSPC and AUDIPC were not observed in any other trials where NCLB is of greater importance.

The use of fungicides can be an effective tool to manage SCR and NCLB in terms of reduced disease severity and increased yields (2, 25, 28). In this study, fungicide efficacy as measured by AUDSPC and AUDIPC values, total plot necrosis and yield was more evident in later-planted trials where disease intensities from SCR, NCLB and SCLB were much higher than in earlier- planted trials. Nonetheless, treatment differences were observed in early-planted trials

when disease occurred before all fungicide treatments had been applied. Significant differences in AUDPC for SCR among fungicide treatments were generally observed when SCR was first detected on, or prior to, the date of the final fungicide application. An exception to this was observed in the late-planted Tifton trial in 2012 where the final SCR severity on P33M57 and P33M52 were 45% and 14%, respectively (Fig 2.4). In this trial, AUDPC for SCR did not differ among fungicide treatments; however, differences were significant at the  $\alpha=0.1$  level. As for SCR, calculated AUDPC values for NCLB were significantly different among fungicide treatments except when disease pressure was low.

Pyraclostrobin (Headline) and pyraclostrobin + metconazole (Headline AMP) were effective for management of SCR, NCLB and SCLB. However, pyraclostrobin + metconazole appeared to be more effective in managing NCLB as compared to pyraclostrobin alone. This was most apparent at Citra in 2013 where the lowest AUDPC values were observed with multiple applications of pyraclostrobin + metconazole. There was often no significant difference between fungicides in terms of their impact on yield. However, yields that were significantly different than the untreated control tended to be numerically higher for applications of pyraclostrobin + metconazole compared to pyraclostrobin alone. At Attapulgis in 2013, treatments of pyraclostrobin + metconazole had significantly higher combined yield than did treatments of pyraclostrobin. In the late-planted Tifton trial in 2012, most treatments with yields significantly higher than the control were from pyraclostrobin + metconazole treatments. Greater efficacy of pyraclostrobin + metconazole in managing diseases and improving yields may be that metconazole is more systemic and has curative properties. The combination of two modes of action may also be more active against foliar disease of corn. More importantly, using

fungicides with different modes of action should be considered by growers for fungicide resistance management.

Disease monitoring is one of the strategies used to judiciously time applications of fungicides for foliar diseases management in corn and other crops. Early warning of these diseases enables growers to initiate timely management programs and to avoid unnecessary fungicide applications. In this study, fungicide applications coinciding with onset of disease tended to result in lower disease ratings and higher yields than those that were applied 14 days or longer after first detection. The beneficial effect of single fungicide applications was more evident when onset of both SCR and NCLB occurred at the same time. However, onset for these diseases generally did not occur at the same time and single fungicide applications were usually not equal to the highest yielding treatments. Additionally, significantly lower necrosis values were more apparent in plots with multiple fungicide applications. Multiple fungicide applications to manage disease and maximize yield were appropriate when disease onset occurred during vegetative growth or when the environment was favorable for disease development. Three fungicide applications oftentimes resulted in the lowest AUDPC values and highest yields but these yields were not significantly different from two timely applications. In late-planted trials, fungicides applied at VEG + EREP were not significantly different and sometimes even numerically higher than were three applications.

A key factor for the effectively managing foliar diseases with fungicides is attention to the timing of the application. Although timing a fungicide application at the early reproductive stages was usually successful in this study and may be more convenient for growers, it may not be the most effective application strategy. Fungicide applications at the vegetative growth stages were valuable especially when the crop was at greater risk to foliar diseases especially to

NCLB and SCLB. This was most apparent in the late-planted trials and especially at Citra in 2013 where a single fungicide application at the vegetative growth stage significantly improved yield over the untreated control. However, yield from a single application of fungicide was not as great as treatments that included a second fungicide application. Similar results were reported from a field trial conducted in Alabama (10). One of the most important results from this study was the evidence that in later planted corn and where NCLB and/or SCLB were a consideration, a fungicide program initiated during the vegetative growth stages and continuing with an additional application might be the most effective strategy for maximizing yields. Two factors that impact this risk are hybrid selection and planting date.

Significant yield increases for fungicide treatments over the untreated control from 6 to 41% were typically observed in late-planted trials. Out of five trials with significant yield data, the trial at Attapulcus in 2013 showed the lowest yield increases of only up to 6%. This may be because other diseases such as ear rot negatively impacted yield at the end of the season. However, yield benefits of fungicide applications were not limited to late-planted trials. At Citra in 2011, nine out of fourteen treatments produced significantly greater yields than did the control although diseases were not severe. Also, yields in other early-planted trials were increased from 3 to 14%, although these values were not significantly different than the control.

From this study, P33M52 still provided an effective measure of resistance to SCR and fungicide combinations were effective to control SCR and NCLB. From these results it seems that a fungicide program for management of SCR is not needed where a variety with the *Rpp9* gene is sown although use of fungicides may be justified on later-planted corn, especially where NCLB or SCLB are present. However, further studies are needed to monitor the efficacy of previously rust-resistant hybrids and determine the efficacy of fungicide treatments in other corn-



producing states since disease spectrum and disease intensity may vary every year and by region. Growers will likely question whether it would be better to plant P33M52 (with apparent lower yield potential than P33M57) without use of fungicides versus planting P33M57 and protecting with fungicides. From the 2013 field study in Attapulgis where severity of SCR was high and severity of other diseases was low, there was no statistical difference in yield between the untreated control for P33M52 and P33M57 that received three fungicide applications (Appendix E). Finally, disease scouting is very important in making fungicide recommendations. Fungicides applied either too early or too late with reference to the onset of disease are likely less effective. Additionally, it will be important to consider economic analysis to determine which fungicide programs bring the greatest financial return to the corn producers.

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Table 2.1. Field locations, plot description and fungicide timings used to manage northern corn leaf blight (NCLB) and southern corn rust (SCR)

Planting date	Location <sup>a</sup>	Plot size (m)	Spacing (m)	Blocks	Days after planting						
					Fungicide application <sup>b</sup>			First disease detection			
					VEG	ERE	MREP	P33M52		P33M57	
								<u>NCLB</u>	<u>SCR</u>	<u>NCLB</u>	<u>SCR</u>
27 Apr 2011	Citra, FL	1.8 × 7.6	0.8	4	29	56	70	63	107	63	107
30 Mar 2012	Tifton, GA	1.8 × 7.6	0.9	4	33	67	81	61	95	61	95
17 Apr 2012	Attapulgus, GA	1.8 × 7.0	0.9	6	35	58	72	64	86	64	72
18 May 2012	Tifton, GA	1.8 × 7.6	0.9	4	32	55	76	32	55	32	55
09 Jul 2012	Citra, FL	1.8 × 7.6	0.8	4	36	52	70	36	50	36	50
24 Aug 2012	Attapulgus, GA	1.8 × 6.1	0.9	4	33	62	77	33	47	33	47
03 Apr 2013	Tifton, GA	1.8 × 7.6	0.9	4	33	69	82	85	104	85	91
08 May 2013	Attapulgus, GA	1.8 × 7.6	0.9	4	22	55	72	55	55	55	55
18 Jun 2013	Citra, FL	1.8 × 7.6	0.6	4	37	64	83	37	37	37	37

<sup>a</sup> Florida location was the University of Florida, Plant Science Research and Education Unit, Tifton location was the University of Georgia Coastal Plain Experiment Station and Attapulgus location was the University of Georgia Attapulgus Research and Education Center

<sup>b</sup> Fungicide timings based upon growth stages of corn. VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3.

Table 2.2. Weather conditions at field sites for corn growing seasons from 2011 to 2013

Year	Location	Month								
		Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Monthly rainfall (mm)										
2011	Citra, FL <sup>a</sup>	95	60	42	126	75	166	99	172	47
2012	Tifton, GA <sup>b</sup>	120	31	88	133	169	340	76	40	33
	Attapulgis, GA <sup>c</sup>	125	41	63	147	221	370	105	22	23
	Citra, FL	33	101	106	381	149	287	119	57	0.3
2013	Tifton, GA	80	113	66	338	147	221	79	16	76
	Attapulgis, GA	132	121	22	110	303	158	83	16	102
	Citra, FL	12	75	44	165	238	202	106	40	135
Mean Monthly Temperature										
2011	Citra, FL	18.1	22.1	24.0	26.7	27.0	27.7	25.3	19.6	17.2
2012	Tifton, GA	19.2	19.7	23.8	24.7	27.5	25.8	24.3	19.5	13.3
	Attapulgis, GA	19.3	19.7	23.9	25.1	27.2	26.0	24.3	20.5	13.2
	Citra, FL	20.2	21.2	24.2	25.2	26.3	25.8	25.2	21.3	15.0
2013	Tifton, GA	11.5	18.3	21.2	26.0	26.1	26.6	24.5	19.9	13.8
	Attapulgis, GA	11.9	18.2	21.3	26.2	25.4	26.3	24.8	19.9	14.1
	Citra, FL	13.7	20.8	22.2	26.0	25.6	26.5	25.5	21.7	17.8

<sup>a</sup> Archived weather data in Plant Science Research and Education Unit retrieved from the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu>).

<sup>b</sup> Historical data in Coastal Plain Experiment Station retrieved from the Georgia Automated Environmental Monitoring Network (<http://www.georgiaweather.net/>).

<sup>c</sup> Historical data in Attapulgis Research and Education Center retrieved from the Georgia Automated Environmental Monitoring Network (<http://www.georgiaweather.net/>).

Table 2.3. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield in Citra, FL in 2011

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>b</sup>		Yield
		FDS <sup>a</sup>	FDI <sup>a</sup>	AUDSPC <sup>b</sup>	AUDIPC <sup>b</sup>	(kg/ha)
Hybrid						
Pioneer 33M57		0.07 B	0.02 A	90.8 A	914.6 A	11188.1 A <sup>c</sup>
Pioneer 33M52		0.15 A	0.02 A	93.2 A	935.2 A	10343.3 B
P( $\alpha$ =0.05)		0.0444	0.6280	0.6790	0.6110	0.0006
Treatment						
Fungicide <sup>c</sup>	Application <sup>d</sup>					
PYR	VEG	0.04 a	0.01 a	110.3 a	1131.8 ab	10546 a-e
PYR	EREP	0.21 a	0.01 a	119.9 a	1181.4 a	11644 ab
PYR	MREP	0.05 a	0.02 a	84.2 a	865.0 c	10335 b-f
PYR	VEG+EREP	0.21 a	0.04 a	82.8 a	860.8 c	10250 c-f
PYR	VEG+MREP	0.20 a	0.02 a	92.0 a	923.3 c	9630 ef
PYR	EREP+MREP	0.24 a	0.04 a	71.0 a	741.2 c	11624 ab
PYR	VEG+EREP+MREP	0.09 a	0.02 a	80.4 a	812.5 c	11653 a
PYR+MET	VEG	0.04 a	0.01 a	112.4 a	1318.6 a	10863 a-e
PYR+MET	EREP	0.06 a	0.02 a	99.2 a	892.5 c	10513 a-f
PYR+MET	MREP	0.15 a	0.02 a	79.9 a	921.0 bc	11179 a-d
PYR+MET	VEG+EREP	0.05 a	0.01 a	86.1 a	798.2 c	9982 def
PYR+MET	VEG+MREP	0.13 a	0.01 a	86.5 a	876.7 c	11246 a-d
PYR+MET	EREP+MREP	0.04 a	0.02 a	89.6 a	856.5 c	11384 abc
PYR+MET	VEG+EREP+MREP	0.09 a	0.01 a	93.10 a	796.7 c	11043 a-d
Untreated Control		0.06 a	0.01 a	92.2 a	897.5 c	9573 f
P( $\alpha$ =0.05)		0.0679	0.224	0.1769	<0.0001	0.0073

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessments dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction.

Table 2.4. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield in early-planted trial conducted in Tifton, GA in 2012.

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Hybrid</b>							
Pioneer 33M57		0.6 A	71.4 A	17.2 A	510.7 A	48.6 A	16998 A
Pioneer 33M52		0.2 A	20.6 B	17.3 A	558.4 A	42.5 A	16332 B
<i>P</i> ( $\alpha=0.05$ )		0.1621	<0.0001	0.9357	0.0783	0.0672	0.004
<b>Treatment</b>							
Fungicide <sup>b</sup>	Application <sup>c</sup>						
PYR	VEG	2.8 a	100.6 a	24.3 a <sup>d</sup>	612.9 ab	48.1 a	16911 a
PYR	EREP	0.1 a	30.6 a	20.2 a-e	634.4 a	38.1 a	15474 a
PYR	MREP	0.6 a	84.6 a	12.6 d-g	431.0 de	60.6 a	17019 a
PYR	VEG+EREP	0.0 a	21.9 a	18.9 a-g	611.3 abc	48.8 a	16447 a
PYR	VEG+MREP	0.0 a	29.2 a	11.1 fg	465.6 cde	41.9 a	16122 a
PYR	EREP+MREP	0.0 a	29.2 a	16.6 b-g	521.0 a-e	50.0 a	16390 a
PYR	VEG+EREP+MREP	0.0 a	5.8 a	12.4 efg	468.5 b-e	38.1 a	16857 a
PYR+MET	VEG	1.0 a	89.0 a	25.0 a	595.0 abc	50.0 a	16513 a
PYR+MET	EREP	0.1 a	14.6 a	15.1 c-f	495.6 a-e	46.9 a	17280 a
PYR+MET	MREP	0.1 a	42.3 a	17.6 a-g	559.0 a-e	47.5 a	16247 a
PYR+MET	VEG+EREP	0.1 a	33.5 a	20.9 a-d	546.7 a-e	46.3 a	16675 a
PYR+MET	VEG+MREP	0.2 a	54.0 a	22.0 abc	571.0 a-d	45.6 a	17144 a
PYR+MET	EREP+MREP	0.2 a	39.4 a	12.1 efg	479.2 b-e	40.6 a	17391 a
PYR+MET	VEG+EREP+MREP	0.2 a	23.3 a	10.0 g	422.1 e	35.6 a	17047 a
Untreated Control		1.0 a	91.9 a	19.5 a-e	604.58 abc	45.0 a	16461 a
<i>P</i> ( $\alpha=0.05$ )		0.0769	0.0709	0.0026	0.0429	0.5114	0.233

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with three assessment dates for SCR and five assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction.

Table 2.5. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield in early-planted trial conducted in Attapulugus, GA in 2012

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Hybrid</b>							
Pioneer 33M57		12.5 A	486.2 A	66.7 A	1479.8 A	36.2 A	10641 A
Pioneer 33M52		0.4 B	27.5 B	56.4 B	1474.0 A	23.2 B	10456 A
<i>P</i> ( $\alpha=0.05$ )		<0.0001	<0.0001	0.0042	0.8940	<0.0001	0.0423
<b>Treatment</b>							
Fungicide <sup>b</sup>	Application <sup>c</sup>						
PYR	VEG	15.2 ab	350.0a	66.5 abc	1393.8 def	37.9 a	10330 a
PYR	EREP	4.3 bc	276.5 abc	80.0 ab	1667.6 abc	33.8 ab	10955 a
PYR	MREP	14.9 ab	392.8 a	63.4 abc	1555.6 b-d	28.3 cde	10750 a
PYR	VEG+EREP	7.4 abc	308.7 abc	58.7 c	1380.8 def	29.6 bcd	10434 a
PYR	VEG+MREP	2.9 bc	246.5 abc	49.4 cd	1397.3 def	26.3 de	10642 a
PYR	EREP+MREP	0.7 c	153.6 c	78.0 ab	1792.8 a	26.3 de	10596 a
PYR	VEG+EREP+MREP	1.7 c	149.7 c	49.8 cd	1325.4 ef	25.0 de	10268 a
PYR+MET	VEG	17.0 a	366.0 a	62.5 bc	1331.9 ef	35.4 a	11117 a
PYR+MET	EREP	7.0 abc	287.8 abc	82.5 a	1749.6 ab	35.0 ab	10488 a
PYR+MET	MREP	3.0 bc	181.8 bc	82.5 a	1582.0 a-d	29.6 bcd	10569 a
PYR+MET	VEG+EREP	3.7 bc	297.5 abc	58.7 c	1416.9 def	28.3 cde	10258 a
PYR+MET	VEG+MREP	1.6 c	164.3 c	48.7 cd	1329.2 ef	26.7 de	10269 a
PYR+MET	EREP+MREP	1.4 c	151.2 c	56.4 cd	1538.8 b-e	25.4 de	10685 a
PYR+MET	VEG+EREP+MREP	1.3 c	178.4 c	37.6 d	1201.2 f	23.3 e	10078 a
Untreated Control		14.5 ab	348.1 ab	65.9 abc	1490.1 cde	34.6 ab	10784 a
<i>P</i> ( $\alpha=0.05$ )		0.0430	0.0137	<0.0001	<0.0001	<0.0001	0.5896

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with five assessment dates for SCR and six assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction



Table 2.6. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield in late-planted trial conducted in Tifton, GA in 2012

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Hybrid</b>							
Pioneer 33M57		330.9 A <sup>d</sup>	2122.5 A	154.5 A	2060.8 A	53.3 A	8966 B
Pioneer 33M52		78.3 B	1540.2 A	147.5 A	2036.1 A	27.7 B	9846 A
<i>P</i> ( $\alpha=0.05$ )		<0.0001	<0.0001	0.239	0.2564	<0.0001	0.0118
<b>Treatment</b>							
Fungicide <sup>b</sup>	Application <sup>c</sup>						
PYR	VEG	354.0 a	2107.8 a	139.8cde	2021.2 cde	47.5 a-d	10449 abc
PYR	EREP	129.9 cd	1646.9 a	175.8 ab	2114.7 abc	51.3 ab	8039 de
PYR	MREP	249.2 abc	1706.3 a	173.8 ab	2114.0 abc	50.8 abc	8726 c-e
PYR	VEG+EREP	151.2 bcd	1818.5 a	122.7 de	1980.6 efg	28.1 fg	10645 a
PYR	VEG+MREP	279.2 a	2094.6 a	136.0 cde	2035.0 b-e	41.9 b-e	9321 a-e
PYR	EREP+MREP	132.3 cd	1875.2 a	181.2 a	2166.9 a	31.3 ef	9287 a-e
PYR	VEG+EREP+MREP	89.3 d	1508.0 a	123.7 de	1988.1 ef	35.3 def	8677 cde
PYR+MET	VEG	304.4 a	1901.4 a	146.2 bcd	1997.9 c-f	56.3 a	8502 cde
PYR+MET	EREP	130.9 cd	1755.3 a	174.2 ab	2147.1 ab	45.8 a-e	8774 b-e
PYR+MET	MREP	337.0 a	1902.8 a	160.7 abc	2151.2 ab	48.3 a-d	10068 abc
PYR+MET	VEG+EREP	116.4 d	1783.3 a	116.6 de	1869.4 g	38.3 b-f	10449 abc
PYR+MET	VEG+MREP	267.7 ab	2048.3 a	131.4 cde	1994.6 def	36.3 c-f	9473 a-d
PYR+MET	EREP+MREP	140.1 cd	1901.0 a	183.6 a	2144.8 ab	24.4 fg	10565 ab
PYR+MET	VEG+EREP+MREP	84.2 d	1569.0 a	113.8 e	1893.0 fg	16.5 g	10790 a
Untreated Control		303.3 a	1851.7 a	185.7 a	2108.7 a-d	56.3 a	7598 e
<i>P</i> ( $\alpha=0.05$ )		<0.0001	0.0801	<0.0001	<0.0001	<0.0001	0.0072

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with four assessment dates.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, (ii) EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction.

Table 2.7. Effect of hybrid and fungicide treatment on southern corn rust (SCR), northern corn leaf blight (NCLB) and southern corn leaf blight (SCLB) epidemics, necrosis and corn yield in Citra, FL in 2012

Variable <sup>f</sup>		SCR <sup>a</sup>			NCLB <sup>a</sup>		SCLB <sup>a</sup>		Necrosis		Yield
		AUDSPC	AUDIPC		AUDSP	AUDIPC	AUDSPC	AUDIPC	(%)	(kg/ha)	
<b>Hybrid</b>											
Pioneer 33M57		59.1 A <sup>d</sup>		1664.7 A	15.5 A	946.6 A	29.1 A	1411.3 A	47.1 A		5791 B
Pioneer 33M52		3.2 B		459.5 B	15.3 A	972.8 A	31.4 A	1380.7 A	26.1 B		6752 A
<i>P</i> (α=0.05)		<0.0001		<0.0001	.07612	0.4754	0.3316	0.4755	<0.0001		<0.0001
<b>Treatment</b>											
Fungicide <sup>b</sup>	Application <sup>c</sup>										
		<u>P33M57</u>	<u>P33M52</u>						<u>P33M57</u>	<u>P33M52</u>	
PYR	VEG	93.4 bc	2.5 a	1105.9 b-e	13.5 a	943.4 a	39.2 abc	1484.4 abc	81.3 a	43.8 ab	5222 e
PYR	EREP	48.5 de	1.5 a	1155.9 a-d	16.8 a	1049.4 a	39.0 abc	1626.9 ab	50.0 cde	30.0 bcd	6791 ab
PYR	MREP	106.0 a	3.1 a	1267.8 abc	15.2 a	940.9 a	47.3 a	1549.7 ab	66.3 abc	26.3 bcd	6121 b-e
PYR	VEG+EREP	23.1 efg	1.7 a	799.1 fg	13.2 a	880.6 a	18.3 efg	1235.9 de	28.8 fg	18.8 cd	6754 ab
PYR	VEG+MREP	71.6 cd	2.1 a	1128.4 a-f	15.3 a	980.6 a	30.8 b-e	1408.4 a-d	61.8 bc	50.0 a	6601 abc
PYR	EREP+MREP	31.6 efg	2.4 a	1120.6 b-e	16.4 a	1024.7 a	32.5 bcd	1454.7 a-d	31.3 efg	13.8 d	6441 abc
PYR	VEG+EREP+MREP	10.2 g	1.5 a	765.3 g	15.4 a	941.3 a	12.4 g	1030.0 e	15.0 g	10.8 d	6339 a-d
PYR+MET	VEG	70.4 cd	3.0 a	1072.8 b-g	15.5 a	948.4 a	25.6 def	1400.9 bcd	66.3 abc	33.8 abc	5389 de
PYR+MET	EREP	46.2 def	2.7 a	1137.8 a-e	16.4 a	960.6 a	30.1 cde	1415.0 a-d	38.8 def	20.5 cd	6211 a-e
PYR+MET	MREP	127.4 a	4.2 a	1482.8 a	19.0 a	1047.2 a	43.5 ab	1574.4 ab	72.5 ab	25.0 bcd	5692 cde
PYR+MET	VEG+EREP	14.8 fg	0.9 a	863.4 d-g	12.2 a	878.1 a	20.9 d-g	1255.9 cde	36.3 def	18.8 cd	6758 ab
PYR+MET	VEG+MREP	18.0 efg	1.1 a	811.9 efg	14.5 a	884.1 a	13.9 fg	1135.0 e	12.5 g	12.0 d	7212 a
PYR+MET	EREP+MREP	36.2 efg	4.4 a	866.2 d-g	14.7 a	945.0 a	33.6 bcd	1472.2 abc	31.3 efg	23.8 cd	6628 abc
PYR+MET	VEG+EREP+MREP	42.3 d-g	1.2 a	964.9 c-g	13.5 a	887.6 a	23.3 d-g	1261.3 cde	53.3 bcd	27.5 bcd	6242 a-e
Untreated Control		135.6 a	4.5 a	1408.4 ab	19.6 a	1083.4 a	43.7 ab	1635.3 a	62.5 abc	37.5 abc	5674 cde
<i>P</i> (α=0.05)		<0.0001	1.0000	0.0686	0.5926	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0044

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessment dates.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i./ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i./ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction

Table 2.8. Effect of hybrid and fungicide treatment on southern corn rust (SCR) northern corn leaf blight (NCLB) and southern corn leaf blight (SCLB) epidemics, necrosis and corn yield in late-planted trial conducted in Attapulga, GA in 2012

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		SCLB <sup>a</sup>	
		AUDSPC	AUDIPC	AUDSPC	AUDIPC	AUDSPC	AUDIPC
<b>Hybrid</b>							
Pioneer 33M57		1.1 A	337.5 A	374.6 A	2990.7 A	1.4 A	350.5 A
Pioneer 33M52		1.4 A	373.0 A	357.3 A	2959.5 A	1.3 A	335.0 A
<i>P</i> ( $\alpha=0.05$ )		0.3954	0.3655	0.1624	0.4041	0.531	0.4433
<b>Treatment</b>							
Fungicide <sup>b</sup>	Application <sup>c</sup>						
PYR	VEG	0.2 b	187.5 e	282.8 gh	2909.1 bc	0.8 bcd	330.0 c-f
PYR	EREP	1.6 ab	491.3 ab	422.9 abc	3043.8 ab	1.7 ab	371.3 b-e
PYR	MREP	2.7 a	431.3 a-d	438.1 ab	3073.8 ab	2.4 a	420.0 ab
PYR	VEG+EREP1	0.4 b	288.8 a-e	334.5 d-h	3058.1 ab	0.7 cd	303.8 d-g
PYR	VEG+,MREP	0.4 b	285.0 b-e	396.7 a-d	2925.9 bc	0.7 cd	240.0 fg
PYR	EREP+MREP	2.0 ab	505.0 a	388.1 a-e	2978.9 bc	2.0 a	513.8 a
PYR	VEG+EREP+MREP	0.3 b	247.5 de	349.8 b-h	3079.0 ab	0.8 bcd	322.5 d-f
PYR+MET	VEG	0.6 b	318.8 a-e	310.6 e-h	2824.1 c	1.5 abc	255.0 f-g
PYR+MET	EREP	2.5 a	495.0 ab	468.1 a	3190.6 a	1.5 abc	300.0 d-g
PYR+MET	MREP	1.4 ab	416.3 a-d	355.8 b-g	2914.7 bc	1.8 ab	461.3 ab
PYR+MET	VEG+EREP	0.5 b	198.8 e	292.4 fgh	2772.8 c	0.7 cd	300.0 d-g
PYR+MET	VEG+MREP	0.4 b	262.5 cde	343.7 e-h	2896.6 bc	0.4 d	210.0 g
PYR+MET	EREP+MREP	1.9 ab	468.8 abc	376.6 b-f	3059.7 ab	1.8 ab	465.0 ab
PYR+MET	VEG+EREP+MREP	1.0 ab	286.3 a-e	268.6 h	2841.6 c	1.0 bcd	266.3 efg
Untreated Control		2.7 a	468.8 abc	423.0 abc	3058.1 ab	2.1a	382.5 b-d
<i>P</i> ( $\alpha=0.05$ )		0.0169	0.0119	<0.0001	0.0028	0.0006	<0.0001

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with two assessment dates for SCR and three assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction.

Table 2.9. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield in Tifton, GA in 2013

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Hybrid</b>							
Pioneer 33M57		6.4 A <sup>d</sup>	343.4 A	3.6 A	163.9 A	57.6 A	14955 A
Pioneer 33M52		0.1 B	47.6 B	2.7 A	136.9 A	54.6 A	14628 A
<i>P</i> ( $\alpha=0.05$ )		<0.0001	<0.0001	0.0832	0.0433	0.1649	0.1203
<b>Treatment</b>							
Fungicide <sup>b</sup>	Application <sup>c</sup>						
PYR	VEG	8.5 a	301.5 a	4.4 a	190.8 a	56.3 a	14879 e
PYR	EREP	1.2 a	154.4 a	4.4 a	207.3 a	52.5 a	16543 a
PYR	MREP	1.9 a	142.9 a	5.0 a	151.3 a	57.5 a	15090 a
PYR	VEG+EREP	1.5 a	203.8 a	2.2 a	119.6 a	48.0 a	14956 a
PYR	VEG+MREP	4.0 a	167.5 a	2.3 a	119.6 a	54.4 a	14554 a
PYR	EREP+MREP	0.2 a	97.7 a	2.3 a	131.3 a	51.9 a	14586 a
PYR	VEG+EREP+MREP	1.3 a	161.7 a	3.7 a	149.0 a	57.6 a	14554 a
PYR+MET	VEG	11.3 a	332.1 a	3.7 a	170.2 a	65.0 a	13699 a
PYR+MET	EREP	1.6 a	200.6 a	3.1 a	160.0 a	53.1 a	15269 a
PYR+MET	MREP	2.8 a	172.9 a	3.1 a	151.3 a	58.8 a	14722 a
PYR+MET	VEG+EREP	2.3 a	245.2 a	2.4 a	135.2 a	53.1 a	15591 a
PYR+MET	VEG+MREP	2.4 a	205.0 a	2.5 a	128.1 a	61.2 a	14670 a
PYR+MET	EREP+MREP	0.6 a	159.2 a	2.4 a	146.0 a	57.5 a	15180 a
PYR+MET	VEG+EREP+MEWP	3.8 a	157.5 a	3.1 a	136.3 a	50.4 a	15110 a
Untreated Control		5.4 a	230.0 a	3.4 a	160.2 a	64.4 a	14161 a
<i>P</i> ( $\alpha=0.05$ )		0.3994	0.1060	0.6525	0.5098	0.1278	0.1482

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with four assessment dates for SCR and six assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction

Table 2.10. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield in Attapulugus, GA in 2013

Variable <sup>f</sup>		SCR <sup>a</sup>			NCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC		AUDSPC	AUDSPC	AUDIPC		
Hybrid								
Pioneer33M57		172.7 A <sup>d</sup>		1982.8 A	21.4 A	833.1 A	67.3 A	10105 A
Pioneer 33M52		11.5 B		846.4 B	18.6 A	857.7 A	36.7 B	10285 A
P(α=0.05)		<0.0001		<0.0001	0.0865	0.613	<0.0001	0.1446
Timing of Application								
Fungicide <sup>b</sup>	Application <sup>c</sup>	P33M57	P33M52					
PYR	VEG	361.9 a	9.8 a	1661.9 ab	23.1 a	900.8 a	64.0 a	9512 e
PYR	EREP	132.9 e-i	13.5 a	1324.6 eh	28.0 a	1054.3 a	54.4 abc	10165 a-e
PYR	MREP	135.7 e-i	7.4 a	1339.6 d-h	20.0 a	900.2 a	45.0 cde	9539 e
PYR	VEG+EREP	207.7 cde	2.6 a	1380.2 c-e	20.1 a	833.1 a	62.5 ab	10144 a-e
PYR	VEG+MREP	170.1 c-g	14.8 a	1561.7 a-d	17.5 a	804.4 a	49.4 cde	9846 de
PYR	EREP+MREP	76.1 hi	1.7 a	1130.2 gh	17.1 a	787.5 a	45.6 cde	10290 a-d
PYR	VEG+EREP+MREP	90.7 ghi	7.5 a	1252.5 fgh	14.7 a	797.5 a	41.9 de	10589 abc
PYR+MET	VEG	307.1 ab	58.7 a	1749.2 a	25.3 a	834.4 a	62.2 ab	10179 a-e
PYR+MET	EREP	156.3 d-h	7.1 a	1401.0 b-e	26.6 a	933.8 a	54.4 abc	10696 a
PYR+MET	MREP	182.5 c-e	12.8 a	1549.2 a-e	25.4 a	1054.6 a	50.0 cde	9933 de
PYR+MET	VEG+EREP	112.8 f-i	5.7 a	1338.5 e-h	20.3 a	803.1 a	56.4 abc	10398 a-d
PYR+MET	VEG+MREP	252.8 bc	6.0 a	1535.4 a-e	12.9 a	636.5 a	48.3 cde	10281 a-d
PYR+MET	EREP+MREP	107.8 g-i	5.2 a	1282.5 e-h	11.6 a	787.5 a	45.6 cde	10666 ab
PYR+MET	VEG+EREP+MREP	64.6 i	1.7 a	1085.6 h	16.7 a	838.4 a	39.3 e	10683 a
Untreated Control		230.8 bcd	17.4 a	1626.7 abc	20.5 a	809.6 a	63.1 ab	10022 b-e
P(α=0.05)		<0.0001	0.9991	<0.0001	<0.0001	0.1594	<0.0001	0.0032

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with five assessment dates.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction

Table 2.11. Effect of hybrid and fungicide treatment on southern corn rust (SCR) northern corn leaf blight (NCLB) and southern corn leaf blight (SCLB) epidemics, necrosis and corn yield in Citra, FL in 2013

Variable <sup>f</sup>		SCR <sup>a</sup>		NCLB <sup>a</sup>		SCLB <sup>a</sup>		Necrosis (%)	Yield (kg/ha)
		AUDSPC	AUDIPC	AUDSPC	AUDIPC	AUDSPC	AUDIPC		
Hybrid									
Pioneer 33M57		42.8A <sup>a</sup>	1947.1A	227.7 A	2374.4 A	56.4 A	2184.2 A	66.3 A	3939 A
Pioneer 33M52		4.4 B	819.6 B	244.8 A	2367.9 A	52.6 A	2184.4 A	52.6 B	4070 A
P(α=0.05)		<0.0001	<0.0001	0.3471	0.8265	0.248	0.9951	<0.0001	0.3397
Timing of Application									
Fungicide <sup>b</sup>	Application <sup>c</sup>								
PYR	VEG	13.7bcd	1047.3d	144.9 f	2294.6 de	71.5 a	2303.1 ab	54.4 de	3861 b-f
PYR	EREP	32.3abc	1539.8abc	370.5 a	2479.4 a	44.9 a	1997.5 d	65.6 abc	3226 efg
PYR	MREP	35.0ab	1632.4ab	315.1 abc	2475.4 ab	54.1 a	2278.5 ab	70.6 ab	3458 d-g
PYR	VEG+EREP	8.9d	1211.9bcd	154.1 f	2378.1 a-d	57.1 a	2339.2 a	44.4 f	4577 ab
PYR	VEG+MREP	25.5bcd	1194.0bcd	208.6 c-f	2424.8 a-d	48.5 a	2105.0 bcd	66.3 ab	4009 b-d
PYR	EREP+MREP	17.6bcd	1344.5a-d	291.6 a-d	2465.4 abc	56.7 a	2218.5 abc	65.6 abc	4247 a-d
PYR	VEG+EREP+MREP	9.7cd	1087.0cd	182.8 ef	2326.4 b-e	59.5 a	2251.8 abc	49.4 def	4557 ab
PYR+MET	VEG	33.6abc	1484.9a-d	227.5 c-e	2313.4 cde	55.1 a	2106.0 bcd	67.3 ab	3970 b-d
PYR+MET	EREP	30.1a-d	1702.9a	336.9 ab	2526.9 a	50.1 a	2283.8 ab	63.8 bc	3593 ac-g
PYR+MET	MREP	27.9bcd	1700.2a	310.1 abc	2475.4 abc	47.3 a	2185.0 a-d	70.6 ab	3078 fg
PYR+MET	VEG+ERP	13.7bcd	1235.8bcd	168.1 f	2180.5 ef	55.8 a	2067.3 cd	49.4 def	4303 abc
PYR+MET	VEG+MREP	22.2bcd	1294.8a-d	144. f	2207.5 ef	65.6 a	2277.9 ab	48.1 def	5053 a
PYR+MET	EREP+MREP	17.2bcd	1623.6ab	270.1 b-e	2523.3 a	56.7 a	2131.5 a-d	56.9 cd	4400 abc
PYR+MET	VEG+EREP+MREP	13.3bcd	1136.7cd	139.2 f	2060.8 f	48.2a	2002.9 d	45.6 ef	4753 ab
Untreated Control		51.9a	1519.8abc	279.5 a-e	2435.2 a-d	57.2 a	2216.5abc	73.5 ab	2982 g
P(α=0.05)		0.0189	0.0177	<0.0001	<0.0001	0.2468	0.0161	<0.0001	<0.0001

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with three assessment dates.

<sup>c</sup> PYR = pyraclostrobin (109 g a.i/ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i/ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP =single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid-treatment interaction.

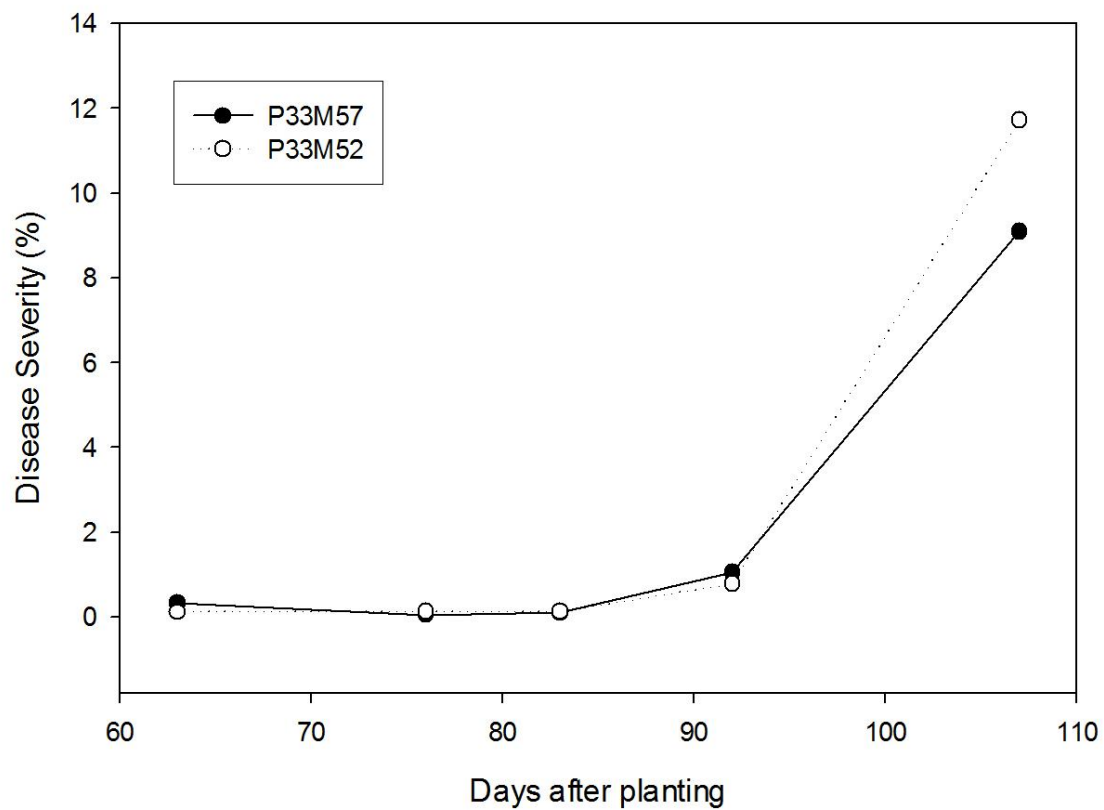


Figure 2.1. Disease progress curve for northern corn leaf blight epidemics in the untreated control plots of two hybrids in Citra, FL on August 27, 2011.

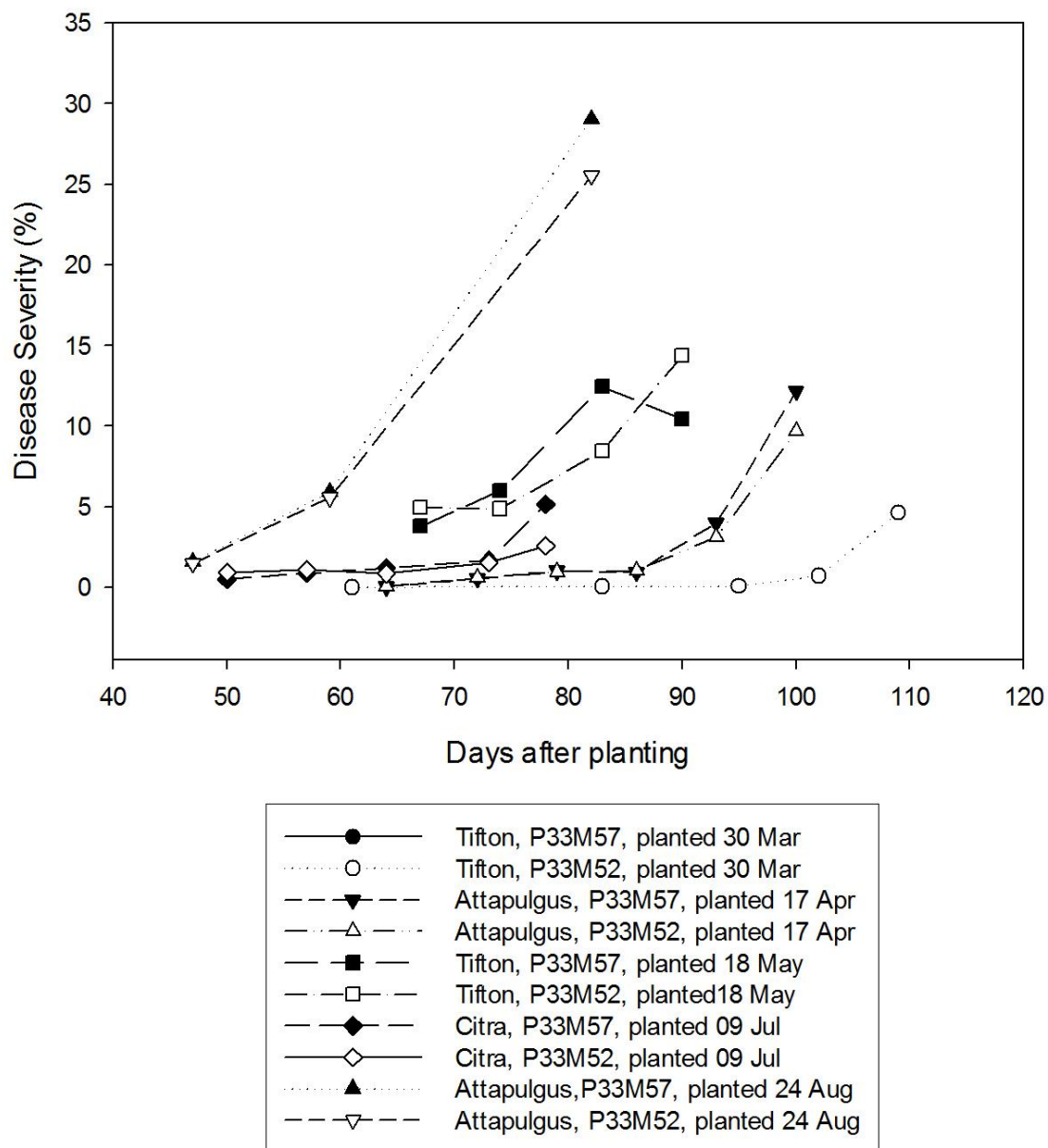


Figure 2.2. Disease progress curve for northern corn leaf blight epidemics in the untreated control plots of two hybrids in all field experiments conducted in 2012.



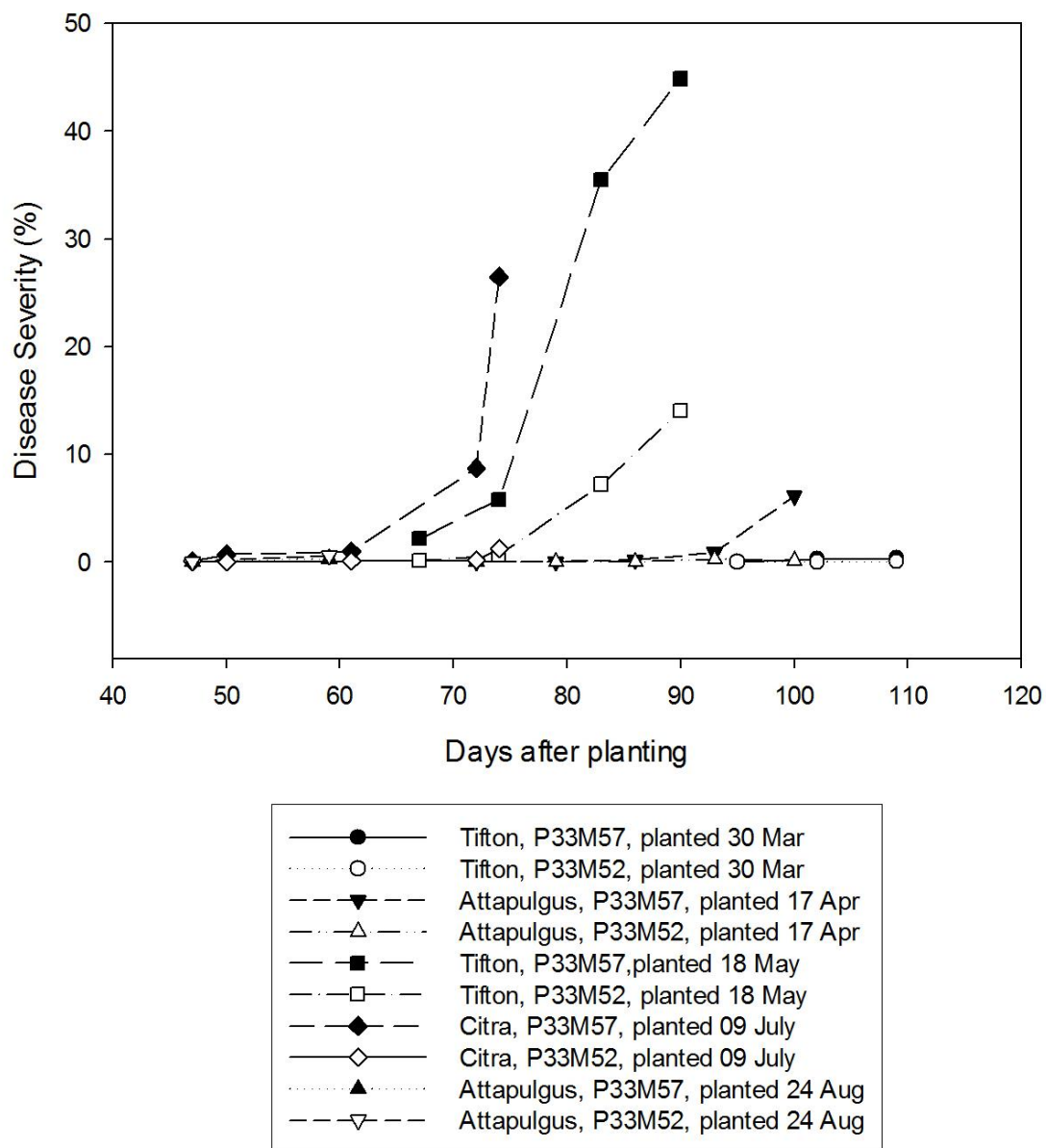


Figure 2.3. Disease progress curve for southern corn rust epidemics in the untreated control plots of two hybrids in all field experiments conducted in 2012.

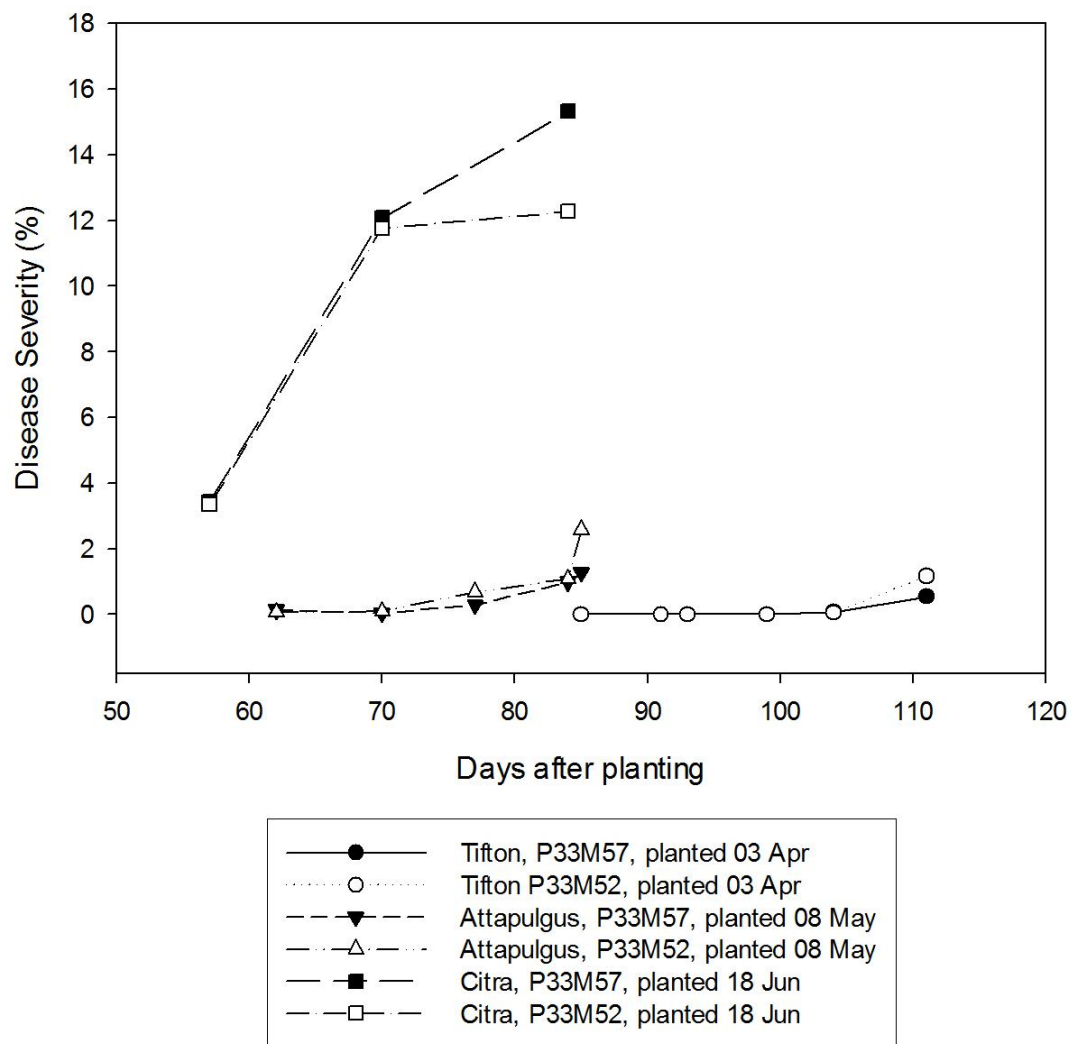


Figure 2.4. Disease progress curve for northern corn leaf blight epidemics in the untreated control plots of two hybrids in all field experiments conducted in 2013.

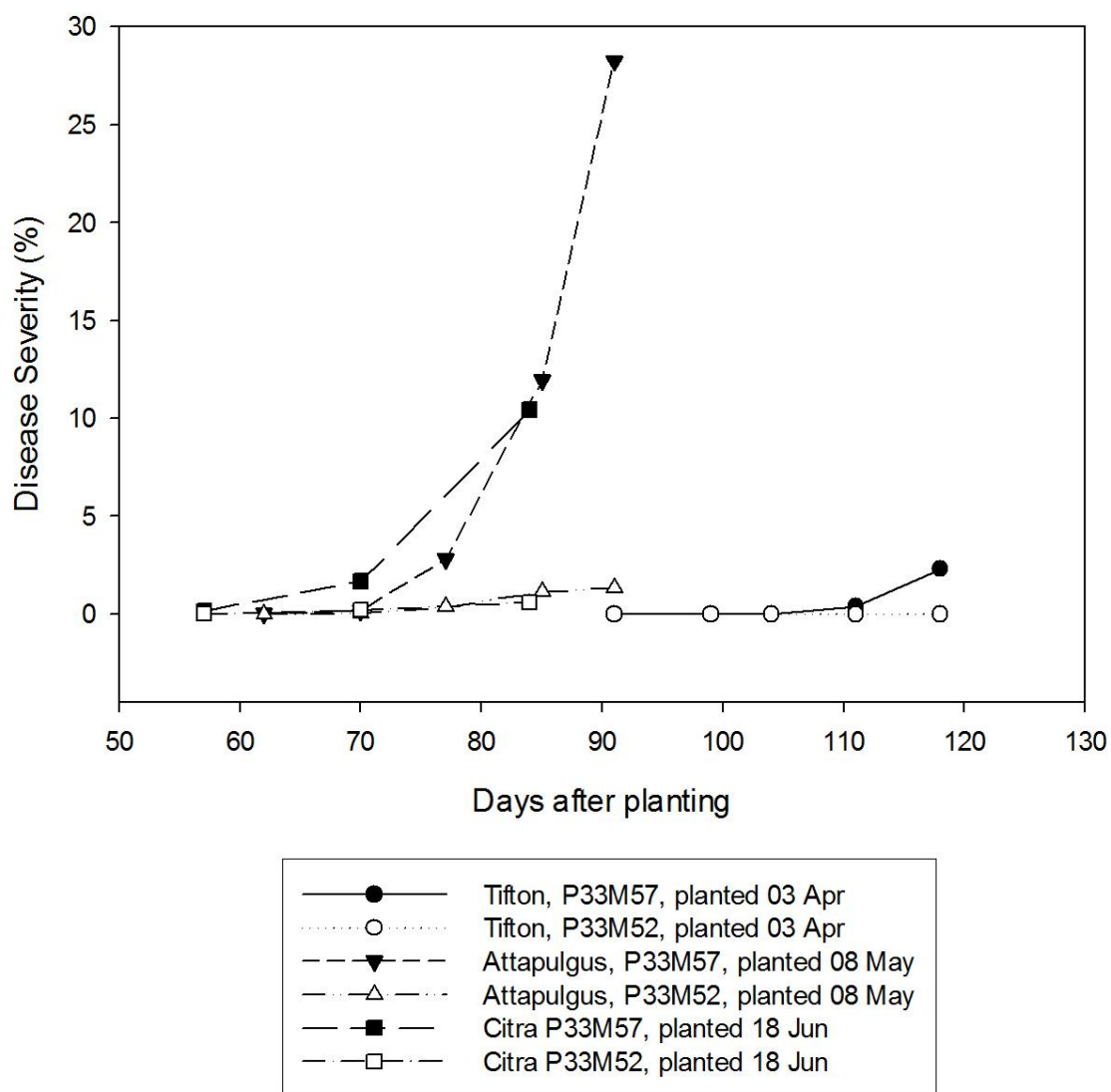


Figure 2.5. Disease progress curve for southern corn rust severity in the untreated control of two hybrids in all field experiments conducted in 2013.

CHAPTER 3

COMPARATIVE EFFICACY OF PYRACLOSTROBIN TO A PRE-MIX OF  
PYRACLOSTROBIN AND FLUXAPYROXAD FOR MANAGEMENT OF FOLIAR  
DISEASES OF CORN

<sup>1</sup>Arcibal, S. S., Kemerait, R. C., Stevenson, K. L. and Newsom, L. J. and 2013. To be submitted to *Plant Disease*.

## **Abstract**

A number of quinone outside inhibitor (QoI) fungicides is currently labeled to manage important foliar diseases such as southern corn rust and northern corn leaf blight. A recently released fungicide product is a premix of a QoI and a succinate dehydrogenase inhibitor (SDHI). The objectives of this study were to compare the efficacy of QoI versus QoI-SDHI fungicides and the timing of application on disease epidemics and yield. Field trials were conducted in Georgia (Tifton and Attapulgus) and Citra, Florida from 2011 to 2013 using the rust-susceptible hybrid Pioneer 33M57 (P33M57). Treatments at each location included pyraclostrobin and pyraclostrobin + fluxapyroxad applied at different timings. Fungicides provided effective disease control and yield increases with timely applications. Significant differences for disease control were observed between fungicides in some trials. Single applications reduced disease intensity but significantly higher yields were only achieved with multiple applications of fungicides. Greatest yield improvement was usually obtained with three applications of fungicide but yields were not significantly different from a well-timed double application. Fungicide treatments increased yields by as much as 28% in Tifton, 9% in Attapulgus and 80% in Citra over the untreated control. Two other diseases, southern corn leaf blight and ear rot, also may have impacted yield. No significant yield differences were observed between the two fungicides used in this study.

## **Introduction**

The two most prevalent foliar diseases of corn in the southern United States are southern corn rust (SCR) caused by *Puccinia polysora* Underwood and northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. Both diseases can cause significant reduction of quality and yield in corn. Yield losses of up to 50% and 70% have been reported for

SCR and NCLB, respectively (8, 17). However, it may be difficult to estimate yield losses separately when both diseases are present.

Foliar diseases of corn can be managed by adjusting planting date, planting resistant hybrids, burying crop residues by tillage, rotation to a non-host crop and applying fungicides at the appropriate time (10, 19). No known commercial varieties are completely resistant to SCR and NCLB due to the presence of virulent races capable of overcoming resistance genes (4, 8, 11). Severe disease epidemics can be avoided by planting early; however, corn growers may still plant partially resistant and susceptible hybrids later in the season to meet market demands. Systemic fungicides have become more popular than protectant fungicides due to longer effective period and curative properties (2, 12).

Fungicides were rarely used in the past to control foliar diseases of hybrid corn. The use of fungicides has increased rapidly since the introduction of quinone outside inhibitor (QoI) fungicides, sometimes referred to as “strobilurins”. Aside from disease control, QoI fungicides have been determined to have beneficial physiologic effects on plants in laboratory and greenhouse experiments (3, 10). One of the most widely used active ingredients on corn is pyraclostrobin (Headline; BASF Corporation, Research Triangle Park, NC). Pyraclostrobin is labeled for control of numerous foliar diseases and also for improvement of plant health. Some believe that the impact of plant health alone will result in an increase in yield. The claim of yield increase regardless of disease pressure has attracted many corn growers to spray fungicides (3). However, the physiological effects of QoI fungicides to yield enhancement has been inconsistent in previous research trials in Georgia (9).

The sterol biosynthesis inhibitors (SBI) and QoI classes are the two most widely used fungicide classes for managing plant diseases. Most fungicides labeled to manage foliar disease

of corn are QoIs, demethylation inhibitors (DMI) in the SBI group and QoI-DMI premix fungicides. Due to extensive use of fungicides in other crops, a number of pathogens have developed resistance to QoIs and reduced sensitivity shifts to DMIs (5, 6, 15). This is one of the main reasons for the successful adoption of the succinate dehydrogenase inhibitor (SDHI) fungicides. SDHIs inhibit fungal respiration by binding to the ubiquinone binding site (Q-site) of the mitochondrial complex II (16). SDHI fungicides are marketed as either solo active ingredient products or premixed with QoIs (16). One of the newly labeled fungicides is Priaxor (BASF Corporation, Research Triangle Park, NC) which is a pre-mix of pyraclostrobin in the QoI class and fluxapyroxad in the SDHI class. However, SDHI resistance has been determined in 14 fungal pathogens within two years of introduction (1, 5, 16). Therefore, SDHI fungicides should be carefully monitored and used according to the label instructions to delay fungicide resistance.

The efficacy of fungicides to control foliar diseases varies. Although registered fungicides can have broad-spectrum activity, their efficacies are also determined by the timing of application. If not timed properly, fungicides may be ineffective and incur additional costs to corn growers without increasing yields. Application of fungicides is usually recommended between the tasseling (VT) and blister (R2) stages (10). In disease-conducive environments, however, diseases may be initiated during the vegetative stages and continue to develop throughout later reproductive stages. There is little research in field corn on the value of applying fungicides during vegetative growth stages and following with a second application at some later time (7, 13). Judicious selection and use of fungicide classes are important to effectively manage SCR and NCLB. The objectives of this study were to compare the efficacies of pyraclostrobin and pyraclostrobin + fluxapyroxad and the timing of application for management of foliar diseases of corn.

## Materials and Methods

*Locations and experimental design.* A total of eight field trials were conducted in Georgia (University of Georgia Coastal Plain Experiment Station in Tifton and Attapulgus Research and Education Center) and Florida (University of Florida Plant Science Research Unit in Citra) from 2011 to 2013. The hybrid Pioneer 33M57, known to be susceptible to both SCR and NCLB, was planted at all locations. Experiments were arranged in a randomized complete block design (RCBD) with four to five replications. Treatments included applications of pyraclostrobin (PYR) (109 g a.i. ha<sup>-1</sup> Headline SC or 110 Headline EC; BASF Corporation, Research Triangle Park, NC) and pyraclostrobin + fluxapyroxad (PYR+FLX) (120 + 60 g a.i. ha<sup>-1</sup>, Priaxor; BASF Corporation, Research Triangle Park, NC). Fungicide timings are represented as follows: (i) VEG (single application between V5 and V7 stages), (ii) EREP (single application during early reproductive stages between VT and R1), (iii) MREP (single application during mid-reproductive stage between R2 and R3), (iv) VEG + EREP, (v) VEG + MREP, (vi) EREP + MREP and (vii) VEG + EREP + MREP. Planting dates, plot dimensions and fungicide application dates varies for each location (Table 3.1). Plots were maintained by using established management practices in each experimental station. All plots were treated with recommended rates of fertilizer, herbicide and insecticide.

*Fungicide applications.* Spray equipment and nozzle tips varied for each location. In Tifton, fungicides were applied using a Lee Spider Spray Trac (Lee Company, Idalou, TX) sprayer with four TeeJet 8003 (Spraying Systems Co., Wheaton, IL) flat fan nozzle tips in 2012 and CO<sub>2</sub> pressurized backpack sprayer with four TeeJet 8002 (Spraying Systems Co., Wheaton, IL) flat fan nozzle tips in 2012 and 2013. In Attapulgus, Lee Spider Spray Trac and CO<sub>2</sub> backpack sprayers with four TeeJet 8002 flat fan nozzle tips were used in 2012 and 2013. In



Citra, a CO<sub>2</sub> backpack sprayer with two TeeJet 8004E (2011) and four Teejet 8002 flat fan nozzle tips (2012 and 2013) was used. For all locations, all nozzle tips were spaced 0.46 m apart and the spray volume was between 187 to 280 liters of water per hectare

*Disease Assessment.* Northern corn leaf blight (NCLB) and SCR were assessed in all field trials. Southern corn leaf blight (SCLB) caused by *Cochliobolus heterostrophus* (syn. *Bipolaris maydis*) was also assessed at Citra in 2012 and 2013. Foliar diseases were visually assessed by arbitrarily and non-destructively selecting 30 leaves on each sampling date from each plot. In 2012, the number of leaves assessed in the late planted trials in Citra and Attapulugus was reduced to 20 leaves per plot due to distance and time to assess trials with high disease pressures. Disease severities were determined by visually estimating the percent leaf area affected for each disease from each leaf. Those assisting with disease assessment were trained prior to assessing field trials. Disease incidences were calculated by dividing the number of infected leaves by the total number of sampled leaves on a per plot basis. In 2012 and 2013, necrosis was also determined at the final assessment date by estimating the percentage of canopy affected by all foliar diseases. All disease assessments were based on a continuous scale from 0 to 100%. Most field trials were assessed weekly and this resulted in a total of between four and seven assessment dates. Two field trials, Attapulugus in 2012 and Citra in 2013, were assessed every two weeks for a total of three rating dates. Initial observations of SCR and NCLB in each trial were recorded based on days after planting (DAP).

Area under the disease severity progress curves (AUDSPC) and area under the disease incidence progress curves (AUDIPC) were calculated from repeated severity and incidence assessments using the trapezoidal method. The formula used to calculate AUDSPC and AUDIPC is the formula for area under the disease progress curve

$$\text{AUDPC} = \sum_{i=1}^{n-1} \left( \frac{y_i + y_{i+1} + 1}{2} \right) (t_{i+1} - t_i)$$

Here  $t$  is the time in days after planting for each assessment,  $y$  is the reading of the variable that was assessed and  $n$  is the number of assessments. In 2011, final severity (%) and incidence (%) were used for SCR since disease symptoms were only observed on the last assessment date.

Yields were obtained in pounds per plot and then converted to kilograms per hectare ( $\text{kg ha}^{-1}$ ).

Area under the disease progress severity curves (AUDSPC) and area under the disease incidence progress curves (AUDIPC) were calculated from repeated severity and incidence assessments to determine the amount of disease over the field season. In 2011, final severity and incidence values were used for analysis of SCR ratings since the disease was observed only during the last assessment date. Data for this study were expressed as transformed proportion relative to the untreated control (%). For example, relative yield was calculated by dividing the yield of a fungicide treatment over the yield of the untreated control then multiplied by 100.

*Statistical analysis.* Data were analyzed separately by trial due to differences in planting dates, disease pressures and environmental conditions for each year. The generalized linear mixed model procedure (PROC GLIMMIX) in SAS (version 9.3, SAS Institute, Cary, NC) was used to determine the effect of fungicide, timing of fungicide application and interaction between the two factors on AUDSPC, AUDIPC, final disease severity, final disease incidence, total plot necrosis and yield. Data were pooled when there was no significant interaction between hybrids and fungicide treatment for each variable. Results of treatments were based on pairwise comparisons of least square means ( $P=0.05$ ). Since data were analyzed as a factorial, statistical comparisons for disease epidemics and yield between fungicide-treated plots and untreated plots could not be performed.

## Results

*Disease detection and disease progress curves.* Foliar diseases were detected sooner after planting in trials planted later during the growing season than in those planted earlier in the season (Table 3.1). However, the highest disease severities were not always found in later-planted trials. The disease severity progress curves of SCR and NCLB from the untreated checks of all field trials are presented by year. In 2012, the highest mean severities for NCLB (17%) and SCR (60%) in the untreated control were observed at Attapulugus and Citra, respectively (Fig. 3.2 and 3.3). In 2013, the highest mean severities for NCLB (15%) and SCR (43%) in the untreated control were observed at Citra and Attapulugus, respectively (Figs. 3.4 and 3.5). Only one trial (Citra) that was conducted in 2011 is reported in this study. In this trial, NCLB was already present at the time of the first disease assessment date (Fig. 3.1) but SCR was not detected until the last assessment date.

*Weather conditions.* Environmental conditions varied over the three growing seasons (Table 3.2). The growing season in 2011 was characterized by prolonged periods of hot and dry weather. Tropical storm Irene did not affect the southeastern United States until late August. The 2012 growing season was more favorable for disease development. Temperatures were warm to hot and rainfall was abundant. Two tropical storms (Beryl and Debby) brought rainfall in late May and late June. Heavy rainfall in August was associated with hurricane Isaac. The growing season in 2013 was initially cold and dry but dramatically shifted to warm and humid beginning in June. Abundant rainfall in June and July was the result of tropical storms Andrea and Dorian, respectively.

*2011 Field Season: Citra.* In this trial, NCLB was first observed at 63 DAP while SCR was not detected until 107 DAP (Table 3.1). The effects of fungicides and the timing of

application are presented in Table 3.3. No significant differences in disease development were observed between PYR and PYR+FLX. Final severities and incidences of SCR did not differ significantly between fungicides or among timings of application. For NCLB, significant differences among fungicide timings were only observed for AUDIPC. Applications at MREP or combined with MREP usually resulted in lower AUDIPC values. Yields associated with fungicide timings were not significantly different from each other but were 101 to 113% of the untreated control.

*2012 Field Season.* In Tifton, NCLB was first observed at 61 DAP and SCR was not found until 95 DAP (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.4. No significant differences in disease development were observed between PYR and PYR+FLX at any disease rating. For SCR, significant differences among fungicide timings were observed among AUDIPC values for timing of application but not for AUDSPC. Single applications at stage EREP or stage MREP, and multiple applications that included MREP resulted in the lowest AUDIPC values. For NCLB, significant differences in AUDSPC were observed among fungicide timings but not for AUDIPC values. Fungicides applied at least once at the MREP stage resulted in the lowest AUDIPC values. No significant differences in total plot necrosis or yield were found among fungicide timings; however, although yields varied numerically between 116 to 119% of the untreated control.

In Attapulgus, NCLB and SCR were detected at 64 DAP and 72 DAP, respectively (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.5. No significant differences in disease development were observed between PYR and PYR+FLX. For SCR, fungicides applied at least once at the MREP stage resulted in the lowest AUDSPC values. There was a fungicide by timing interaction for AUDIPC. Applications that included at

the MREP stage usually resulted in lower AUDIPC values for SCR. However, differences in AUDIPC were only observed when PYR+FLX was applied at EREP and EREP + MREP stages. For NCLB, single fungicide applications at the MREP stage resulted in significantly higher AUDSPC values than all other timings. Multiple applications that included MREP resulted in the lowest percent total plot necrosis. There were no significant differences in yield among fungicide timings although yields were up to 104% of the untreated control. Yield may also have been impacted by ear rot present near the end of the growing season.

In Citra, NCLB and SCR were first observed at 36 DAP and 50 DAP, respectively (Table 3.1). Also, SCLB was first detected at 36 DAP. The effects of fungicides and the timing of application are presented in Table 3.6. Significant differences between PYR and PYR+FLX were only observed in AUDSPC values calculated for NCLB. For NCLB, PYR+FLX applications resulted in significantly lower AUDSPC values than did applications of PYR. However, there were no significant differences in AUDSPC or AUDIPC values among timings of fungicide application. For SCR, fungicides applied at least once at the EREP stage resulted in significantly lower AUDSPC values than all other timings. There was a fungicide by timing interaction for values of AUDIPC. Applications that included both VEG and EREP stages resulted in the lowest AUDIPC values. Application of PYR at the VEG + EREP stages resulted in a AUDIPC that was significantly lower than that of PYR+FLX. However, three applications of PYR resulted in higher AUDIPC values than for PYR+FLX. For SCLB, fungicide applications applied at least once at the VEG stage resulted in significantly lower AUDSPC and AUDIPC values. Multiple applications that included the EREP stage had the lowest total plot necrosis values. Applications that included the VEG + EREP stages resulted in the highest yields. Yield from plots treated with

fungicides at the VEG + EREP stages (175% of the untreated) was as good as those that received three applications (180% of the untreated).

*2013 Field Season.* In the early-planted Tifton trial, both SCR and NCLB were not detected until 91 DAP (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.7. Significant differences between PYR and PYR+FLX were only observed for NCLB. For NCLB, values of AUDSPC and AUDIPC were significantly lower for PYR compared to that of PYR+FLX. However, there were no significant differences in AUDSPC or AUDIPC values among timings of application. For SCR, single fungicide applications at the VEG stage had significantly higher AUDSPC and AUDIPC values than at other timings. There were no significant differences in total plot necrosis and yield among fungicide timings. However, yields of fungicide treatments were up to 106% of the untreated control.

In Attapulgus, both NCLB and SCR were first observed at 55 DAP (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.8. No significant differences in disease development were observed between PYR and PYR+FLX at any disease ratings. For SCR, fungicides applied at least once at the EREP stage resulted in significantly lower AUDSPC and AUDIPC values. For NCLB, neither AUDSPC nor AUDIPC differed significantly among fungicide timings. Multiple applications that included stages EREP + MREP had the lowest total plot necrosis values. Yields did not differ significantly among fungicide timings but varied numerically from 102 to 109% of the untreated control. Yield may also have been impacted by ear rot present near the end of the growing season.

In the late-planted Tifton trial, both NCLB and SCR were first observed at 52 DAP (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.9. No significant differences in disease development were observed between PYR and PYR+FLX

at any disease rating. For SCR, fungicides applied at least once at the EREP stage resulted in lower AUDSPC and AUDIPC values. For NCLB, single fungicide applications at MREP and multiple applications that included stage EREP + MREP resulted in lower AUDSPC and AUDIPC values. Multiple applications that included EREP + MREP had the significantly lower total plot necrosis and higher yields compared to all other treatments. Yield from plots treated with fungicides at the EREP + MREP stages (124% of the untreated) was as good as those that received three applications (128% of the untreated).

At Citra, SCR, SCLB and NCLB were first detected at 37 DAP (Table 3.1). The effects of fungicides and the timing of application are presented in Table 3.10. Significant differences between PYR and PYR+FLX were only observed for AUDIPC values of SCLB. For SCLB, values of AUDSPC and AUDIPC were significantly lower for PYR+FLX compared to that of PYR. No significant differences among fungicide timings were observed for AUDSPC or AUDIPC values of any disease. Nevertheless, apparent trends for AUDSPC values of SCR and NCLB were observed. Fungicides applied at least once at the VEG and EREP stages tended to reduce AUDSPC values of SCR and NCLB, respectively. Applications that included EREP and MREP stages typically reduced total plot necrosis. Yields did not differ significantly among fungicide timings although yields were up to 134% of the untreated control.

## **Discussion**

Fluxapyroxad is one of the newest active ingredients that belong to the SDHI class. The QoI-SDHI premix pyraclostrobin + fluxapyroxad (Priaxor) received full registration for disease management of corn and other crops in 2012. This is the first comprehensive study to evaluate the efficacy of pyraclostrobin + fluxapyroxad compared to the established fungicide pyraclostrobin (Headline) for management of foliar diseases of corn. In this study, disease

assessments and yield data were analyzed as a proportion of the respective untreated control. Such was to directly compare the efficacy of the two fungicides and determine the most appropriate timing of fungicide applications.

Both fungicides were effective for managing foliar diseases and increasing yields of corn. The differences between AUDSPC and AUDIPC values for these fungicides were usually not statistically significant. At Attapulgis in 2012, however; significant differences in SCR AUDIPC among timings were only observed for pyraclostrobin + fluxapyroxad applications. Significantly higher NCLB AUDPC values were observed in PYR+FLX than PYR in early-planted Tifton trial in 2013. However, final NCLB severity of the untreated control was less than 1%. Differences in AUDPC values were not observed in any other trials where NCLB levels were severe. Significantly lower NCLB AUDSPC and SCLB AUDIPC values for pyraclostrobin + fluxapyroxad as compared to pyraclostrobin alone were also found in field trials conducted at Citra in 2012 and 2013, respectively. Improved disease control observed for pyraclostrobin + fluxapyroxad may not be due to the addition of fluxapyroxad but rather the result of higher amounts of pyraclostrobin in this pre-mix product as compared to the individual product. However, significant yield differences between fungicides were not observed throughout the study.

Timing of fungicide application impacts the efficacy of fungicides to control foliar diseases. The most effective fungicide timings vary depending on the onset and development of foliar diseases. Similar results were shown in the previous chapter and in several other field trials (13, 14, 18). In early-planted Tifton and Attapulgis trials, fungicide applications at the mid-reproductive stages resulted in significantly lower AUDSPC and AUDIPC values for SCR and NCLB. However the diseases in these trials were not severe enough to demonstrate significantly



improved yields among fungicide timings. The efficacy of fungicide applied during vegetative growth stages was shown in later-planted trials. Fungicide application at the early reproductive stages was most successful for management of SCR. Applications during the vegetative growth stages were more effective for management of NCLB and SCLB. However, a single application was not always sufficient since two or more foliar diseases were present in the field so multiple fungicide applications may be needed. The highest yields were usually obtained with three applications of fungicides. However, fewer applications resulted in similar yields when timed near disease onset. Yields from treatments that included well-timed fungicide applications were as much as 180% of the untreated control.

The effect of fungicide application timing was oftentimes not significantly different among treatments when fungicides were applied well before onset of disease. At Tifton in 2013, however, fungicides applied at the early vegetative stage had significantly higher AUDSPC value for SCR than the remaining timings. Aside from this trial, numerically higher AUDSPC and AUDIPC values relative to the untreated control were observed in other early-planted trials. However, these values were only observed in early-planted trials and it is unclear why this occurred.

From this study, pyraclostrobin and pyraclostrobin + fluxapyroxad were effective for disease control and yield improvement. Although pyraclostrobin + fluxapyroxad was more effective for reducing SCR and SCLB in some trials, yields were not significantly different from pyraclostrobin alone. However, the QoI-SDHI premix formulation of pyraclostrobin + fluxapyroxad can be an important tool in the practice of fungicide resistance management. More studies are needed to determine the efficacy of pyraclostrobin + fluxapyroxad compared to other

active ingredients. Fungicides should only be used when needed; this is especially for plant pathogens that have high risks of developing resistance to QoI and SDHI fungicides (6, 16).

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Table 3.1. Field locations, plot details and fungicide timings used to manage northern corn leaf blight (NCLB) and southern corn rust (SCR)

Planting Date	Location	Plot size (m)	Spacing (m)	Blocks	Days after planting				
					Fungicide application			First disease detection	
					VEG	EREP	MREP	NCLB	SCR
27 Apr 2011	Citra, FL	1.8×7.6	0.8	4	29	56	70	63	107
30 Mar 2012	Tifton, GA	1.8×7.6	0.9	4	33	67	81	61	95
17 Apr 2012	Attapulgus, GA	1.8×7.0	0.9	5	35	58	72	64	72
09 Jul 2012	Citra, FL	1.8×7.6	0.8	4	36	52	70	36	52
03 Apr 2013	Tifton, GA	1.8×7.6	0.9	4	33	69	82	91	91
08 May 2013	Attapulgus, GA	1.8×7.6	0.9	4	22	55	72	55	55
20 May 2013	Tifton, GA	1.8×7.0	0.9	4	22	53	64	52	52
18 Jun 2013	Citra, FL	1.8×7.6	0.8	4	37	64	83	37	37

<sup>a</sup> Florida location was the University of Florida, Plant Science Research and Education Unit, Tifton location was the University of Georgia Coastal Plain Experiment Station and Attapulgus location was the University of Georgia Attapulgus Research and Education Center

<sup>b</sup> Fungicide timings based upon growth stages of corn. VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3.

Table 3.2. Weather conditions at field sites for corn growing seasons from 2011 to 2013

Year	Location	Month							
		Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Monthly rainfall (mm)									
2011	Citra, FL <sup>a</sup>	95	60	42	126	75	166	99	172
2012	Tifton, GA <sup>b</sup>	120	31	88	133	169	340	76	40
	Attapulgis, GA <sup>c</sup>	125	41	63	147	221	370	105	22
	Citra, FL	33	101	106	381	149	287	119	57
2013	Tifton, GA	80	113	66	338	147	221	79	16
	Attapulgis, GA	132	121	22	110	303	158	83	16
	Citra, FL	12	75	44	165	238	202	106	40
Mean Temperature (°C)									
2011	Citra, FL	18.1	22.1	24.0	26.7	27.0	27.7	25.3	19.6
2012	Tifton, GA	19.2	19.7	23.8	24.7	27.5	25.8	24.3	19.5
	Attapulgis, GA	19.3	19.7	23.9	25.1	27.2	26.0	24.3	20.5
	Citra, FL	20.2	21.2	24.2	25.2	26.3	25.8	25.2	21.3
2013	Tifton, GA	11.5	18.3	21.2	26.0	26.1	26.6	24.5	19.9
	Attapulgis, GA	11.9	18.2	21.3	26.2	25.4	26.3	24.8	19.9
	Citra, FL	13.7	20.8	22.2	26.0	25.6	26.5	25.5	21.7

<sup>a</sup> Archived weather data in Plant Science Research and Education Unit retrieved from the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu>).

<sup>b</sup> Historical data in Coastal Plain Experiment Station retrieved from the Georgia Automated Environmental Monitoring Network (<http://www.georgiaweather.net/>).

<sup>c</sup> Historical data in Attapulgus Research and Education Center retrieved from the Georgia Automated Environmental Monitoring Network (<http://www.georgiaweather.net/>).

Table 3.3 Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield as a proportion shown as percentage of the untreated control in Citra, FL in 2011

Variable <sup>g</sup>	SCR <sup>a</sup>		NCLB <sup>b</sup>		Yield <sup>f</sup>
	FDS	FDI	AUDSPC	AUDIPC	
<b>Fungicide<sup>c</sup></b>					
PYR	49.1 A	113.7 A	88.6 A	108.3 A	108.9 A
PYR+FLX	90.8 A	111.3 A	80.8 A	97.5 A	106.7 A
<i>P</i> ( $\alpha=0.05$ )	0.2262	0.9327	0.2705	0.1035	0.5324
<b>Timing<sup>d</sup></b>					
VEG	135.6 a	155.6 a	71.4 a	81.8 b	109.0 a
EREP	39.0 a	100.0 a	103.0 a	118.8 a	109.8 a
MREP	95.9 a	83.0 a	74.9 a	92.2 b	112.7 a
VEG+EREP	95.9 a	83.5 a	91.3 a	125.4 a	108.5 a
VEG+MREP	67.1 a	166.5 a	87.2 a	102.0 ab	108.2 a
EREP+MREP	11.0 a	49.5 a	81.3 a	84.4 b	105.4 a
VEG+EREP+MREP	45.2 a	149.5 a	83.9 a	117.3 a	101.1 a
<i>P</i> ( $\alpha=0.05$ )	0.5198	0.2475	0.2872	0.0018	0.6591

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessment dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 10473 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.4. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in Tifton, GA in 2012

Variable <sup>g</sup>	SCR		NCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	45.8 A	55.3 A	61.5 A	78.4 A	80.1 A	118.0 A
PYR+FLX	26.2 A	63.3 A	57.3 A	79.4 A	85.4 A	117.6 A
<i>P</i> ( $\alpha$ =0.05)	0.3832	0.4491	0.5861	0.8599	0.4796	0.8070
<b>Timing<sup>d</sup></b>						
VEG	134.9 a	87.1a	88.6 a	91.7 a	82.3 a	116.4 a
EREP	48.0 a	65.1abc	65.7 abc	91.5 a	95.8 a	117.4 a
MREP	7.0 a	44.7bc	47.0 bc	76.4 a	86.5 a	118.9 a
VEG+EREP	15.6 a	92.5a	75.5 ab	83.9 a	96.9 a	117.4 a
VEG+MREP	19.7 a	66.1ab	55.7 bc	73.6 a	85.4 a	118.1 a
EREP+MREP	25.7 a	31.6bc	42.0 c	67.5 a	57.3 a	119.0 a
VEG+EREP+MREP	11.7 a	28.0c	44.3 bc	67.7 a	75.0 a	117.4 a
<i>P</i> ( $\alpha$ =0.05)	0.1925	0.0079	0.0172	0.0833	0.1126	0.9877

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with three assessment dates for SCR and five assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 17495 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.5 Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in Attapulgis, GA in 2012

Variable <sup>g</sup>	SCR		NCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	19.8 A	50.1 A	82.0 A	93.4 A	68.7 A	102.3 A
PYR+FLX	11.7 A	43.0 A	81.2 A	94.5 A	64.3 A	102.0 A
<i>P</i> ( $\alpha$ =0.05)	0.1049	0.4170	0.9027	0.6795	0.3291	0.8762
<b>Timing<sup>d</sup></b>						
		<u>PYR</u>	<u>PYR+FLX</u>			
VEG	28.7 a	68.7 a	78.5 a	78.8 b	68.7 a	82.3 ab
EREP	23.7 ab	81.5 a	14.1 c	83.6 b	81.5 a	83.1 a
MREP	7.8 bc	43.5 a	20.4 bc	113.3 a	43.5 a	66.1 bc
VEG+EREP	26.3 ab	40.8 a	84.6 a	75.5 b	40.9 a	76.6 ab
VEG+MREP	17.4 abc	38.3 a	66.4 ab	69.1 b	38.3 a	55.6 cd
EREP+MREP	4.1 c	50.1 a	17.5 c	75.2 b	50.1 a	54.8 cd
VEG+EREP+MREP	2.4 c	28.01 a	19.5 c	76.0 b	28.0 a	46.8 d
<i>P</i> ( $\alpha$ =0.05)	0.0148	0.2762	0.0049	0.0174	0.1946	<0.0001

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with five assessment dates for SCR and six assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 10708 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.



Table 3.6. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in Citra, FL in 2012

Variable <sup>g</sup>	SCR		NCLB		SCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>								
PYR	45.8 A	67.8 A	90.2 A	82.5 A	57.1 A	80.1 A	62.0 A	149.8 A
PYR+FLX	52.0 A	71.0 A	77.8 A	77.8 A	55.9 A	75.2 A	61.0 A	152.3 A
<i>P</i> ( $\alpha=0.05$ )	0.3801	0.3733	0.0404	0.3840	0.8688	0.2388	0.8409	0.7657
<b>Timing<sup>d</sup></b>								
		<u>PYR</u>	<u>PYR+FLX</u>					
VEG	93.1 a	64.8 b	74.5 ab	93.3 a	84.5 a	37.4 c	65.4 b	116.4 a
EREP	33.9 b	84.5 a	82.2 a	88.9 a	85.6 a	86.2 b	100.8 a	51.9 c
MREP	95.3 a	102.5 a	90.9 a	93.1 a	81.7 a	113.8 a	100.4 a	96.2 b
VEG+EREP	19.9 b	34.4 d	58.3 b	87.0 a	81.2 a	19.3 c	50.6 b	26.9 d
VEG+MREP	69.6 a	56.4 bc	78.6 a	72.9 a	76.8 a	35.6 c	65.0 b	88.5 b
EREP+MREP	16.5 b	86.6 a	79.5 a	75.1 a	75.5 a	81.4 b	101.0 a	23.1 d
VEG+EREP+MREP	14.0 b	45.1 cd	33.1 c	77.7 a	75.8 a	21.9 c	60.2 b	27.5 d
<i>P</i> ( $\alpha=0.05$ )	<0.0001	<0.0001	<0.0001	0.5434	0.9101	<0.0001	<0.0001	<0.0001

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessment dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 3658 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.7. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in early-planted trial at Tifton, GA in 2013

Variable <sup>g</sup>	SCR		NCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	69.9 A	47.2 A	87.0 B	71.4 B	96.2 A	101.3 A
PYR+FLX	64.4 A	49.1 A	276.1A	178.3A	96.9 A	102.2 A
<i>P</i> ( $\alpha$ =0.05)	0.8844	0.9383	0.0493	0.0072	0.9002	0.7636
<b>Timing<sup>d</sup></b>						
VEG	274.9 a	152.4 a	241.1 a	140.3 a	90.4 a	104.6 a
EREP	65.0 b	47.6 b	327.3 a	230.7 a	100.0 a	106.3 a
MREP	16.2 b	35.5 b	201.1 a	87.5 a	100.0 a	102.8 a
VEG+EREP	16.2 b	14.5 b	115.5 a	125.0 a	97.1 a	100.7 a
VEG+MREP	32.6 b	71.0 b	237.5 a	122.9 a	85.6 a	104.0 a
EREP+MREP	16.2 b	4.0 b	27.8 a	37.5 a	106.7 a	93.5 a
VEG+EREP+MREP	48.7 b	12.1 b	120.3 a	130.3 a	95.8 a	100.3 a
<i>P</i> ( $\alpha$ =0.05)	0.0131	0.0402	0.6825	0.2566	0.5393	0.3579

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with four assessment dates for SCR and six assessment dates for NCLB.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 13605 kg/ha. <sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.8. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in Attapulgus, GA in 2013

Variable <sup>g</sup>	SCR		NCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	55.8 A	86.5 A	121.5 A	116.3 A	70.6 A	107.9 A
PYR+FLX	53.8 A	86.4 A	113.3 A	107.7 A	76.8 A	104.9 A
<i>P</i> ( $\alpha$ =0.05)	0.7122	0.9810	0.5662	0.2460	0.1645	0.1224
<b>Timing<sup>d</sup></b>						
VEG	80.8 a	94.6 a	116.9 a	115.2 a	94.9 a	102.0 a
EREP	51.5 bc	84.0 bc	125.8 a	118.2 a	81.7 ab	109.2 a
MREP	64.1 ab	93.1 ab	103.4 a	101.8 a	76.9 b	104.8 a
VEG+EREP	41.3 c	77.2 c	128.5 a	110.3 a	74.8 b	109.0 a
VEG+MREP	71.6 ab	94.5 a	103.6 a	110.8 a	78.9 ab	102.3 a
EREP+MREP	37.3 c	82.8 c	130.7 a	114.2 a	55.4 c	108.2 a
VEG+EREP+MREP	36.8 c	79.0 c	113.0 a	113.5 a	53.3 c	109.2 a
<i>P</i> ( $\alpha$ =0.05)	0.0002	0.0013	0.8936	0.9370	<0.0001	0.1231

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with five assessment dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 9488 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.9 Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in late-planted trial at Tifton, GA in 2013

Variable <sup>g</sup>	SCR		NCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	33.3 A	91.7 A	69.4 A	90.5 A	58.5 A	114.3 A
PYR+FLX	30.7 A	93.7 A	69.2 A	90.7 A	59.9 A	114.6 A
<i>P</i> ( $\alpha=0.05$ )	0.6290	0.4781	0.9874	0.9761	0.6370	0.9146
<b>Timing<sup>d</sup></b>						
VEG	87.0 a	100.7 a	108.2 a	111.7 a	99.6 a	100.2 d
EREP	21.2 bc	87.7 d	75.6 ab	102.2 ab	71.2 b	107.1 cd
MREP	35.3 b	99.2 ab	53.4 b	79.9 bc	55.0 c	110.9 cd
VEG+EREP	22.1 bc	86.4 d	73.1 ab	97.5 ab	71.2 b	114.4 bc
VEG+MREP	35.6 b	98.2 abc	77.4 ab	97.5 ab	52.3 c	116.4 abc
EREP+MREP	12.5 c	88.0 cd	55.2 b	78.6 bc	32.3 d	124.4 a
VEG+EREP+MREP	10.0 c	89.1 bcd	42.4 b	66.6 c	32.7 d	127.7 a
<i>P</i> ( $\alpha=0.05$ )	<0.0001	0.0180	0.0303	0.0361	<0.0001	0.0006

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with seven assessment dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 8089 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

Table 3.10. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics, necrosis and corn yield as a proportion shown as percentage of the untreated control in Citra, FL in 2013

Variable <sup>g</sup>	SCR		NCLB		SCLB		Necrosis	Yield <sup>f</sup>
	AUDSPC <sup>a</sup>	AUDIPC <sup>b</sup>	AUDSPC	AUDIPC	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>								
PYR	31.8 A	84.7 A	83.5 A	96.5 A	77.8 A	97.3 A	73.95 A	110.0 A
PYR+FLX	49.7 A	91.8 A	93.5 A	100.1 A	59.4 A	92.5 B	77.31 A	109.0 A
<i>P</i> ( $\alpha$ =0.05)	0.0970	0.2056	0.2601	0.1093	0.0521	0.0003	0.3355	0.8964
<b>Timing<sup>d</sup></b>								
VEG	53.5 a	88.5 a	82.3 a	98.2 a	53.4 a	95.8 a	87.5 a	110.3 a
EREP	26.8 a	96.5 a	101.5 a	102.9 a	86.0 a	96.0 a	74.3 bc	102.8 a
MREP	61.2 a	93.8 a	112.6 a	97.8 a	69.8 a	95.5 a	83.1 ab	103.7 a
VEG+EREP	47.4 a	87.4 a	89.3 a	99.7 a	52.5 a	95.1 a	74.3 bc	112.1 a
VEG+MREP	37.7 a	87.4 a	68.5 a	95.3 a	76.8 a	95.4 a	72.8 bc	101.0 a
EREP+MREP	35.2 a	84.1 a	91.4 a	97.1 a	71.9 a	95.5 a	75.7 ab	102.4 a
VEG+EREP+MREP	23.0 a	81.7 a	73.9 a	96.7 a	69.8 a	91.3 a	61.8 c	134.3 a
<i>P</i> ( $\alpha$ =0.05)	0.4187	0.7719	0.1314	0.6265	0.4437	0.4371	0.0117	0.2884

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with three assessment dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3).

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 2759 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

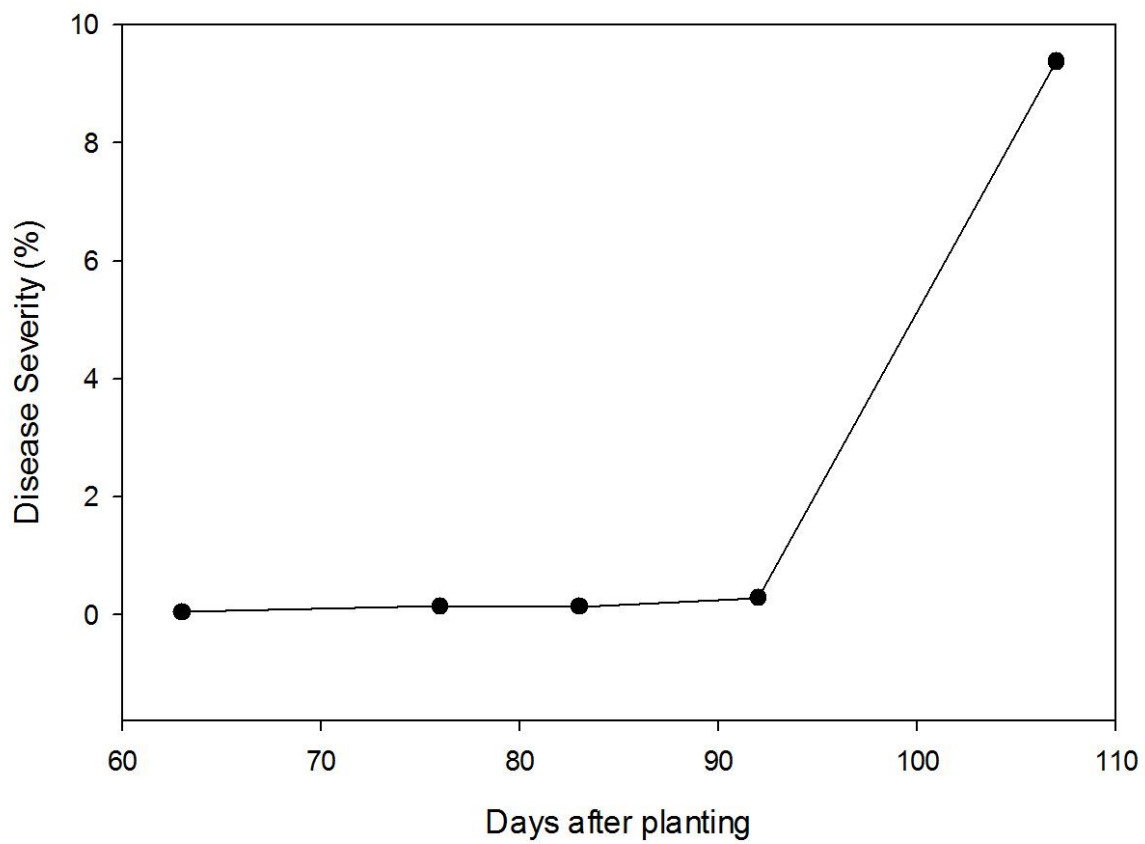


Fig. 3.1. Disease progress curve for northern corn leaf blight the untreated control in Citra, FL on April 27, 2011.

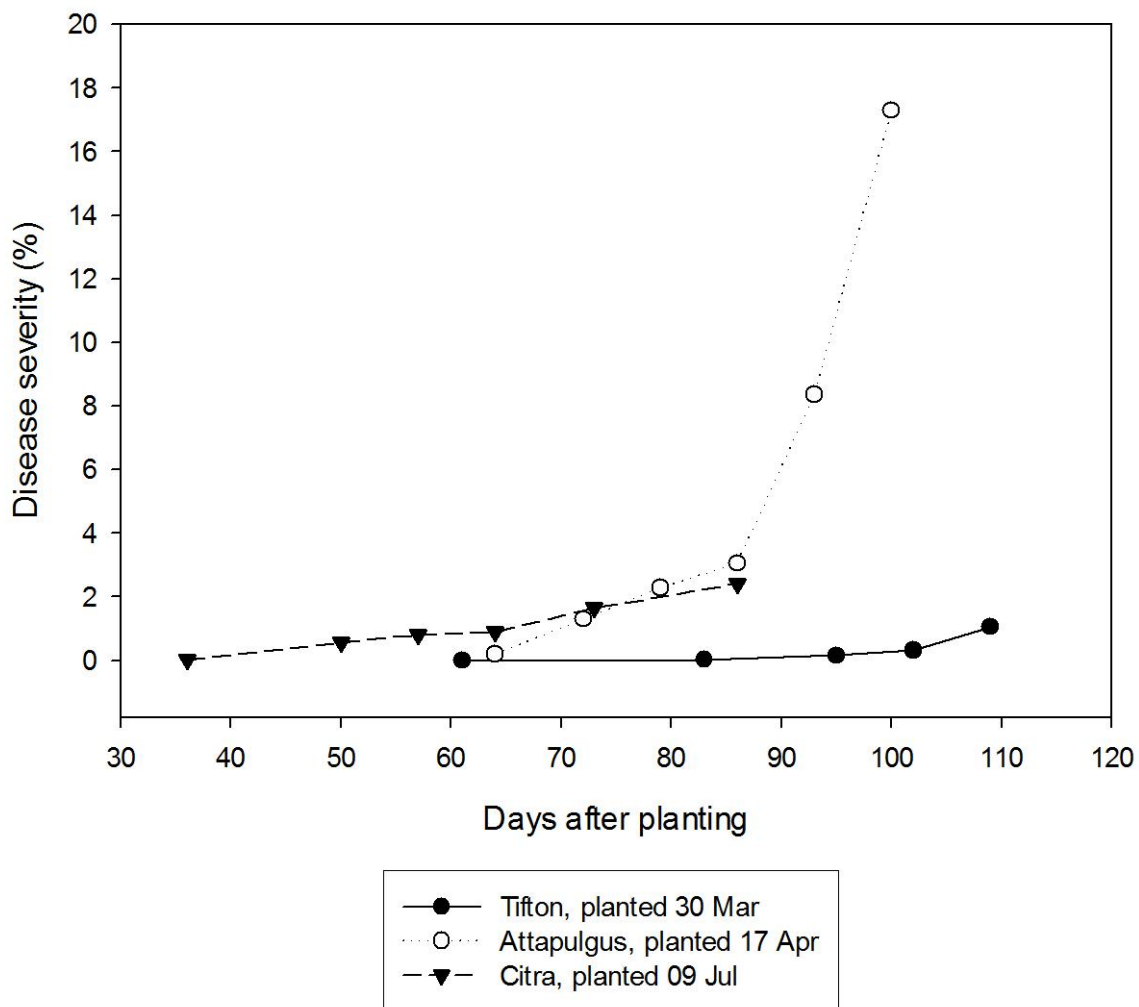


Figure 3.2. Disease progress curves for northern corn leaf blight in the untreated control plots for all field experiments conducted in 2012.

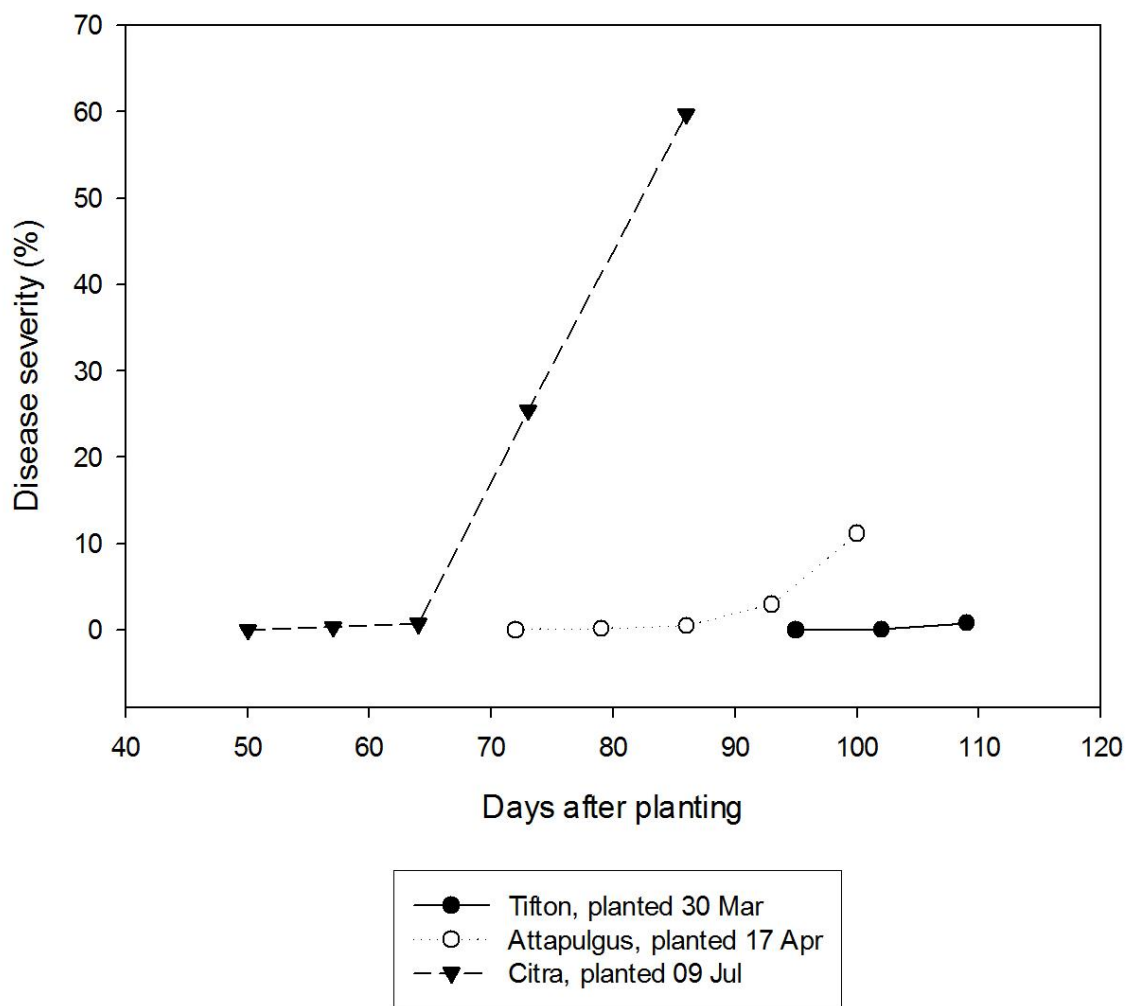


Figure 3.3. Disease progress curves for southern corn rust in the untreated control plots for all field experiments conducted in 2012.



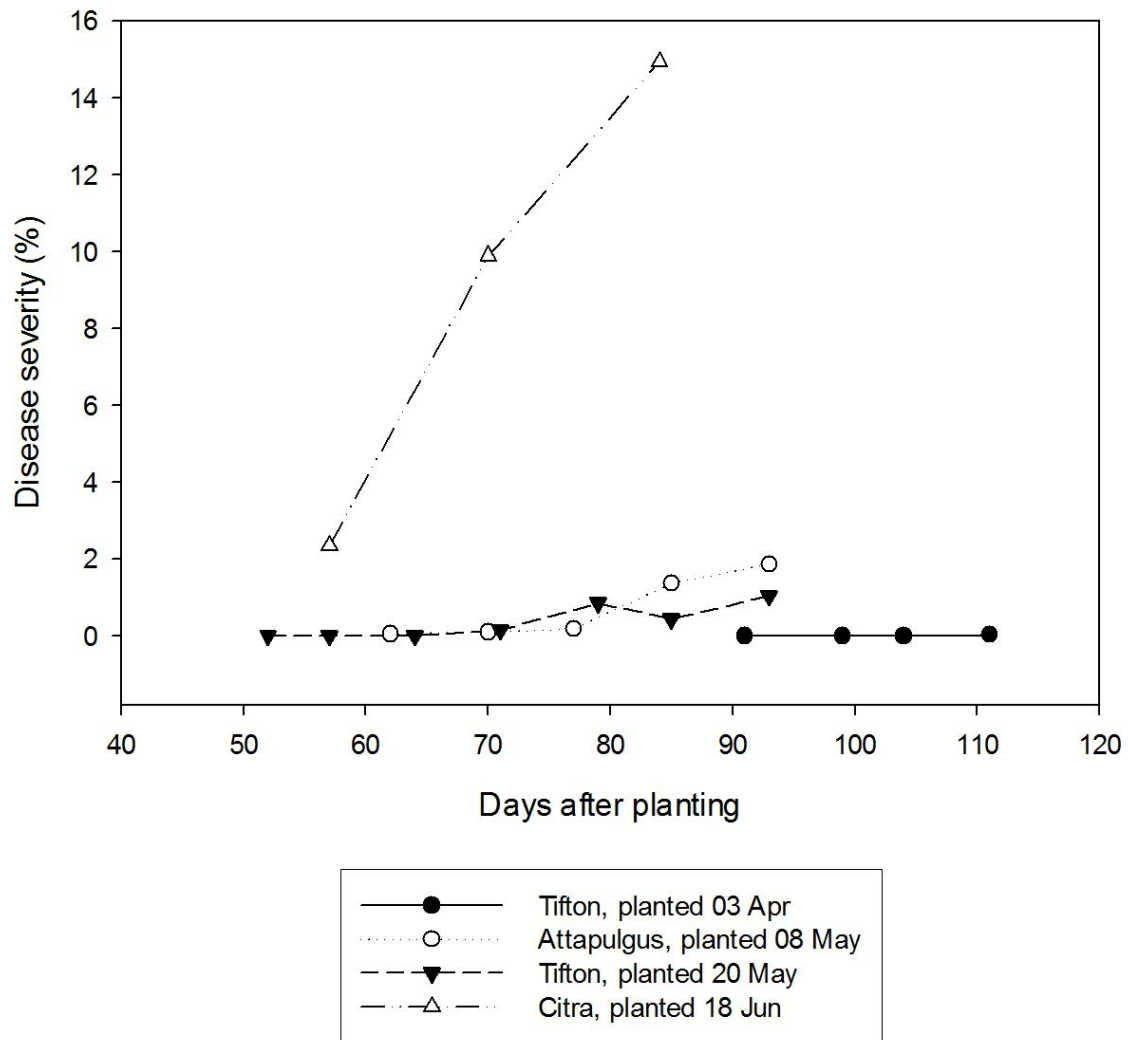


Figure 3.4. Disease progress curve for northern corn leaf blight in the untreated plots for all field experiments conducted in 2013.

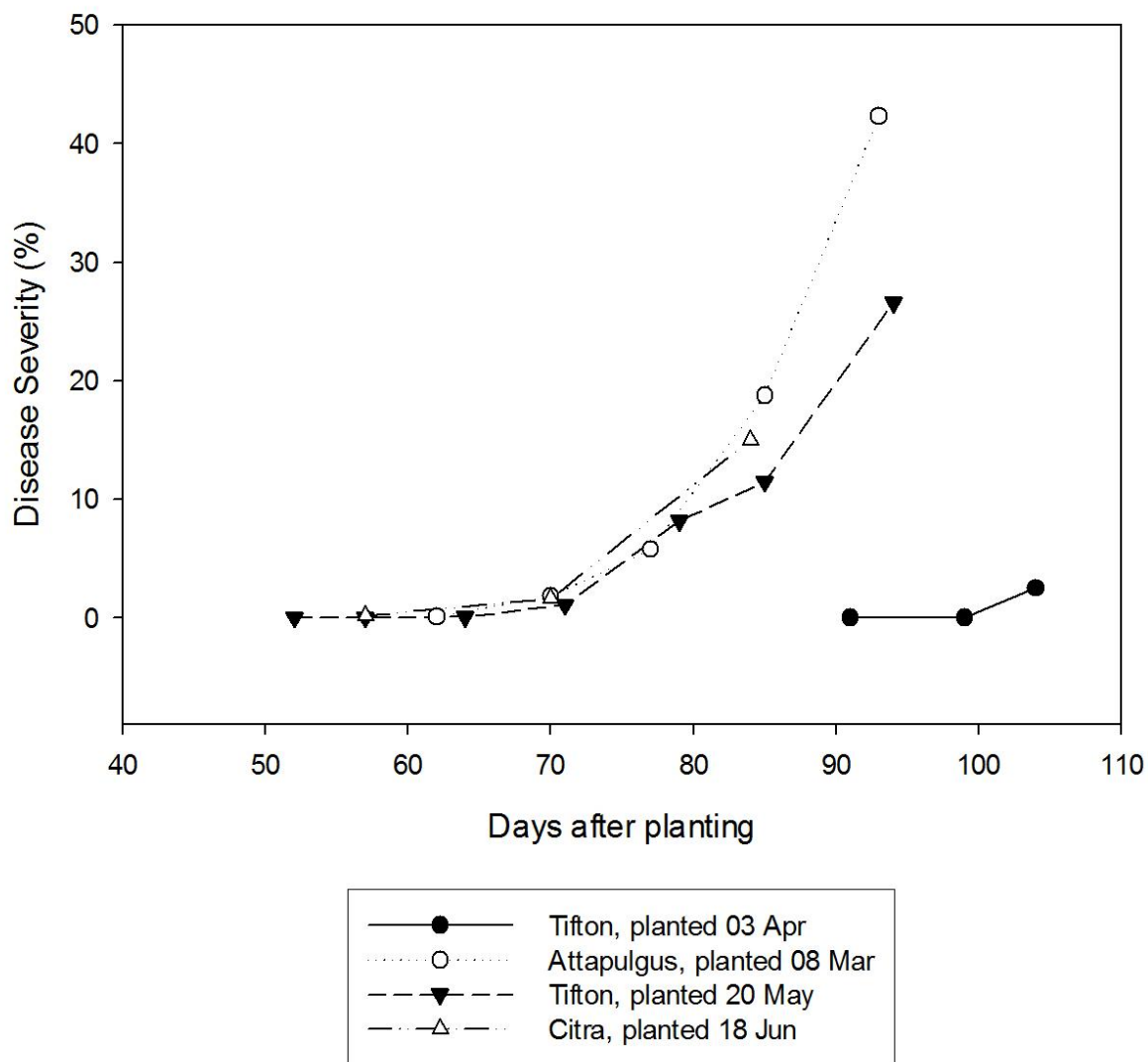


Figure 3.5. Disease progress curves for southern corn rust in the untreated control plots for all field experiments conducted in 2013.

CHAPTER 4

SENSITIVITY OF *EXSEROHILUM TURCICUM* TO THE DEMETHYLATION INHIBITOR  
METCONAZOLE

<sup>1</sup>Arcibal, S. S., Stevenson, K. L. and Kemerait, R. C. 2013. To be submitted to *Plant Health Progress*.

## Abstract

*Exserohilum turcicum*, the causal agent of northern corn leaf blight (NCLB), can cause significant yield loss in the United States. Severe NCLB outbreaks were reported in Georgia in 2008 and 2009 and the use of fungicides was common among growers. Demethylation inhibitor (DMI) fungicides have been used to manage NCLB for many years in the United States. Metconazole is one of the newest active ingredients in the DMI class. DMIs are marketed as solo active ingredients or premixed with quinone outside inhibitors (QoI). Fungicide resistance has not been reported in *E. turcicum* to date. To facilitate fungicide resistance monitoring, 25 isolates of *E. turcicum* were collected from fields and tested in vitro using metconazole-amended medium to determine the effective concentration at which 50% of mycelial growth is inhibited ( $EC_{50}$ ).  $EC_{50}$  values ranged from 0.008 to 0.155  $\mu\text{g/ml}$  and the mean and median values were 0.026 and 0.017, respectively. These  $EC_{50}$  values will be used to determine possible shifts in sensitivity of *E. turcicum* populations to DMI fungicides.

## Introduction

Northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, is one of the most important foliar diseases of corn in Georgia. Northern corn leaf blight was recognized as a serious problem for growers in Georgia when severe outbreaks occurred in 2008 and 2009. Historical yield losses of up to 70% were reported on a susceptible hybrid inoculated with *E. turcicum* (28). NCLB and other foliar diseases of corn can be managed by planting resistant hybrids, burying crop residues by tillage, rotation to a non-host crop and by applying foliar fungicides (20, 21, 28). Northern corn leaf blight has been primarily managed by host resistance; however, complete resistance is impossible due to the presence of virulent *E. turcicum* races (22).

Fungicides have been used for many years to control NCLB on sweet and dent corn inbreds. The trend of fungicide use on corn shifted from protectant to systemic fungicides since the late 1980s. Protectant fungicides such as chlorothalonil, maneb and mancozeb were traditionally used to control NCLB. These fungicides were most effective when applied prior to disease onset and then weekly through the remainder of the growing season. Systemic fungicides have longer effective periods. Thus, systemic fungicides offer a more cost-effective choice for corn producers because these fungicides have longer effective period (3, 23). The use of systemic fungicides has become more popular because these fungicides provide disease control and potential plant health benefits. Some of these fungicides are marketed to have physiological effects which results in a yield increase even in the absence of plant diseases. This reason alone attracted many corn producers to apply foliar fungicides even when disease levels are low (4, 20).

Fungicides currently labeled for use on corn are within the quinone outside inhibitor (QoI) and demethylation inhibitor (DMI) classes. Registered DMI fungicides for use on corn include metconazole, propiconazole, prothioconazole, tebuconazole and tetraconazole. These DMI fungicides are marketed as individual active ingredient products or as products premixed with QoI fungicides. DMI fungicides marketed as individual active ingredient products are propiconazole (e.g. Tilt and Propimax), prothioconazole (Proline) tebuconazole (e.g. Folicur) and tetraconazole (Domark). While propiconazole and prothioconazole are marketed as both individual and premixed products, metconazole is marketed for use on corn only as a premixed product. Metconazole is currently marketed as a solo product (Caramba, BASF Corporation, Research Triangle Park, NC) but for use on other crops. As QoI-DMI products, propiconazole is premixed with azoxystrobin (Quilt and Quilt Xcel, Syngenta Crop Protection, Greensboro, NC)

or trifloxystrobin (Stratego, Bayer CropScience, Research Triangle Park, NC). Prothioconazole is premixed with trifloxystrobin (Stratego YLD, Bayer CropScience, Research Triangle Park, NC) and metconazole is premixed with pyraclostrobin (Headline AMP, BASF Corporation, Research Triangle Park, NC).

As a class of sterol biosynthesis inhibitor (SBI) fungicides, DMIs interfere with the C14-demethylase of the ergosterol biosynthesis pathway resulting in membrane leakage (5). Introduced in the 1970s, DMIs have broad-spectrum activity against many fungal pathogens and are currently registered for use on many crops. Although DMIs have traditionally been used to control many foliar diseases of corn (2, 23), resistance in *E. turcicum* has not yet been reported. According to the Fungicide Resistance Action Committee (FRAC), DMIs have moderate resistant risk and resistance to DMIs has been reported in over 30 DMI-resistant fungal pathogens (9) including *Fusicladosporium effusum* (syn. *Cladosporium caryigenum*) (25), *Monilinia fructicola* (19), *Mycosphaerella graminicola* (18) and *Venturia inequalis* (15).

With the increase of DMI fungicides labeled for management of NCLB, there is a potential for resistance development in *E. turcicum* populations. Although DMIs have been used for many years on corn in the United States, no true baseline sensitivities of *E. turcicum* populations have been reported. However, it is still important to initiate monitoring programs to detect shifts in pathogen sensitivity. The objective of this research was to determine current in vitro sensitivities of *E. turcicum* isolates to metconazole. The current sensitivity profile will serve as a basis for detecting potential shifts in sensitivity as part of an overall monitoring program.

## Materials and Methods

*Collection of E. turcicum isolates.* Twenty six single-spore isolates of *Exserohilum turcicum* were obtained from infected corn leaves from six counties in Georgia and one county in Florida during the 2012 growing season (Table 4.1). Fungicide trials were conducted in three out of six the locations. Of these locations, two (Attapulgus and Citra) have a history of metconazole use as a QoI-DMI premix in 2011 and 2012 and one (Tifton) during the 2012 growing season only. Remaining fields were presumably exposed to DMIs. Small sections of tissues, approximately 0.5 cm<sup>2</sup>, were cut from the margin of one lesion on each leaf. The leaf tissues were surface-disinfested with 0.6% NaOCl, rinsed twice in sterile water and placed onto PDA amended with antibiotics (50 µg/ml each of streptomycin, tetracycline and chloramphenicol) and incubated at 24°C for 7 days and periodically transferred to obtain pure cultures. The isolates were then stored on filter paper at -20°C until needed.

*Mycelial growth assay.* Technical grade metconazole (97% a.i.; BASF Corporation, Research Triangle Park, NC) was dissolved in acetone to obtain a stock solution of 30 mg/ml. The stock solution was serially diluted in acetone and added to autoclaved PDA cooled to 55°C to obtain eight different concentrations (0.001, 0.003, 0.01, 0.03, 0.01, 0.3, 1 and 3 µg/ml). Sensitivity to metconazole was determined by in vitro mycelial growth assay on fungicide-amended and non-amended (acetone only) PDA. Two replications of each isolate and fungicide concentration were prepared. Mycelial plugs were removed from the margin of 7- to 10- day-old cultures using a 6 mm diameter cork borer and placed upside down on the center of fungicide amended and non-amended PDA plates. Cultures were incubated at 24°C for 7 days in the dark. Following incubation, the diameter of each fungal colony was measured and corrected by subtracting the diameter of the plug (6 mm). Each isolate was assayed in at least two trials.

Relative growth (RG) was calculated as the proportion of the corrected colony diameter on fungicide-amended medium and the corrected colony diameter on non-amended medium.

*Data analysis.* The effective concentration at which mycelial growth was inhibited by 50% (EC<sub>50</sub>) value for each isolate was estimated based on linear regression of probit-transformed relative inhibition (1 - RG) on log<sub>10</sub>-transformed fungicide concentration. The frequency distribution of log<sub>10</sub>-transformed EC<sub>50</sub> values was tested for normality using four tests (PROC UNIVARIATE) in SAS (version 9.3; SAS Institute Inc., Cary, NC). Paired *t*-tests were performed to compare the mean log<sub>10</sub>-transformed EC<sub>50</sub> values among trials. Coefficient of variability (standard error/mean) of log<sub>10</sub>-transformed EC<sub>50</sub> values for each isolate among trials was calculated as a measure of reproducibility.

## **Results**

The coefficient of variation of log<sub>10</sub>-transformed EC<sub>50</sub> values among trials ranged from 0.1 to 2.5%. The coefficient of variation were considerably less than 20% indicating that the log<sub>10</sub>-transformed EC<sub>50</sub> values of each isolate were consistent among trials. Thus, data of individual isolates across trials were combined to calculate mean EC<sub>50</sub> values. Frequency distribution of mean EC<sub>50</sub> values was not log-normal on three out of four normality tests. The EC<sub>50</sub> values ranged from 0.008 to 0.155 µg/ml and the mean and median values were 0.026 and 0.017 µg/ml, respectively (Fig. 4.1).

## **Discussion**

Fungicide resistance monitoring programs are important to detect shifts in pathogen sensitivity to fungicides, confirm efficacy of registered fungicides and update resistance management recommendations. Headline AMP (pyraclostrobin + metconazole) received full registration in 2009 and is currently used by corn growers for management of NCLB in Georgia.



As an individual active ingredient product (Caramba), metconazole is not labeled for use on corn but is labeled for use on other economically important crops such as wheat, sugar beet and almond since 2007.

The range and means of EC<sub>50</sub> values of *E. turcicum* isolates was relatively narrow and similar to baseline sensitivity range in *Fusarium oxysporum* (EC<sub>50</sub> 0.0058 µg/ml to 0.080 µg/ml, mean 0.038 µg/ml), *Fusarium graminearum* (EC<sub>50</sub> 0.006 µg/ml to 0.080 µg/ml, mean 0.031 µg/ml), *Fusarium sp. nov.* (EC<sub>50</sub> 0.007 µg/ml to 0.084 µg/ml, mean 0.0187 µg/ml) (6) and *Alternaria spp.* (0.014 to 0.224 µg/ml, mean 0.108 µg/ml) (7) but unlike the broader range of EC<sub>50</sub> values reported in *Fusicladosporium carphophilum* (EC<sub>50</sub> 0.013 to 3.85 µg/ml, mean 0.496 µg/ml) (7) and *Sclerotinia sclerotiorum* (EC<sub>50</sub> 0.05 to 1.64 µg/ml) isolates (1). The relatively narrow range of *E. turcicum* isolates suggests that there is limited sensitivity shift to metconazole. The risk of developing fungicide resistance is not fully determined by the shape of the distribution; however, a skewed distribution to the less sensitive end serves a warning that resistance is possible (26). The narrow range of EC<sub>50</sub> values and the skewed distribution may also be due to few counties sampled and the small number of isolates sampled and limited sampling locations.

Fungicide resistance can result from point mutation and high selection pressure on a resistant pathogen population due to the continuous and large scale use of fungicides within the same class. Mechanisms of reduced sensitivity to DMIs in plant pathogens include the alteration of target site CYP51 gene coding the sterol 14 $\alpha$ -sterol demethylase and overexpression of efflux transporters. Substitution of isoleucine to valine at position 381 (I381V) is one of several mechanisms involved in reduced sensitivity to azole fungicides in *Mycosphaerella graminicola*. The I381V mutation is responsible for reduced sensitivity of *M. graminicola* populations to

tebuconazole and metconazole (8, 17). Cross resistance between tebuconazole and metconazole was also reported in *Fusarium graminearum* (27). Positive (13) cross resistance among DMI fungicides was reported (14, 25) but a lack of cross resistance has been observed in a number of pathogens (16, 18, 19). Although reports of cross resistance are inconsistent, FRAC recommends that it is wise to assume that cross resistance occurs between DMI fungicides used to control the same pathogen (10).

Although not yet reported in *E. turcicum*, shifts in sensitivities to fungicides were observed in closely related species have been reported. *Helminthosporium halodes*, causal agent of sugarcane leaf spot, developed in vitro resistance to mancozeb (24). Polyoxin-resistant mutants of southern corn leaf blight pathogen *Cochliobolus heterostrophus* were also produced in the laboratory (11). Recently, field resistance of *Helminthosporium solani* to thiabendazole and thiophanate-methyl was reported in the Columbia basin (12). Thus, proper fungicide resistance management strategies must be followed and *E. turcicum* populations should be monitored for reduced sensitivity to registered fungicides.

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Table 4.1. Collection information for *Exserohilum turcicum* isolates from corn fields in 2012.

Isolate	County	State
NL-5	Cook	GA
NL-6	Cook	GA
NL-8	Cook	GA
NL-9	Cook	GA
NL-15	Tift	GA
NL-21	Macon	GA
NL-22	Macon	GA
NL-28	Macon	GA
NL-30	Macon	GA
NL-31	Benhill	GA
NL-32	Benhill	GA
NL-36	Benhill	GA
NL-39	Benhill	GA
NL-40	Benhill	GA
NL-46	Decatur	GA
NL-48	Decatur	GA
NL-49	Decatur	GA
NL-51	Decatur	GA
NL-52	Decatur	GA
NL-53	Decatur	GA
NL-55	Decatur	GA
NL-57	Erwin	GA
NL-58	Erwin	GA
NL-42	Marion	FL
NL-43	Marion	FL
NL-44	Marion	FL

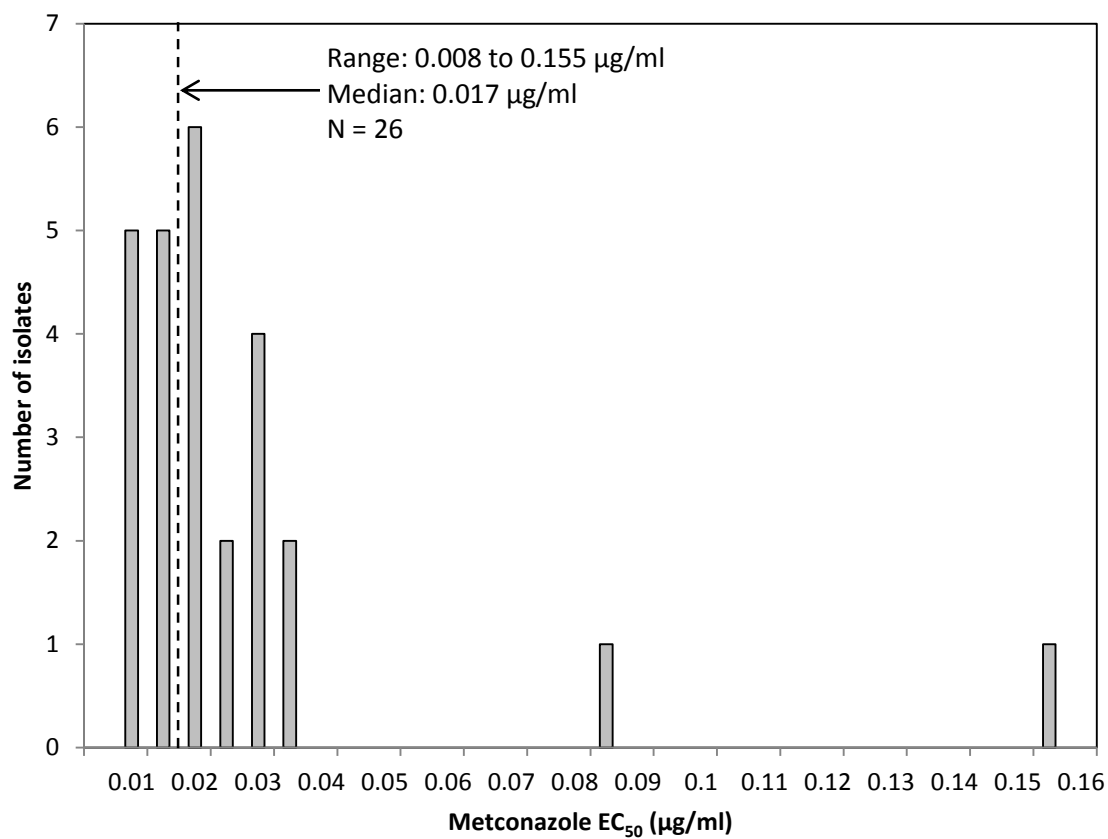


Fig. 4.1. Frequency distribution of EC<sub>50</sub> values for isolates of *Exserohilum turcicum* to metconazole.

## CHAPTER 5

### SUMMARY

Southern corn rust (SCR) caused by *Puccinia polysora* and northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* are the most important foliar diseases of corn in the southern United States. Sources of SCR resistance were stable for two decades until a new *Rpp9*-virulent race was identified in Georgia in 2008 (1). Concurrently, NCLB was also severe in 2008 and became more destructive in 2009. In Georgia in 2010, SCR and NCLB caused a combined damage and cost of control of 8.3 and 2.3 million dollars, respectively (4). With the lack of management options, fungicides may be necessary to control foliar diseases and increase yields. Therefore, field characterization of both virulent and avirulent races of *P. polysora* is crucial for management of SCR. Integration of disease resistant corn hybrids and timely application of fungicides is crucial for management of SCR and NCLB.

A total of nine field trials were conducted at three locations from 2011 to 2013 to determine the effect of hybrids, fungicides and timing of fungicide application to manage SCR and NCLB. In one study, field trials were planted to “rust-resistant” Pioneer 33M52 (P33M52) and rust-susceptible Pioneer 33M57 (P33M57). Pyraclostrobin (Headline) and pyraclostrobin + metconazole (Headline AMP) were applied at seven different timings based on corn growth stages. First detection of SCR symptoms in P33M52 was delayed by up to two weeks compared to P33M57 in most early-planted trials. However, SCR was first detected on both hybrids on the same assessment date in late-planted trials. Based upon field observations, uredinia in P33M57

was typically larger and denser in distribution than those observed on P33M52. Area under the disease severity progress curve (AUDSPC) and area under the disease incidence progress curve (AUDIPC) values were calculated from repeated disease severity and incidence assessments over time. Significant differences in AUDSPC, AUDIPC values of SCR, total plot necrosis and yield were observed between hybrids. In most cases, AUDSPC and AUDIPC values of SCR and total plot necrosis were significantly lower in P33M52 than in P33M57. Yields were usually higher in P33M52 when SCR was severe, but yields were higher in P33M57 when SCR levels were low.

Fungicides were effective for foliar disease control and yield improvement. Yields were usually not significantly different between fungicides although combined yields of pyraclostrobin + metconazole tended to be significantly greater than pyraclostrobin in two late-planted trials. The efficacy of a given fungicide treatment usually depended on the onset and development of foliar diseases. A single fungicide application could significantly reduce AUDSPC and AUDIPC values and increase yields but this may be insufficient in many situations since foliar diseases usually occur at different growth stages and over time. Three fungicide applications oftentimes resulted to the lowest AUDSPC and AUDIPC values and highest yields but such were not significantly different from treatments with fewer applications of fungicides when timed near disease onset. Fungicide application during the early reproductive stages were usually effective, but was not always the most successful for disease control and yield improvement. Fungicides applied at the vegetative stage followed by a second application were the most effective application strategy. Fungicides were not needed to manage SCR where hybrids with the *Rpp9* gene, e.g., P33M52 were sown. However, a fungicide application program may be justified on such hybrids where NCLB and SCLB are of great importance.



Most fungicides labeled to manage foliar disease of corn are quinone outside inhibitor (QoI) and demethylation inhibitors (DMI). A number of plant pathogens affecting other crops have developed resistance to QoIs and sensitivity shifts to DMIs have occurred. Fungicide resistance is one of the reasons that led to the adoption of succinate dehydrogenase inhibitor (SDHI) fungicides (3). In this study, pyraclostrobin and newly registered QoI-SDHI premix pyraclostrobin + fluxapyroxad were applied in seven different timings to Pioneer 33M57. Similar to the first study, the effects of fungicides and timing of fungicide application were closely examined by conducting a total of eight trials in three locations over three growing seasons. In this study, data were analyzed as proportions of the untreated checks to directly compare differences between fungicides and among timings of fungicide applications. Fungicides provided effective disease control and yield increase with timely applications. Single applications reduced disease intensities but significantly higher yields were achieved with multiple applications. Highest yield increase was usually obtained with three applications but was not significantly different from a well-timed double application. There were few instances that fungicides differed in AUDSPC and AUDIPC values and no significant yield differences were observed between fungicides. However, pyraclostrobin + fluxapyroxad may be an important tool to include in a fungicide program to facilitate fungicide resistance management.

Fungicide resistance could result from extensive use of fungicides within the same class. A number of demethylation inhibitor (DMI) fungicides are available for use on corn. Metconazole is one of the five DMIs registered for managing corn foliar diseases. Although resistance in *E. turcicum* has not yet been reported, the Fungicide Resistance Action Committee (FRAC) listed over 30 DMI-resistant fungal pathogens (2). With the increased use of fungicides in recent years, it is important to initiate fungicide resistance monitoring. Sensitivity to

metconazole was determined by conducting a mycelial growth assay in 26 isolates collected from six counties. The EC<sub>50</sub> values ranged from 0.008 to 0.155 µg/ml and the mean and median values were 0.026 and 0.017, respectively. These EC<sub>50</sub> values will be used to determine possible shifts in sensitivity of *E. turcicum* populations to DMI fungicides. Frequency distribution of log-transformed EC<sub>50</sub> values was not log-normal. The distribution does not prove that there was a shift in sensitivity in *E. turcicum* isolates although it may be possible. Thus, fungicides must be judiciously used to manage diseases and prevent fungicide resistance development.

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## APPENDICES

APPENDIX A. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield in Tifton, GA in 2011

Variable		SCR <sup>a</sup>		NCLB <sup>b</sup>		Yield (kg/ha)
		FDS	FDI	AUDSPC	AUDIPC	
<b>Hybrid</b>						
Pioneer 33M57		0.21	0.06 A <sup>c</sup>	4.5 A	186.4 B	19487 A
Pioneer 33M52		0.06	0.03 B	4.3 A	216.4 A	19261 B
<i>P</i> ( $\alpha$ =0.05)		0.0892	0.0007	0.7806	0.038	0.0013
<b>Treatment</b>						
Fungicide <sup>c</sup>	Application <sup>d</sup>					
PYR	V5	0.37 a	0.04 bcd	6.4 a	226.9 a	19411 a
PYR	R1	0.02 a	0.03 d	3.3 a	203.5 a	19456 a
PYR	R2	0.03 a	0.05 bcd	3.8 a	228.1 a	19395 a
PYR	V5+R1	0.30 a	0.12 a	5.4 a	248.2 a	19326 a
PYR	V5+R2	0.18 a	0.04 bcd	3.4 a	172.6 a	19395 a
PYR	R1+R2	0.04 a	0.03 d	3.2 a	195.0 a	19375 a
PYR	V5+R1+R2	0.02 a	0.04 cd	5.1 a	238.5 a	19285 a
PYR+MET	V5	0.65 a	0.1 ab	4.2 a	195.0 a	19220 a
PYR+MET	R1	0.01 a	0.02 d	3.3 a	135.5 a	19537 a
PYR+MET	R2	0.03 a	0.02 d	3.4 a	170.6 a	19558 a
PYR+MET	V5+R1	0.04 a	0.02 d	7.2 a	236.6 a	19105 a
PYR+MET	V5+R2	0.03 a	0.03 d	4.3 a	178.8 a	19475 a
PYR+MET	R1+R2	0.02 a	0.03 d	5.2 a	206.2 a	19436 a
PYR+MET	V5+R1+R2	0.12 a	0.06 bcd	3.6 a	183.5 a	19456 a
Untreated Control		0.12 a	0.08abc	4.6 a	202.0 a	19175 a
<i>P</i> ( $\alpha$ =0.05)		0.3853	0.0064	0.6599	0.2620	0.5162

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessments dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i./ha, Headline AMP, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3)

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different.

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid by treatment interaction.

APPENDIX B. Effect of hybrid and fungicide treatment on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield in Attapulga, GA in 2011

Variable		NCLB <sup>a</sup>		Yield (kg/ha)
		AUDSPC	AUDIPC	
<b>Hybrid</b>				
Pioneer 33M57		30.1 A	286.6 A	5120.6 A
Pioneer 33M52		35.5 A	258.7 A	5605.6 A
<i>P</i> (α=0.05)		0.3781	0.4369	0.2265
<b>Treatment</b>				
Fungicide <sup>b</sup>	Application <sup>c</sup>			
PYR	VEG	23.8 a	183.2 bcd	5322 a
PYR	EREP	15.9 a	216.7 bcd	4283 a
PYR	MREP	51.0 a	353.4 ab	5154 a
PYR	VEG+EREP	2.3 a	55.5 d	4229 a
PYR	VEG+MREP	51.0 a	353.3 ab	5821 a
PYR	EREP+MREP	29.6 a	293.4 abc	5924 a
PYR	VEG+EREP+MREP	41.6 a	328.8 abc	5037 a
PYR+MET	VEG	25.1 a	247.7 a-d	5129 a
PYR+MET	EREP	33.2 a	316.6 abc	5154 a
PYR+MET	MREP	51.0 a	426.5 abc	5598 a
PYR+MET	VEG+EREP	41.7 a	324.4 abc	6253 a
PYR+MET	VEG+MREP	16.6 a	140.9 cd	5170 a
PYR+MET	EREP+MREP	51.8 a	371.2 a	6789 a
PYR+MET	VEG+EREP+MREP	36.7 a	243.2 a-d	4381 a
Untreated Control		21.1 a	235.4 a-d	5373 a
<i>P</i> (α=0.05)		0.0658	0.0233	0.5716

<sup>a</sup> FDS = final disease severity (%), FDI = final disease incidence (%).

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with four assessments dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) PYR+MET = pyraclostrobin + metconazole (107 + 40 g a.i./ha, Headline AMP, BASF Corporation)

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3)

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different.

<sup>f</sup> Data are combined across hybrids and across fungicide treatments when there are no hybrid by treatment interaction.

APPENDIX C. Effect of fungicide and timing of application control on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield as a proportion of the untreated control in Tifton, GA in 2011

Variable	SCR <sup>a</sup>		NCLB <sup>b</sup>		Yield <sup>f</sup>	
	FDS	FDI	AUDSPC	AUDIPC		
<b>Fungicide<sup>c</sup></b>						
PYR	166.0 A	95.0 A	118.7 A	97.1 A	99.9 A	
PYR+FLX	175.4 A	94.1 A	99.2 A	103.4 A	99.9 A	
( $\alpha=0.05$ )	0.8677	0.9772	0.2317	0.3660	0.9464	
<b>Timing of Application<sup>d</sup></b>						
					PYR	PYR+FLX
VEG	101.0 a	69.5 a	149.6 a	126.9 a	100.7 ab	99.7 a
EREP	138.9 a	109.9 a	93.5 a	89.5 b	99.8 bc	99.9 a
MREP	32.0 a	39.2 a	111.4 a	103.8 ab	100.3 abc	99.7 a
VEG+EREP	242.8 a	106.6 a	93.4 a	99.0 b	99.4 cd	100.8 a
VEG+MREP	308.0 a	99.8 a	102.7 a	85.3 b	100.3 abc	99.9 a
EREP+MREP	178.4 a	129.6 a	85.4 a	88.5 b	98.4 d	99.7 a
VEG+EREP+MREP	194.0 a	106.4 a	126.6 a	108.5 ab	101.2 a	99.5 a
( $\alpha=0.05$ )	0.1905	0.7329	0.3628	0.0340	0.0002	0.5126

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with six assessments dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3)

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 19660 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

APPENDIX D. Effect of fungicide and timing of application on southern corn rust (SCR) and northern corn leaf blight (NCLB) epidemics and corn yield as a proportion of the untreated control in Attapulgis, GA in 2011

Variable	NCLB <sup>a</sup>		Yield <sup>f</sup>	
	AUDSPC	AUDIPC		
<b>Fungicide<sup>b</sup></b>				
PYR	72.8 A	91.2 A		77.7 A
PYR+FLX	85.3 A	101.1 A		81.7 A
<i>P</i> ( $\alpha=0.05$ )	0.2020	0.1824		0.0810
<b>Timing of Application<sup>c</sup></b>				
			P	P + F
VEG	97.6 a	113.5 a	72.1 a	88.2 ab
EREP	87.3 a	103.4 a	81.5 a	69.6 cd
MREP	80.7 a	98.4 a	84.2 a	75.8 a-d
VEG+EREP	58.2 a	88.8 a	71.8 a	80.8 bcd
VEG+MREP	83.6 a	95.8 a	81.6 a	77.6 bcd
EREP+MREP	62.9 a	77.4 a	76.8 a	87.3 ab
VEG+EREP+MREP	88.4 a	95.7 a	76.6 a	93.0 a
<i>P</i> ( $\alpha=0.05$ )	0.3301	0.2562	0.9853	0.1212

<sup>a</sup> FDS = final disease severity, FDI = final disease incidence.

<sup>b</sup> Area under the disease progress severity curve (AUDSPC) and area under the disease progress incidence curve (AUDIPC) were calculated with four assessments dates.

<sup>c</sup> PYR = pyraclostrobin (110 g a.i./ha, Headline, BASF Corporation) Priaxor, PYR+FLX = pyraclostrobin + fluxapyroxad (120 + 60 g a.i./ha, Priaxor, BASF Corporation).

<sup>d</sup> Growth stages of corn (VEG = single application between V5 and V7 stages, EREP = single application between VT and R1 stages, MREP = single application between R2 and R3)

<sup>e</sup> Means in the same column followed by the same uppercase letter or lowercase letter are not significantly different ( $\alpha=0.05$ ).

<sup>f</sup> Mean yield of the untreated control was 12024 kg/ha.

<sup>g</sup> Data are combined across fungicides and across timings when there are no fungicide by timing interaction.

APPENDIX E. Effect of hybrid and fungicide treatment on southern corn rust (SCR) epidemics and corn yield in Attapulgis, GA in 2013

Fungicide	Application	Yield (kg/ha)	
		P33M57	P33M52
PYR	VEG	9337 e	9689 cde
PYR	EREP	10115 a-e	10246 a-e
PYR	MREP	10162 a-d	10172 a-d
PYR	VEG + EREP	10388 a-d	11007 a
PYR	VEG + MREP	9469 de	9611 cde
PYR	EREP + MREP	9858 cde	10010 b-e
PYR	VEG + EREP + MREP	9806 cde	10485 abc
PYR+ MET	VEG	10559 abc	10240 a-d
PYR+ MET	EREP	9978 b-e	9717 cd
PYR+ MET	MREP	10344 a-d	10222 a-e
PYR+ MET	VEG + EREP	10421 a-d	10162 a-e
PYR+ MET	VEG + MREP	10353 a-d	10982 a
PYR+ MET	EREP + MREP	10293 a-e	10851 ab
PYR+ MET	VEG + EREP + MREP	10889 ab	10478 abc
Untreated Control		9622 cde	10425 abc

<sup>a</sup> Means in the same column followed by the same letter are not significantly different ( $\alpha=0.05$ ).