Foliar Fungicide Effects on Gray Leaf Spot and Yield of Hybrid Corn as Influenced by Application Timing, Hybrid Characteristics and Production Practices

THESIS

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Ohio ranks 9\textsuperscript{th} among leading U.S. corn-producing states in total grain corn acreage. The corn crop adds an estimated $3 billion to Ohio’s economy each year. Since the 1980s and 1990s when gray leaf spot, caused by \textit{Cercospora zeae-maydis}, provoked widespread yield and economic losses in Ohio, foliar diseases have only been a concern in localized areas of the state. In most years, producers effectively manage foliar diseases by practicing some form of crop residue management, rotating corn with soybean, and planting resistant hybrids. However, without a major corn disease epidemic in more than a decade, the focus has shifted away from production practices geared to minimized disease-related losses. Since 2006, growers across the Corn Belt have been investing heavily in fungicide application. This is due in part to recent corn prices and the potential for greater disease development in a continuous-corn conservation-tillage cropping system, but most importantly, to industry claims of substantial yield increases due to the use of foliar fungicide, particularly the Quinone Outside Inhibitors (QoI). In Ohio, more than 650,000 hectares acres of corn were sprayed with fungicide during 2007. At an estimated application cost of $57 per hectare, this represented a total expenditure on fungicide of $37 M. Based on grain prices in that year, it would have required an estimated 375-501 kg/ha increase in yield to offset the cost of fungicide application. Results from university-based replicated trials suggest that the effect of fungicide
application on yield is less clear-cut than industry data seem to suggest. In some university trials, positive yield responses were associated with disease control, in others there were no yield benefits even when diseases were controlled, yet in other trials positive yield responses were observed in the absence of high disease pressure. Since production practices have changed substantially since the available disease management recommendations were developed, there is a need to revisit these recommendations and develop sustainable management guidelines suitable for current production systems. Hence the objectives of the research presented in this thesis were to: investigate the effects of hybrid gray leaf spot resistance and yield potential, and fungicide application (product and timing) on the development of gray leaf spot and grain yield and yield components in a minimum-till/continuous-corn and a corn-soybean rotation cropping systems.

A total of five field trials were conducted in Ohio in 2009 and 2010. In two of those trials, the effects of hybrid characteristics and application timing of a QoI and a DMI (Demethylation Inhibitor) fungicide on GLS severity, grain yield and yield components were evaluated. Four hybrids with different yield potential, maturity, and GLS resistance profiles were used. Plots were treated with either Domark (20.5% tetraconazole; a DMI), Headline (23.6% pyraclostrobin, a QoI), or left untreated. Applications were either made at the silking (R1), blister (R2) or milk (R3) growth stage, with each product being applied at label-recommended rate, along with a nonionic surfactant. In the remaining three trials, the effects of cropping sequence (crop rotation) and fungicide application timing were evaluated. Plots were established in sections of
fields previously planted with corn or soybean, and a QoI/DMI combination fungicide, Stratego (10.8% prothioconazole + 32.3% trifloxystrobin) or Stratego YLD (11.4% propiconazole + 11.4% trifloxystrobin), was applied at label-recommended rates at growth stage R1, R2, or R3, along with a nonionic surfactant. Untreated checks were used as references. In all five trials, a randomized complete block design was used, with a split-plot arrangement of hybrid or previous crop (whole plot) and fungicide application timing (sub-plot). Plots in the first two trials were inoculated with C. zeae-maydis, while those in the last three were not. GLS severity was assessed between silking and maturity in all plots. Grain yield was estimated and yield components determined by weighing five ears and then counting the number of kernel rows and the number of kernels in two arbitrarily selected rows on each of the five ears. Linear mixed models were used to analyze the data from all trials. GLS severity varied among trials, with only nominal levels of disease developing in one of the uninoculated trials. In general, fungicide treatments had a significant effect on GLS severity, with applications made a R1 resulting in the best control of GLS relative to the check. The R3 applications were not significantly different from the check. Fungicide effects on GLS, however, did not translate into an effect on grain yield, since the effects of fungicide treatment on yield was not statistically significant. However, hybrid, previous crop, and the interaction between previous crop and fungicide treatment had significant effects on yield in at least one trial. One of the late-maturing hybrids with high yield potential out-yielded all other hybrids in one of the trials. Plots planted following soybean yielding significantly more than plots planted following corn in one of the cropping sequence x fungicide treatment
trials, whereas in another, the response depended on the fungicide treatment. For plots planted into corn residue, only the R2 treatment resulted in significantly higher yield than the check, whereas for plots planted after soybean, the R1 and R3 treatments yielded significantly more than the check. Yield components varied substantially among trials, hybrids, and fungicide treatments, with the hybrid x fungicide interaction being statistically significant for all yield components in at least one of the two trials in which these factors were treated. Cropping sequence did not affect yield components. In general, hybrid maturity and yield potential had a greater influence on yield components than fungicide application (product or timing). However, neither the main effects of hybrid nor its interaction with fungicide timing were consistent between trials or among yield components. For a given yield component, the most effective treatment or treatment combination in one trial was not necessarily the most effective in another trial. Moreover, the most effective treatment or combination for one yield components within or between trials was not necessarily as effective for another yield component. The results from this investigation clearly emphasize the complexity of yield response to fungicides and serve to reiterate the fact that such a response is highly variable, unreliable, and as such, may not always be profitable.
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Publications


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CHAPTER 1: CORN PRODUCTION, GROWTH, AND DEVELOPMENT

The United Stated is the largest producer of corn in the world. In 2010, corn growers in the U.S produced an estimated 316.2 million metric tons of grain corn. Ohio ranks 9th in total corn acreage among leading U.S. corn producing states, behind Iowa, Illinois, Nebraska, Minnesota, Indiana, South Dakota, Kansas, and Wisconsin. Nearly 16% of the U.S. corn crop was exported in 2010, with the top five destinations being Japan, Mexico, South Korea, Taiwan, and Egypt (76). An estimated 51% of the crop is used for domestic feed and residual use (79). This includes whole-corn and fractionated products made by either the dry- or wet-milled methods. (18)

Over the last decade, both yield and grain corn production acreage have increased in Ohio. Average yield in the state was 10.3 MT/ha in 2010 compared to 8.8 MT/ha in 2001. Acreage has increased from 1.3 million hectares in 2006 to 1.5 million hectares in 2011, and projections are for further increases over the next few years (79). This increase in acreage is very consistent with the national trend, being triggered largely by higher grain prices ($79/MT in 2006 compared to $277/MT in 2011) and the prospect of large-scale ethanol production, which currently accounts for 34 to 35 percent of corn use (79).

In Ohio, field corn is usually planted between April 15 and May 10 in the North and between April 10 and May 10 in the southern half of the State (74, 78). Depending on the planting date, weather conditions, and hybrid characteristics, physiological maturity is usually reached between late-August and mid-September. Grain yield is determined
throughout the development of the crop, beginning at a very early growth stage, with the
different yield components defined at various growth stages. According to Abendroth et al. (1), at the V3 growth stage (the stage at which the plant has three visible leaf collars),
all leaves and ear shoots are initiated within the corn plant, and by V5 (the 5-leaf stage),
the tassel is initiated. The number of rows of kernels per ear is determined starting at the
V9 growth stage and continues until V12. The number of kernels per row is determined
from the V12 growth stage, continuing until one week before silking (R1) or V17. The
V15 growth stage marks the beginning of the most crucial period of plant development in
terms of grain yield.

Yield loss may result from stresses (nutritional, moisture, pest and disease, etc.) at
any time during the development of the crop, but the greatest yield reductions result from
stress at silking (74, 80). From VT to R1, the plant is more vulnerable to damage than at
any other growth stage. At R1, the number of ovules that will be fertilized is determined,
and at R2, starch begins to accumulate in the watery endosperm of the kernels (1). This
marks the beginning of rapid and steady dry matter accumulation, also known as grain-
fill, which continues until close to R6. During the R3 growth stage, cell division in the
endosperm is essentially complete, so growth is mostly due to cell expansion and starch
accumulation in the cells.

Final yield depends on the plant population, the size of the ear, the number of
kernels on the ear, and the final size of the kernels. All of these yield components are
influenced by the genetics of the hybrid, weather conditions, and crop
management/production practices. Stresses between R1 and R3 may have profound
effects on yield by reducing both kernel number and size. For instance, stresses at R1
may reduce pollination and fertilization, whereas at R2 and R3, kernel abortion may occur if the supply of carbohydrates to the ear is reduced (1). The loss of photosynthetic leaf area due to adverse environmental conditions, pest and disease problems or inappropriate cultural practices can lead to yield losses as high as 100 percent, depending on the extent of foliar damage and the growth stage at which the damage occurs (74, 80). The greatest reductions in yield are observed when more than 50 percent of the leaf area is destroyed and when the damage occurs close to VT/R1 (74, 80). Stress during the R4 and R5 growth stage may reduce yields by reducing kernel weight, but not kernel number.

GRAY LEAF SPOT

Diseases are a primary cause of yield loss in corn in Ohio. Depending on the disease and the plant part affected, all major yield components may be affected. The most common stalk diseases in Ohio, Gibberella, Fusarium, Diplodia, and Anthracnose stalk rots, impact grain yield indirectly by reducing the plant population (final stand). Frequently observed ear diseases include Gibberella, Fusarium, and Diplodia ear rots, which reduce both the yield and quality of the grain. However, the most important yield-limiting diseases in the state are foliar diseases, including northern corn leaf blight, eyespot, rust, and gray leaf spot (GLS). Not only do these diseases reduce the photosynthetic area of the plant, causing direct yield loss, they may also reduce stalk quality, predisposing plants to stalk rot, and consequently, indirect yield and quality losses. Of the foliar diseases, GLS is the single most important disease in Ohio, based on year-to-year prevalence and severity.
**GLS Etiology and Epidemiology:**

Gray leaf spot, caused by *Cercospora zeae-maydis* Tehon & Daniels, has been the foliar disease of greatest concern in corn since first becoming a problem in the state in the 1980s and 1990s (36, 37). Since first being described in Illinois in 1925, GLS has been a consistent cause of yield loss in corn in warmer corn-growing regions of the world (16, 35, 82). GLS was formally thought to be caused by two sibling species, recognized as Groups I and II (26), with Group I being predominant in the U.S. and occurring elsewhere in the world, and Group II, which is genetically and phenotypically distinct from Group I, occurring in the U.S., Africa and possibly elsewhere (20, 81). However, more recent research has shown that differences in the internal transcribed spacers (ITS1 and ITS2) region suggest that Groups I and II are two distinct species (20), with Group I considered as *Cercospora zeae-maydis* and Group II as *Cercospora zeina* (20, 26, 29, 81). *C. zeina* causes GLS in southern Africa (20).

*C. zeae-maydis* survives in corn residue left on the soil surface (23), thus residue in the field serves as the main source of primary inoculum in the spring (23, 24). The first lesions usually develop on the lower leaves, producing conidia that serve as secondary inoculum for the upper leaves. The appearance of lesions usually occurs following a latent period of approximately 14-28 days (62, 83). Initially, immature lesions are not easily distinguished from lesions caused by other foliar pathogens of corn. These early lesions usually appear as small, tan spots, about 1 to 3 mm long, that are rectangular to irregular in shape, with chlorotic borders. Initial lesions are most easily observed when leaves are viewed through transmitted light. Mature GLS lesions, however, are readily distinguished from those of other foliar diseases of corn; they are gray to tan in color and
are distinctly rectangular in shape, with dimensions ranging from 5 to 70 mm long by 2 to 4 mm wide. These lesions typically run parallel to the leaf veins. Sporulating lesions produce conidia on conidiophores growing through the stomata from stromata located in the sub-stomatal cavity of the infected tissues (5, 85). These conidia are then wind or splash-disseminated from one plant to another or from one leaf to another on the same plant and the infection cycle resumes in accordance with the polycyclic nature of the disease (5, 85).

Environmental factors have a tremendous impact on the rate of in-season GLS spread. GLS development is favored by extended periods of overcast days, warm temperatures and high relative humidity (5, 65). High relative humidity, suitable air temperatures, a susceptible host and the presence of a source of inoculum are necessary for GLS epiphytotics to occur (5, 51, 65). Paul (55) reported that the number of hours of daytime air temperature between 20 and 30 C and the night-time relative humidity > 90% for the period between growth stages V4 and V12 were more highly correlated with GLS severity than overall mean temperature and relative humidity during the growing season. The greatest rates of lesion expansion and subsequent spore production occur at temperatures between 25 and 30 C, at high relative humidity (55).

Light (quality and intensity) also affects GLS development (7, 4). _C. zeae-maydis_ produces cercosporin, a photosensitizing secondary metabolite that is toxic to a diverse array of organisms (22). Light-activated cercosporin reacts with oxygen to generate singlet oxygen and superoxide, which induce lipid peroxidation and damage cell membrane, resulting in leakage of cytoplasmic contents and eventually, cell death (22). The most compelling evidence for the involvement of cercosporin in pathogenesis was
provided by Upchurch et al. (77) in their work with *Cercospora kikuchii*, a related species that affects soybean. Ultra-violet-induced mutants of *C. kikuchii* selected for defects in cercosporin production on agar media were found to be unable to incite typical necrotic lesions on soybean leaves and could not be isolated from small chlorotic spots (77). Shim and Dunkle (67) created cercosporin-deficient *C. zeae-maydis* mutants by disrupting the CZK3 gene and found that these mutants grew faster on agar media than the wild type but were deficient in conidiation and caused only small chlorotic spots on inoculated maize leaves compared with rectangular necrotic lesions incited by the wild type (67).

Early and extensive blighting of leaves reduce the photosynthetic area of the plant, resulting in yield loss (the difference between attainable yield and actual yield [43]). Donahue et al. (25), Ayers, et al. (4), and Beckman et al. (5) reported losses due to GLS to be 10-25% of the yield potential. Gevers et al. (28) observed up to 40% yield reduction due to GLS, whereas Ward and Nowell (82) and Ward et al. (83) reported losses of up to 50-65 %, with reduction in both yield and quality of silage. Latterell and Rossi (35) reported losses due to GLS approaching 80-100% in epiphytotic years.

**MANAGEMENT OF GLS**

GLS can be effectively managed through a combination of integrated management tactics. Crop rotation with soybean or soybean and wheat can reduce the amount of primary inoculum available to initiate infections in the spring. Tillage, either conventional or minimum-tillage, is another tactic that could reduce in-field inoculum, and consequently GLS severity, by burying infected plant tissues. Choosing hybrids with GLS resistance is another important management strategy, along with foliar fungicide application.
**Crop Rotation and Tillage**

GLS and several other economically important diseases of corn are residue-borne, and as such, managed fairly effectively with tillage and crop rotation. In Ohio, a 2-year rotation of soybeans and corn, or alternatively, a 3-year rotation of corn, soybean, and wheat is recommended in order to reduce the build-up of surface residue, and consequently, primary inoculum of fungal foliar pathogens such as *Cercospora zeae-maydis*, *Exoserhilum turcicum*, and *Aureobasidium zeae*, causal agents of gray leaf spot, northern corn leaf blight, and eyespot, respectively. In addition, there is an association between the severity of these diseases and the use of conservation tillage (9). *C. zeae-maydis*, *E. turcicum*, and *A. zeae*, foliar pathogens of relatively low competitive saprophytic ability, have become major pathogens in areas where conservation tillage practices are used (24, 35). Widespread adoption of conservation tillage combined with continuous-corn and high levels of surface residue are believed to be responsible for the prevalence and severity of GLS throughout the Corn Belt (35, 43).

Bhatia and Munkvold (6) and Paul and Munkvold (55) showed that maize surface residue was an important predictor of GLS severity, as demonstrated by the fact that percent corn surface residue and distance to nearest corn residue were found to have significant effects on GLS. *C. zeae maydis* overwinters best in crop residue left on the soil surface (23, 35, 85), leading to earlier GLS onset and greater disease intensity in no-till fields than in tilled fields (57, 82). In Ohio, de Nazareno et al. (24) showed that any tillage method that left residue on the soil surface favored gray leaf spot development and that disease intensity increased as the amount of surface residue increased. In North Carolina, a comparison of no-till and other tillage systems that result in various amounts
of residue on the soil surface indicated that there were more airborne conidia trapped over no-till plots than over tilled plots. Furthermore, plants in no-till plots had a higher number of lesions per leaf than those in plowed and disked plots (57).

**Resistance:**

Despite the fact that several GLS resistant germplasms have been identified, resistance has not been incorporated into all commercial hybrids (4, 83). This is partly because of the complex nature of GLS resistance. Resistance to *C. zeae-maydis* is quantitative (partial resistance) and additive effects are predominant, but dominance is significant as well (31). Most resistant genotypes are considered to have two or more factors associated with resistance (60). Genetic mapping experiments have identified quantitative trait loci (QTL) for resistance to *C. zeae-maydis* on all 10 chromosomes of corn (15, 17, 30, 60).

There are commercial hybrids with partial resistance to GLS. When infected by *C. zeae-maydis*, these usually exhibit one or more components of partial resistance such as longer incubation periods, fewer lesions, and smaller lesions (4, 35). Partially resistant GLS hybrids commonly display fleck-type lesions (4, 35), whereas, moderately resistant and susceptible hybrids exhibit chlorotic and necrotic lesions, respectively (4, 33, 55, 63, 66).

**Fungicides:**

Most of the fungicides currently labeled for use on corn contain active ingredients belonging to the Quinone Outside Inhibitor (QoI) and Demethylation Inhibitor (DMI) classes of fungicide chemistries, the so-called strobilurins and triazoles, either as a single active ingredient or as a premix of the two chemistries. To date, the QoI fungicide active
ingredients registered for use on corn in the U.S. include azoxystrobin, pyraclostrobin, and trifloxystrobin. The QoIs are either marketed as solo active ingredient under the trade names Quadris (22.9% azoxystrobin, Syngenta Crop Protection Inc., Greensboro, NC) and Headline (23.6% pyraclostrobin, BASF Corporation Agricultural Products, Research Triangle Park, NC) or as products premixed with the DMIs propiconazole, prothioconazole or metaconazole under the trade names Quilt (7% azoxystrobin + 11.7% propiconazole; Syngenta Crop Protection Inc., Greensboro, NC), Quilt Xcel (13.5% azoxystrobin + 11.7% propiconazole; Syngenta Crop Protection Inc., Greensboro, NC), Stratego (11.4% propiconazole + 11.4% trifloxystrobin; Bayer CropScience, Research Triangle Park, NC), Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin; Bayer CropScience, Research Triangle Park, NC) and Headline AMP (13.64% pyraclostrobin + 5.14% metconazole, BASF Corporation Agricultural Products, Research Triangle Park, NC) (12).

The efficacy, yield benefit, and profitability of fungicide application in field corn is highly variable and depends of several factors, including application timing, the fungicide being applied, the level of disease at the time of application, disease resistance and tolerance of the hybrids being treated, as well as application cost and grain price. Ward et al (84) showed that chemical control of GLS (with Benlate 50% WP [50% benomyl, Du Pont de Nemoirs and Coy] and Punch Xtra [125 g/l flusilazole+ 250 g/l carbendazim, Du Pont de Nemoirs and Coy]) in South Africa was most effective when treatments were initiated as disease severity reached 2 to 3% on the basal five leaves of the plant. Mean yield response to fungicides tends to be higher for trials in which hybrids with fair to poor resistance to gray leaf spot are planted than in those with GLS resistant
hybrids, and higher for trials in which corn is planted after corn than those in which corn is rotated with soybean (40, 41). However, even when there is a positive yield response to fungicide, the response is not always sufficient to offset the cost of making the application. Based on trials conducted in Iowa, Munkvold and colleagues (40) concluded that the probability of a profitable propiconazole (Tilt 3.6E, Novartis Crop Protection Inc., Greensboro, NH) application in corn was strongly influenced by hybrid susceptibility. In a more recent study conducted in Illinois, in which the effect of foliar fungicides was assessed on corn with and without simulated hail damage, Bradley and Ames (11) concluded that neither Quadris nor Headline significantly improve grain yield in either damaged or non-damaged plots, compared with the untreated control.

Research conducted in Ohio between 2008 and 2010 showed mixed results in terms of the effects of fungicides on GLS severity and yield. In a field trial conducted at South Charleston, OH, in 2008, mean GLS severity on the ear leaf in the untreated check was 2.8%, and less than 2% in treated plots, and yield was not significantly affected by any of the treatments, which included picoxystrobin applied at various rates and Headline applied at label-recommended rate at VT. Some of the treated plots even had numerically lower yields than the untreated control (44). In another trial at the same location in the same year, Headline significantly reduced the levels of GLS severity compared to the untreated check, while Envito 4F (40.3% fluoxastrobin, Arysta LifeScience, Cary, NC) did not. However, the effect of fungicide treatment on yield was again not statistically significant (45). Similarly, results from a third trial conducted at the South Charleston in 2008 (46) and a similar trial conducted near Custar, OH (47), showed that none of the tested fungicides (Headline, Stratego 250 EC, Quadris 2.08 SC, and Quilt
1.67 SC) provided a statistically significant yield benefit, with some treatments yielding numerically less than the untreated control. Similar trends were observed in trials conducted in 2009 (48-52) and 2010 (53, 54) in which various fungicides were tested at different rates and application times on different hybrids.

**CHANGING TRENDS IN FUNGICIDE USE IN CORN**

Since 2007, the use of foliar fungicides on corn has increased, and for the most part, this increase has not been due to higher levels of foliar diseases. Estimates of approximately 4.3 to 5.6 million hectares (ha) out of an total of approximately 30.8 million ha of corn in the Midwestern United States were sprayed with a foliar fungicide in 2007 (41). In Ohio alone, more than 648,000 hectares of corn were sprayed with fungicide during the 2007 growing season, with slightly lower estimates for 2008 through 2010. At an estimated application cost (product plus application) of $57/ha, this represents a total expenditure on fungicide of $36.8 M in 2007 alone.

Recent changes in foliar fungicide use in field corn have been due in part to higher grain prices, but more importantly, industry claims of substantial yield increases associated with the use of foliar fungicide, especially those belonging to the Quinone Outside Inhibitor (QoI) group of compounds, the strobilurins. Reports from fungicide industry-based research suggest that the application of fungicides, especially Headline (23.6% pyraclostrobin), leads to yield increases as high as 0.57 t/ha in some years. Most of these reports, however, were provided without a complete characterization of the conditions under which the trials were conducted. Very little information was given about disease onset relative to grain fill, the overall level of disease, environmental and soil
conditions, hybrid susceptibility, and yield potential. Moreover, several of these trials were conducted without adequate replication, making it difficult to ascertain whether the response was due to the fungicide. Results from university-based replicated trials suggest that the effect of Headline application on yield is less clear-cut than industry data seem to suggest. In some university trials, positive yield responses were associated with disease control, in others there were no yield benefits even when diseases were controlled, yet in other trials positive yield responses were observed in the absence of high disease pressure (48-54). Negative yield responses have also been observed both in the presence and absence of high disease pressure.

Paul et al. (56) recently conducted a meta-analysis of hybrid field corn yield response to a single VT/R1 application of four of the most commonly used QoI fungicides. They showed that, a subset of the 212 studies used in the analysis had a negative yield response, meaning that the nontreated check plots had higher mean yields than the fungicide–treated plots. This occurred in 28-48% of the studies, depending on the fungicide being evaluated (56). However, in general, mean grain yield was higher in plots treated with a fungicide than in the nontreated check. Using the mean yield and the between study variance from the meta-analysis and a range of fungicide application cost x grain price combinations, they estimated the probability of the expected yield response in a new randomly selected trial being insufficient to offset the cost of fungicide application. For 205 of the 384 grain price x application cost x fungicide scenarios considered, there was more than a 70% chance of not seeing a return on investment when disease severity was less than 5%. Paul et al. (56) concluded that foliar fungicides on field corn may be warranted and cost effective when disease severity is higher than 5% in
the absence of fungicide, grain prices are very high, and application costs are low. However, their data suggested that there is great uncertainty, even when disease severity is greater than 5%, that a grower would realize a profit in any given year and location when a fungicide is applied between VT and R1 (56).

In addition to hopes of high yield increases, "Plant Health" or “Plant Performance” claims have also contributed to the increase in foliar fungicide application. Some of these claims include better tolerance to drought, heat, cold and ozone; improved plant utilization of nitrogen; increased tolerance to bacterial and viral infections; improved stalk strength and better harvestability; better tolerance to stalk diseases; better tolerance to hail; and more uniform seed size (32). To investigate some of the “Plant Health” claims Bradley et al. (11) conducted a study during the 2007 and 2008 growing seasons in which fungicide treatments were applied to plots with simulated hail-damage. Results from this study showed that although there were reductions in disease levels during the first year, foliar fungicides did not significantly improve yields in either the damaged or non-damaged plots, compared with the non-treated controls (11).

Not only do unnecessary fungicide applications increase production cost without necessarily resulting in profitable increases in grain yield and plant health benefits, prophylactic use of foliar fungicides also put these useful disease management tools at risk for fungicide resistance (12). The Fungicide Resistance Action Committee (FRAC; www.frac.info) characterizes QoI fungicides as having a high risk of resistance being developed (27). To date, over 40 phytopathogenic fungal species have developed field resistance to this group of compounds. This is largely because the QoI fungicides have a single site of action and resistance (or reduced sensitivity) development in fungi to these
products is a single-step process (42). Three single-step amino acid substitutions in the cytochrome b gene of fungi have been characterized as being responsible for shifts in sensitivity to QoI fungicides (42). These substitutions include a change from glycine to alanine at position 143 (G143A), a change from phenalanine to leucine at position 129 (F129L), or a change from glycine to arginine at position 137 (G137R) (42). Resistance to QoI fungicides was found in Cercospora sojina, a close relative of Cercospora zeae-maydis, in 2010 (13, 14). Evaluating the sensitivity of C. zeae-maydis to QoI fungicides, Bradley et al. (12) observed that in vitro germination of C. zeae-maydis conidia was most inhibited by pyraclostrobin and trifloxystrobin than by azosystrobin. However, this does not seem to translate into differences in efficacy between azoxystrobin and pyraclostrobin against gray leaf spot under field conditions (10, 11, 12). Bradley et al. (12) further reported that the distribution of baseline sensitivity for pyraclostrobin was skewed towards the less sensitive end, suggesting that resistance in C. zeae-maydis populations is possible. In contrast, the relatively narrow range of sensitivity of the baseline isolates to the three tested fungicides suggested otherwise. However, they concluded that since the use of baseline sensitivity to assess the risk of resistance development is not the most reliable approach, further monitoring of sensitivity to QoI fungicides in C. zeae-maydis populations over time will be necessary to determine whether there is a shift in sensitivity (12).
THESIS OBJECTIVES

Although crop rotation and tillage have both been shown to be effective management strategies for foliar diseases in corn, there are practical, political and economic limitations to widespread adoption of these practices. For instance, because of soil conservation concerns and government-imposed restrictions on the amount of residue left on the soil surface, conventional tillage practices capable of burying more than 80% of the surface residue have not been used routinely in farm operations since the 1970s (8). High fuel prices are another factor limiting tillage adoption, leading to the increase in conservation tillage. For example, the energy saving for the no-tillage production of a hectare of corn is equivalent to about 33 liters of diesel fuel annually compared to conventional-tillage (59). Conventional-tillage has long been replaced by conservation tillage (“no-till”, “direct drill”, “minimum-till” and “ridge-till”) which leaves 30% or more of the soil surface covered with crop residue (8, 72). It is highly unlikely that growers will return to conventional-tillage.

More areas planted to corn to meet the demands of the ethanol market or in response to higher grain prices will lead to an increase in continuous-corn acreage and could result in relatively fewer acres planted to soybeans and wheat, disrupting a two- or three-crop rotational system that has been a major component of integrated management of corn, wheat and soybeans diseases in Ohio. Because of current trends in corn
production away from crop rotation and conventional tillage, resistance along with fungicide use will play an increasingly important role in disease management in fields planted to continuous corn, especially in situations where conservation tillage is practiced. Moreover, fungicides are currently being used without or with limited regard for the risk of foliar diseases. However, the conditions under which application of these fungicides, especially those belonging to the relatively newer QoI group of compounds, are most profitable are not fully understood. While there is circumstantial and empirical evidence suggesting that foliar fungicide may lead to yield increases, more direct investigations are needed to determine the influence of crop-, environment-, pest-, fungicide- and management-related factors on yield and yield component responses to these products to better determine the conditions under which their use may be most consistent and profitable. Hence the objectives of the research presented in this thesis were to: investigate the effects of hybrid gray leaf spot resistance and yield potential, and fungicide application (product and timing) on the development of gray leaf spot and grain yield and yield components in a minimum-till/continuous-corn and a corn-soybean rotation cropping system.
References


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Ohio ranks 9th among leading U.S. corn-producing states in total grain corn acreage. The total estimated area planted to grain corn in the state has increased over the past 5 years, from 1.3 million hectares in 2006 to 1.5 million hectares in 2011, and projections are for further increases over the next few years (16). This increase is very consistent with the national trend, being triggered largely by higher grain prices ($0.08/kg in 2006, compared to $0.26/kg in 2011 (price on Nov 7, 2011)) and the prospect of large-scale ethanol production (16). More areas planted to corn has led to an increase in continuous-corn acreage and could result in relatively fewer acres planted to soybeans and wheat, disrupting a two- or three-crop rotational system that has been a major component of the crop production system in Ohio.

The increase corn acreage has been accompanied by an increase in the use of foliar fungicides. Since 2007, growers across the Corn Belt have been investing heavily in fungicide application. This is due in part to current corn prices and the potential for greater disease development in a continuous-corn conservation-tillage cropping system, but more importantly, to industry claims of substantial yield increases due to the use of fungicide. Some of these reports suggest that the application of fungicides, especially products belonging to the chemistry class, QoIs, leads to yield increases as high as 22.45 kg/ha in some years. However, results from university-based replicated trials suggest that
the yield response to foliar fungicides is less clear-cut than industry data seem to suggest. In some instances, positive yield responses were associated with disease control, in others there were no yield benefits even when diseases were controlled, yet in other trials positive yield responses were observed in the absence of high disease pressure.

Paul et al. (8) conducted a meta-analysis of field corn yield response to QoI-based fungicide using data from 212 fungicide studies. A subset of the studies used in the analysis had a negative yield response, meaning that the non-treated plots had higher mean yields than the fungicide–treated plots. This occurred in 28-48% of the studies, depending on the fungicide being evaluated (8). However, there was a significant, positive mean yield response across all studies to all four of the fungicide treatments, with a single application between the VT and R1 (8) resulting in 1,821 to 3,034 kg/ha higher mean grain yield than the nontreated check. Paul et al. (8) also used statistics from the meta-analysis along with a range of fungicide application cost and grain prices to estimate the probability of the expected yield response in a new randomly selected trial being insufficient to offset the cost of fungicide application. For 205 of the 384 grain price x application cost x fungicide scenarios that were considered, there was more than a 70% chance of not seeing a return on investment when disease severity was less than 5%. Based on these results, they concluded that foliar fungicides on field corn may be warranted and cost effective when disease severity is higher than 5% in the absence of fungicide, grain prices are very high, and application costs are low. However, based on the fact the a large portion of the between study variability remained unexplained, even after accounting for the effects of foliar disease severity on the yield response, suggest that there is great uncertainty, even when disease severity is greater than 5%, that a
grower would realize a profit in any given year and location when a fungicide is applied between VT and R1 (8).

The findings in Paul et al (8) are comparable to those from other studies which showed that mean yield responses to fungicides were higher for trials in which hybrids with fair to poor resistance to gray leaf spot (GLS) were planted than in those with GLS resistant hybrids, and higher for trials in which corn was planted after corn than those in which corn was rotated with soybean (5, 18). However, final yield is a function of several additional factors (2, 9, 11, 15, 18), including soil and environmental conditions such as compaction, flooding or drought; crop management and cultural practices such as nutrient level, crop rotation and tillage; pest, weed, and disease levels; and complex interactions involving these factors. In the case of fungicides, yield responses appear to be a function of the growth stage at which the product is applied, the amount of disease at the time of application, the length of fungicide control, and effective disease control through to physiological maturity. The fungicide effective period is defined at that period, after application of the fungicide, during which there is minimal disease increase (17). Results from a study conducted by Ward et al (17), indicate that fungicide treatments should be initiated after GLS was observed but before high levels were present, commencing when disease was 2% and present on the basal five leaves.

More direct investigations are needed to determine the influence of crop-, environment-, pest-, fungicide- and management-related factors on yield response to fungicides to better determine the conditions under which the use of foliar fungicides may be most consistent and profitable. The overall goal of this study was to systematically evaluate the effects of foliar fungicides on yield and yield components of
hybrid field corn as influenced by cropping system, hybrid resistance, foliar disease intensity, fungicide application timing, and class of active ingredient. To accomplish this goal, experiments were conducted to specifically: i) determine the individual and combined effects of a DMI and QoI fungicide on yield and yield components; ii) determine whether application of these fungicides at different growth stages during grain development (R1, R2 and R3) affected the responses; iii) determine the effect of hybrid maturity, yield potential and gray leaf spot resistance on responses to fungicides; and iv) evaluate the influence of a corn-soybean and no-till continuous-corn cropping systems on fungicide effects on yield and yield component.

MATERIALS AND METHODS

Experimental design and treatment layout: The Hybrid x Fungicide Treatment study:

Two field experiments were conducted during the 2009 and 2010 growing seasons at the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, to investigate the effects of hybrid characteristics and fungicide treatments on yield and disease intensity. Both experiments were designed as a randomized complete block with four replicate blocks in a split-plot treatment structure. Hybrid served as the whole-plot and fungicide class x application timing combination as the sub-plot. Plots were planted on May 6, 2009, and April 4, 2010, using a Kinze 2100 no-till planter (Kinze Manufacturing, Williamsburg, IA) into a minimal-tilled field previously cropped to corn. The planter was equipped with seed boxes and calibrated to plant at a seeding rate of approximately 74,000 seeds/ha. Each plot consisted of four 7.62-m rows, with 0.635-m between rows. Standard herbicides (Roundup in 2009, Corvus, Atrazine in 2010) were applied at label-recommended rates (1.6 l/ha, 240 ml/ha, and 3.7 l/ha, respectively) and
fertility was adjusted for a potential yield of 11.4 t/ha, based on a soil test, by applying 19-19-19 at a rate of 224 kg/ha in both years and urea at 224 and 336 kg/ha in 2009 and 2010, respectively.

Four Seed Consultant hybrids with different GLS resistance, yield potential, and maturity profiles were selected based on information reported in the Seed Consultant 2007 Annual Seed Catalogue: SC1085 - GLS susceptible, high-yielding, and late-maturing (cumulative relative maturity [CRM] of 108 days); SC1103 - GLS resistant, low-yielding and late-maturing (CRM of 110 days); SC1006 - GLS moderately resistant, moderate-yielding and early-maturing (CRM of 100 days), and SC10RR33 - GLS resistant, high-yielding and early-maturing (CRM of 103 days). In each block, each whole plot was divided into seven sub-plots to which different fungicide treatments were applied. Sub-plots were either treated with: i) Domark (20.5% tetraconazole, Valent, Walnut Creek, CA), a demethylation inhibitor (DMI); ii) Headline (23.6% pyraclostrobin, BASF, Research Triangle Park, NC), a quinone outside inhibitor (QoI), or; iii) left untreated. Applications were either made at growth stage R1, R2, or R3. Domark (treatments 2, 3, and 4) was applied at a rate of 366 ml/ha, and Headline (treatments 5, 6 and 7) at 437 ml/ha. The nonionic surfactant, Induce (Helena Chemical Company, Collierville, TN), was added to each treatment at a rate of 0.125%, vol/vol. Applications were made using a high-clearance sprayer fitted with a 3-m-long boom, with 8 Teejet 8001 EVS flat fan nozzles spaced 0.45-m apart. The sprayer was calibrated to deliver approximately 187 l/ha at 276 kPa. Treatments 2 and 5 were applied on 6 August in 2009 and 13 July in 2010 at growth stage R1; treatments 3 and 6 were applied on 13 August in
2009 and 27 July in 2010 at R2; and treatments 4 and 7 were applied on 24 August in 2009 and 2 August in 2010 at R3.

**The Cropping Sequence x Fungicide Treatment study:** Three additional experiments were conducted during the 2009 and 2010 growing seasons to investigate the effects of cropping sequence (crop rotation) and fungicide application timing on yield and disease intensity. Two of the three experiments were conducted in 2009, one at the OARDC Wooster location and the other at the OARDC Western Research Station, near South Charleston, OH. The third was conducted in 2010 at the OARDC, Wooster. For all three experiments, the design was a randomized complete block, with four replicate blocks in a split-plot arrangement of treatments. The whole-plot was previous crop (corn or soybean) and the sub-plot was fungicide treatment. To establish plots representing different cropping sequences (the whole-plots), adjacent portions of a field were planted with either corn (Pioneer Brand hybrid 38A55, susceptible to gray leaf spot) or soybean (variety Kottman) during the season before the establishment of each experiment (in 2008 for the 2009 experiments and in 2009 for the 2010 experiment). Four pairs of corn/soybean plots were planted, constituting the four blocks. The purpose of using two crops, corn and soybean, was to establish plots with residue that could either contribute (corn residue) or not contribute (soybean residue) to corn foliar disease development. Both crop were harvested in the fall, and in the following spring, the entire field was planted with Pioneer Brand hybrid 38A55 on 30 May in 2009 and on 19 April in 2010 at Wooster, and on 11 May in 2009 at Western. Plots at Wooster were planted and managed as described above, while those at Western were planted using a John Deere 7200 planter, fitted with seed boxes and calibrated to plant at an approximate seeding rate of 79,279
seeds/ha. At Western, herbicides were applied at label recommended rates (Bicep II Magnum at 6 l/ha, AMS at 2.8 kg/ha, Roundup at 1.6 l/ha, and Callisto at 218 ml/ha) and fertility was adjusted for a potential yield of 11.4 t/ha, based on soil tests, by applying 19-19-19 at 224 kg/ha and ammonia nitrate at 202 kg/ha.

Each whole-plot was divided into four sub-plots (each consisting of four 15.2-m rows, with 0.635-m between rows) to which fungicide treatments were randomly assigned. Three of the plots were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin; Bayer CropScience, Research Triangle Park, NC), a premix of a DMI and a QoI, at separate growth stages, R1, R2 or R3, and the fourth was left untreated. At Wooster in 2009 Stratego 250 EC (11.4% propiconazole + 11.4 trifloxystrobin; Bayer CropScience, Research Triangle Park, NC) was used for the R1 application. At Wooster, fungicide applications were made as described above, whereas at Western, treatments were applied using a high-clearance LEE Spider Sprayer (Model 4451-DL/C: West Texas Lee Co., Inc., Lubbock, TX), fitted with a 3-m-long boom with 7 Teejet 8001 flat fan nozzles, spaced 0.38-cm apart and calibrated to deliver approximately 187 liters/ha at 276 kPa. Stratego YLD was applied at a rate of 365 ml/ha, while Stratego 250 EC was applied at a rate of 730 ml/ha, with a nonionic surfactant, Induce (Helena Chemical Company, Collierville, TN) added to each treatment at a rate of 0.125%, vol/vol. R1 treatments were applied on 15 July 2009 and 9 July 2010 at Wooster and on 17 July 2009 at Western; R2 treatments on 27 July 2009 and 20 July 2010 at Wooster and on 24 July 2009 at Western; and R3 treatments on 6 August 2009 and 27 August 2010 at Wooster and on 3 August 2009 at Western.
**Inoculum preparation and Inoculation.**

All plots in the *Hybrid x fungicide study* were artificially inoculated, whereas plots in the *Cropping sequence x fungicide study* were naturally infected. Inoculum consisted of *Cercospora zeae-maydis*-infested millet. *C. zeae-maydis* was isolated onto V8-agar from GLS-affected corn leaves collected from fields in Ohio. Diseased leaf tissues were surface disinfested in 10% Clorox solution for 1 minute and incubated in a moist chamber for 72 hours. With the aid of a stereoscopic microscope (Leica Microsystems S6E, Buffalo Grove, IL) and a dissecting needle, spores were picked from sporulating lesions, transferred to V8-agar, and incubated for 10 days under ultraviolet and white light, with a 12-h photoperiod. *C. zeae-maydis* was identified based on colony and spore morphology, and pure cultures were prepared and stored on V8 agar at 0°C until used for inoculation.

Inoculum was prepared by infesting sterilized millet with five *C. zeae-maydis* isolates. Fresh V8-agar plates were seeded with spores from *C. zeae-maydis* stock cultures and incubated as described above. Ten days after seeding, one plate was cut into 16 sections and added to a spawn bag (Myco Supply, Pittsburgh, PA) containing sterilized millet. Prior to being inoculated, the spawn was prepared by adding 1,400 ml of millet to spawn bags containing 750 ml reverse osmosis (R/O) water and allowing the mixture to soak overnight. Bags were autoclaved for 30 minutes on two consecutive days and seeded with *C. zeae-maydis* the following day. Spawn was then grown at room temperature on the laboratory bench for 10-14 days, then spread on Kraft paper in a greenhouse to air dry for 24 hours before being placed in brown paper bags and stored in a cooler at 6°C until ready for use.
In 2009, plants in the two center rows of each plot were inoculated using Bazooka inoculators (Custom Bio-Products, St. Paul, MN). The inoculator was modified to deliver 1.23 ml of *C. zeae-maydis*-infested millet into the whorl of each plant on 21 July, 28 July and 3 August, corresponding to growth stages V8, V10, and VT, respectively. In 2010, plants in the center two rows of each plot were inoculated with 1.23 ml infested millet into the whorl using a 1/4 teaspoon on 11 Jun, 19 Jun, 25 Jun, and 2 Jul, corresponding to growth stages V6, V8, V10, and V13, respectively. Plots were mist irrigated from 20 Jul to 2 Aug in 2009 and from 21 June to 12 July in 2010 to enhance spore infection and disease development.

**Assessment of disease intensity, stalk strength, lodging and yield component:** Disease severity was assessed on 10 arbitrarily selected plants in the two center rows of each plot by estimating the percent diseased area on the ear leaf, the leaf below the ear leaf, and the leaf above the ear leaf, with the aid of a disease rating scale. A standard area diagram was created by using the Severity Pro 3 software (Ver. 3.0, Dept. of Plant Pathology, University of Iowa, Nutter) to generate colored images of sections of corn leaf with gray leaf spot severity ranging from 5 to 95%. Photoshop CS4 (Adobe Systems Incorporated, San Jose, CA) was used to edit images produced by Severity Pro 3 for severity values below 5% and above 95% (0, <1, 2 and 100%). The images were then used to develop a disease rating scale with seven severity classes: 1 = 0% severity, 2 = <1%, 3 = 2-5%, 4 = 6-20%, 5 = 21-50%, 6 = 51-95%, and 7 = 100% disease severity. Whole-plant disease severity also was rated with the aid of a 1 to 7 whole-plant rating scale developed by Agriculture and Agri-Food Canada (10). Disease severity ranges in the different classes of the whole-plant scale were the same as those in the individual-leaf rating scale.
Assessments in the Hybrid x Fungicide Treatment study were done on 18 August, 27 August, and 2 September in 2009 and on 20 July, 29 July and 16 August in 2010, corresponding to growth stages R2, R3, and R4, respectively. Whole-plant assessments were made on 3 September (at growth stage R4) and 10 September (at R5) in 2009 and on 20 July (at R2), 29 July (at R3) and 16 August (R4). For the Cropping Sequence x Fungicide Treatment study, in 2009, individual-leaf GLS severity was rated every 5-8 days between 17 July (R1) and 11 September (R5) at Wooster and between 19 August (R4) and 1 September (R5) at Western. In 2010, the corresponding assessments were made on 30 August (R3) and September 16 (R4) in Wooster. Whole-plant assessments were made in 2009 at Wooster on 3 July, 17 July, 25 July, 3 September, and 11 September, between growth stages R1 and R5. Whole-plant assessments were not done at Wooster in 2010 or at Western in 2009, due to very low disease intensity.

Stalk strength was assessed with a modified Chatillon Digital Force Gauge (Model DFX-050, AMETEK, Largo, FL). Using the rind penetrometer of the force gauge, the force required to puncture the flat side of the stalk at the center of the first internodal region below the primary ear was determined. Stalk strength was recorded as peak force required to rupture the surface of the stalk to a depth of approximately 5 mm, based on the method described by Martin and Darrah (4, 12, 13). Assessments were done on 10 Aug, 3 Oct, and 12 Nov in 2009 and on 4 Aug and 12 Oct in 2010 in the Hybrid x Fungicide Treatment study and on 7 Aug, 2 Sept and 22 Oct in 2009 and 2 Aug and 15 Oct 2010, at Wooster, and on 25 Aug and 19 Oct 2009 at Western in the Cropping Sequence x Fungicide Treatment study.
Lodging was determined in each plot based on the difference in stand count between the R4 and R6 growth stages. The number of standing plants in the two center rows of each plot was counted at the R4 growth stage, and then at the time of harvest, the number of lodged plants in the same two rows was counted. A lodged plant was defined as one that was broken and bent over below the ear. Percent lodging was estimated by dividing the number of lodged plants at the R6 growth stage by the number of standing plants at the R4 growth stage and multiplying by 100.

At R6, prior to harvesting plots with the combine harvester, the primary ear was hand-harvested from five arbitrarily selected plants from the two center rows of each plot, with no more than three ears being collected from either row at both locations. Yield components were determined by weighing each ear and then counting the number of kernel rows and the number of kernels in two arbitrarily selected rows on each of the five ears. The averages of the five ear weights and row and kernel counts were then used as estimates of mean ear weight, mean number of kernel rows per ear, and mean number of kernels per row for each plot. The number of kernels per ear was estimated as the product of the mean number of kernel rows per ear and number of kernels per row. At Wooster, the two center rows of each plot were harvested using an ALMACO SPC 20 plot combine (ALMACO, Nevada, IA). In 2009, grain harvested from each plot was bagged and weighed using a platform scale (HV-WP series, A&D Co., LTD., Tokyo, Japan), and moisture and test weight were estimated using a Seedburo 1200 series moisture tester and Seedburo model 8800A computer grain scale (Seedburo Equipment Company, Des Plaines, IL), whereas in 2010, plot weight moisture and test weight was measured using a GrainGage weigh system (Juniper Systems, Inc., Logan, UT). For the trial conducted at
Western, plots were harvested and weights and moisture were determined using a Massey Ferguson 8XP combine.

**Data Analysis**

Prior to data analysis, disease severity classes were converted to percent severity by using the midpoint of the range of severity values for each level on the 1-7 scale (8). Severity was then arcsine-square-root-transformed for analysis. The effects of hybrid, fungicide treatment (product x timing), and their interactions on transformed disease severity, yield, and yield components (kernel rows per ear, kernels per row, and kernels per ear) were determined with a generalized linear mixed model. In the analysis, hybrid, treatment, and previous crop were all treated as qualitative factors and considered fixed effects in the model, whereas, block and all interactions involving block were considered random effects. Analyses were done using proc GLIMMIX of SAS. Fixed effects were evaluated with F-tests, and random effects were evaluated with standard normal test statistics. *Estimate, lsmeans*, and *contrast* statements were used to compare treatments and treatment combinations.
RESULTS

The Hybrid x Fungicide Treatment study – Fungicide and hybrid effects on gray leaf spot severity: Gray leaf spot (GLS) intensity varied among years and fungicide treatments, with the overall level of disease being higher in 2010 than in 2009. Averaged across hybrids, mean ear-leaf severity in the untreated check at the R4 growth state was 26% in 2010 compared to 2.3% in 2009. In both years, all treated plots had lower mean levels of GLS severity than the untreated check. Among the fungicide treatments, plots treated with either Domark or Headline at the R1 growth stage generally had the lowest levels of disease severity, both on the ear-leaf and whole-plant rating scale, than those treated at R2 and R3 (Fig. 2.1).

On both rating scales, fungicide treatment had a significant effect on transformed GLS severity in both 2009 and 2010. However, the main effect of hybrid was only significant in 2009, but not in 2010, whereas the hybrid x treatment interaction was not significant in either year (Table 2.1). Based on mean separation tests and pairwise comparisons between treatments, there was generally no evidence of a difference between Domark and Headline at any of the application times (Fig. 2.1). The only exception was for treatments applied at R2 in 2009, where Headline resulted in significantly lower mean disease severity (on the transformed scale) on the ear leaf than Domark. Averaged across fungicides, the R1 and R2 treatments significantly reduced ear leaf GLS severity relative to the check in both years, whereas the R3 treatments were not significantly different from the check. For disease rated on the whole-plant scale, only the R1 treatment consistently had significantly lower mean disease intensity than the check (Fig. 2.1)
**Fungicide and hybrid effects on grain yield and yield component:** Averaged across hybrids and fungicide treatments, mean yields were higher in 2010 than in 2009 (Fig. 2.2). In the first year, mean yields ranged from 3.9 to 5.9 MT/ha, with hybrid SC10RR33 having the lowest and hybrid SC1085 the highest yields, respectively (Fig. 2.2). In year two of the trial, mean yields ranged from 6.5 to 8.3 MT/ha, with hybrid SC1103 and SC10RR33 having the lowest and highest means, respectively. However, the effect of hybrid was only statistically significant in 2009 (Table 2.1), with all pairwise comparisons between hybrids being significant (Fig. 2.2). For both Domark and Headline treatments, yields were numerically higher for treatments applied at R1 than at the other growth stages in 2009, but in 2010, the yield response was very similar among the fungicide treatments (Fig. 2.3). The effects of fungicide and the interaction between fungicide and hybrid were not significant (P < 0.05) in either year.

Fungicide and hybrid effects on individual yield components varied between years and with the component being evaluated (test weight, 100-kernel weight, ear weight, number of kernels per ear, kernels per row, and rows per ear). For test weight, in both 2009 and 2010, the main effect of hybrid was highly significant, but the effects of fungicide treatment and the fungicide x hybrid interaction were not (Table 2.1). In both years, hybrid SC1006 had significantly higher test weight than the other three hybrids, with means of 665 and 722 kg/M³ in 2009 and 2010, respectively, compared to 630 and 691 kg/M³ for SC1085, 641 and 705 kg/M³ for SC10RR33, and 637 and 684 kg/M³ for SC1103 in the two years (Fig. 2.4). In 2009, test weights were not significantly different
among SC1085, SC10RR33 and SC1103. In contrast, in 2010, a significant difference in test weight was observed between SC10RR33 and SC1103 ($P < 0.05$).

The effect of the interaction between fungicide treatment and hybrid was statistically significant for four of the other five yield components (100-kernel weight, number of kernels per ear, number of kernels per row, and number of rows per ear) evaluated in 2010 and for two (number of kernels per ear and number of kernels per row) of the five in 2009 (Table 2.1). In addition, the interaction had a marginal effect on ear weight in both years ($P = 0.058$ and 0.062, respectively). Of all treatment x hybrid combinations, the application of Domark to hybrid SC1085 at R1 (treatment 2) and Headline to the same hybrid at R2 (treatment 6) resulted in the highest mean ear weight in 2009. In contrast, the application of Domark at R3 (treatment 4) and Headline at R2 (treatment 6) to hybrid SC1006 resulted in the highest mean ear weight in 2010. Across hybrids, fungicides, and growing seasons, the R2 application tended to be the most consistent in terms of its effect on ear weight (Fig. 2.5). For instance, when Headline was applied at R2 (treatment 6), the mean weight of the ear was (numerically but not always statistically) higher for five of the eight hybrid-year combinations. A similar trend was observed for four of the eight combinations when comparing R1 and R2 applications of Domark (Fig. 2.5). In both years, averaged across hybrids, the mean ear weight was between 2 and 31 g higher in fungicide treated plots than in the untreated check, with treatments 3 (Domark at R2) and 5 (Headline at R1) in 2009, and treatments 2 (Domark at R1) and 6 (Headline at R2) in 2010 resulting in the greatest increases in ear weight relative to the check.
One-hundred-kernel weight was significantly affected by hybrid in both years, and by the fungicide x hybrid interaction in 2010. The main effect of fungicide treatment was not statistically significant in either year (Tables 2.1), although several treatments resulted in numerically higher 100-kernel weights than the untreated check for three of the four hybrids in both years. In 2009, all pairwise differences between hybrids were statistically significantly. Hybrid SC1085 had the highest mean 100-kernel weight (40 g), followed by SC1006 (36 g), SC1103 (33 g) and SC10RR33 (31 g). Similarly, in 2010, hybrids SC1085 and SC1006 had significantly higher mean 100-kernel weights than SC1103 and SC10RR33 (Fig. 2.6). In 2010, differences among fungicide treatments were significant for hybrids SC1085, SC10RR33, and SC1103. For SC1085, the treatments applied at R3 (treatments 4 and 7) resulted in the highest 100-kernel weights; for SC10RR33, treated plots had numerically lower 100-kernel weight than the untreated check, however, the difference was only statistically significant for treatment 7; and for SC1103, treatment 4 had significantly lower 100-kernel weight than treatments 2 and 3.

In both 2009 and 2010, the effect of fungicide treatment on the number of kernels per ear (KRNL_EAR) and the number of kernels per row (KRNL_ROW) depended on the hybrid (Table 2.1). In 2009, mean KRNL_EAR was numerically higher in fungicide treated plots than in the untreated check for 22 of the 24 comparisons, with the difference ranging from 8 to 176 kernels (Fig. 2.7). For hybrid SC1006, with the exception of treatments 2 and 6, all treatments had significantly higher mean KRNL_EAR than the check. For hybrids SC1085 and SC10RR33, only treatment 6 and treatments 5 and 7, respectively, had significantly higher KRNL_EAR than the check. None of the treatments were significantly different from the check when applied to hybrid SC1103. Somewhat
similar trends were observed in 2010, with most treatments resulting in numerically higher mean KRNL_EAR than the untreated check for all hybrids except for SC1103 (Fig. 2.7). Averaged across treatments, hybrid SC10RR33 had significantly higher KRNL_EAR than the other hybrids in both years.

The same general trends observed for KRNL_EAR were also observed for KRNL_ROW. Treated plots again had numerically higher mean KRNL_ROW than the untreated check for most of the hybrid x treatment combinations (22 of 24 in 2009 and 18 of 24 in 2010) in both years (Fig. 2.8), but the differences were only statistically significant for a few combinations. In 2009, all treatments, except for treatment 6, resulted in significantly higher mean KRNL_ROW than the check for hybrid SC1006. However, none of the treatments led to a significant increase in KRNL_ROW over the check for any of the other hybrids. In 2010, only treatment 6 applied to hybrid SC10RR33 and treatment 2 applied to SC1103 resulted in significantly higher KRNL_ROW than the check. Averaged across treatments, hybrids SC1006 and SC10RR33 had the highest mean KRNL_ROW in both years (Fig. 2.8).

The mean number of rows of kernels per ear (ROW_EAR) varied among hybrids in both years, but for any given hybrid, the response was very similar among fungicide treatments (Fig. 2.9). The main effect of hybrid was highly significant in both 2009 and 2010, whereas the effect of the hybrid x fungicide treatment interaction was only significant in 2010 (Table 2.1). Fungicide alone did not have a significant effect on ROW_EAR in either year. In 2009, only treatment 5 led to a significant increase in ROW_EAR over the untreated check when applied to hybrid SC1085. As was the case in 2009, in 2010, several treatments had numerically higher mean ROW_EAR than the
check on the four hybrids, but the differences are generally not statistically significant. The exceptions were for treatment 4 applied to hybrids SC10RR33 and SC1103 and treatment 6 applied to SC1103. In these cases, the treated plots had significantly lower mean ROW_EAR than the check. Hybrid SC10RR33 had significantly higher mean ROW_EAR than all other hybrids in both years, followed by hybrid SC1103 which had significantly higher ROW_EAR than SC1006 in 2009 and both SC1006 and SC1085 in 2010. A significant difference was also observed between SC1006 and SC1085 in 2009 but not in 2010 (Fig. 2.9).

*Cropping sequence x Fungicide Treatment study – Previous crop and fungicide effects on foliar disease severity*: In this study, foliar disease intensity was extremely low at Wooster in 2010. Mean GLS severity was below 1% in all plots, and not analyzed. However, at Western and Wooster in 2009, higher levels of foliar disease were observed. Mean foliar disease intensity varied among cropping sequence, with plots planted after corn (into corn residue) tending to have higher mean percent leaf area diseased than plot planted after soybean (Fig. 2.10). At Western 2009, mean severity in the untreated check was 10.4% and 5.6% in plots planted into corn and soybean residue, respectively. The corresponding means at Wooster 2009 were 9.8% and 1.4%. For both cropping sequences, treated plots generally had lower levels of disease than the untreated checks (Fig. 2.10).

The main effects of previous crop and fungicide treatment on GLS severity were statistically significant at Wooster in 2009, but not at Western. The interaction effect was not significant in any of the trials (Table 2.2). Disease severity was too low in 2010 to
warrant statistical analysis. In Wooster, averaged across fungicide treatments, mean
disease severity on the ear leaf (on the transformed scale) was significantly lower ($P < 0.05$) in plots planted into soybean residue that in plots planted into corn residue. All
treatments (application timings) resulted in significantly lower whole-plant GLS severity
than the untreated check. Among the fungicide treatments, applications made at R1 led to
a greater reduction in both ear-leaf and whole-plant disease severity than applications
made at R2 and R3.

**Previous crop and fungicide effects on yield and yield components:** Mean yield varied
among trials and among treatments within trial (Fig. 2.11). Averaged across treatments
and cropping sequences mean yield was 11.8 MT/ha at Western 2009, 10.5 MT/ha at
Wooster 2009, and 9.4 at Wooster 2009. Yields were numerically between 0.1 and 0.7
MT/ha higher in fungicide treated plots than in the untreated check at Western 2009,
between 0.32 and 0.86 MT/ha lower in fungicide treated plots as Wooster 2009, and
between 0.30 and 0.51 MT/ha higher in treated plots at Wooster 2010. However, the
effect of fungicide treatment on yield was only statistically significant at Western 2009,
and the effect varied with cropping sequence since the interaction was also statistically
significant (Table 2.2). For plots planted into corn residue, only the R2 treatment resulted
in significantly higher yield than the check, whereas for plots planted after soybean,
treatments 2 and 4, applied at R1 and R3, respectively, yielded significantly more than
the check. The effect of previous crop was statistically significant at Wooster 2009, with
plots planted following soybean yielding significantly more (1.5 MT/ha) than plots
planted following corn (Table 2.2, Fig. 2.11).
Test weights were very similar among treatments and trials (Fig. 2.12), ranging from 747 to 783 kg/m$^3$ at Western 2009, from 696 to 766 kg/m$^3$ at Wooster 2009, and from 700 to 757 kg/m$^3$ at Wooster 2010. The fungicide x previous crop interaction was statistically significant at Western 2009, but not in any of the other trials. Cropping sequence did not have a significant effect on test weight in any of the trials. The main effect of fungicide and its interaction with previous crop were marginally significant at Wooster 2009 (Table 2.2). However, none of the treatments resulted in a significant increase in test weight over the check for any of the trial x previous crop combinations.

Fungicide treatment had a significant effect on 100-kernel weight at Western 2009 and Wooster 2010, and its interaction with previous crop had a marginal effect at Wooster 2010, but the main effect of previous crop was not statistically significant in any of the trials (Table 2.2). Averaged across previous crop, all fungicide treatments resulted in significantly higher 100-kernel weights than the untreated check at Western 2009 (Fig. 2.13). The mean difference ranged from 2.61 g for the R1 treatment to 5.09 g for the R3 treatment. The effect of fungicide treatment on 100-kernel weight was somewhat opposite at Wooster 2010 to that observed at Western 2009. In the 2010 trial, all fungicide-treated plots had numerically lower 100-kernel weight than the untreated check, with the difference relative to the check being greatest and statistically significant for the R1 and R2 treatments when corn followed soybean in the rotation.

The main and interaction effects of fungicide treatment and previous crop were not statistically significant for any of the other yield components in any of the trials (Table 2.2). Mean ear weight, number of kernels per ear, number of kernels per row, and number of rows per ear were very similar among treatments and previous crops in all
trials (Fig. 2.14 and 2.15). In two of the three trials (Western and Wooster 2009), plots planted after soybean had numerically higher mean ear weight, number of kernels per row, and number kernels per ear than plots planted after corn, whereas the opposite was observed in the third trial (Wooster 2010). However, the differences were not statistically significant.
DISCUSSION

Recent trends in corn production in Ohio have led to an increase in conservation-tillage, underutilization of crop rotation, and farmers choosing hybrids based on yield potential and neglecting host resistance. These trends have led to a greater reliance on foliar fungicides for disease management. In addition to fungicide applications for disease control, there is also the recent trend of applying fungicides for ‘plant health’ benefits as well as ‘insurance applications’ in the absence of foliar diseases. Although results from a meta-analysis conducted by Paul et al. (8) clearly showed a yield response to foliar fungicide when disease levels were high, there was considerable unexplained among-study variability in that investigation, suggesting that other factors affected the yield response. In addition, the study by Paul et al. (8) only considered QoI-based fungicide applied at R1, a group of active ingredients known to affect crop physiology (1, 3, 8). However, the QoI fungicides are not the only chemistry currently being used prophylactically in Ohio. Moreover, it is unclear whether the yield responses reported in Paul et al. (8) were fungicide-chemistry- or application-timing dependent, or whether those results were due to a particular yield component or set of yield components.

In this study the effects of hybrid resistance, maturity, and yield potential, and fungicide chemistry and application timing on the development of gray leaf spot (GLS) and yield response of field corn in corn-soybean rotation and minimum till/continuous-corn cropping systems were investigated. Individually, all of the aforementioned factors have been shown to affect GLS intensity and grain yield and quality. However, few studies have investigated the combined effects of these factors on GLS, yield, and yield
components. All fungicides tested, regardless of chemical class and hybrid characteristics (the hybrid x treatment interaction was not statistically significant), led to the greatest reduction in GLS intensity relative to the untreated check when applied at R1 (silk emergence). R2 applications also reduced GLS intensity; however, the overall magnitude of the reduction was lower than that achieved with the R1 application. R3 applications were the least effective, generally having disease intensity comparable to that of the untreated check. Comparisons between the two cropping systems evaluated showed that corn planted after soybean generally had significantly lower mean GLS severity than corn planted after corn. In fact, the untreated check in the corn-soybean rotation generally had lower mean GLS severity than all fungicide-treated plots in the corn-on-corn cropping system. The effect of hybrid on GLS severity was statistically significant in one of the two trials in which hybrid characteristics were evaluated. Averaged across fungicide treatments, as expected, the early-maturing resistant hybrid had significantly lower mean GLS severity than the other three hybrids. The observed effects of fungicide application timing and hybrid resistance and maturity of GLS intensity were consistent with findings from other studies in which older corn hybrids and older fungicides with different active ingredients were evaluated (5, 17).

The effects of fungicide treatment and hybrid resistance on GLS severity did not necessarily translate into an effect on grain yield and yield components. In a few instances, there was some evidence of numerically higher yield in fungicide-treated plots than in the untreated check; however, the treatments that resulted in the greatest levels of GLS control (the R1 applications) did not always result in the highest yield increase. The effect of fungicide treatment on yield was statistically significant in only one of the five
trials, and the fungicide effect was influenced by cropping sequence but not by hybrid characteristics. In the corn-on-corn system, mean yield was significantly higher in plots treated at R2 than in those treated at R1, R3, or the untreated check, despite the fact that the level of GLS severity was similar between the R1 and R2 treatments. In the corn-soybean rotation, on the other hand, the R1 and R3 treatments had significantly higher mean yields than the check.

In the trials in which hybrid characteristics were evaluated, hybrid maturity had a greater effect on yield response than GLS resistance or yield potential. The two late-maturing hybrids had significantly higher yield than the two early-maturing hybrids. Contrary to what was expected based on GLS resistance, one of the most resistant hybrids (SC10RR33) and the one with the lowest level of foliar disease severity had the lowest mean yield response, whereas the most susceptible hybrid (SC1085) had the highest yield response. On the other hand, the hybrid with the highest level of disease, SC1006, did have one of the lowest mean yields. SC1085 and SC10RR33 are considered hybrids with high yield potential, while SC1006 is considered to be of moderate yield potential. SC1085 is late-maturing, whereas SC10RR33 and SC1006 are early-maturing. Hybrid maturity affects the length of the grain fill period, with late-maturing hybrids generally having longer grain fill periods, and consequently, higher yields than early-maturing hybrids (14). Moreover, maturity also affects the timing of disease onset and development relative to grain development and the coincidence of favorable weather with susceptible growth stages of the crop (7). However, these responses are strongly influenced by weather conditions (11, 15). The fact that the effect of hybrid on yield and
the association between hybrid maturity and yield was only observed in 2009 but not in 2010 may be indicative of the influence of weather conditions.

Fungicide treatment did not have a significant effect on test weight in any of the trials, but the effect of hybrid was statistically significant in both of the trials in which this effect was evaluated. However, the test weight response appeared to be independent of disease level and hybrid maturity, or the levels of disease were not high enough to influence the response, since the hybrid with the highest mean GLS severity had significantly higher mean test weight than the other hybrids with lower disease severity.

In one of the fungicide treatment x cropping sequence trials (Western 2009), the interaction had a significant effect on test weight. The R2 application resulted in significantly higher test weight than the R1 application in the continuous-corn system but not in the corn-soybean rotation. Comparing treatments across cropping systems, the R1 application in the corn-soybean rotation resulted in significantly higher mean test weight than the same application in the corn-on-corn system. However, these results were not consistent across location-years.

The effects of hybrid and hybrid x fungicide treatment interaction were statistically significant for several yield components in both years of this study, but the main effect of fungicide treatment alone was generally not significant for any of the components in either year. The observed differences among hybrids in this study were expected given that hybrids are known to be inherently different in these traits and because the hybrids used were specifically selected for this purpose. However, the significant hybrid x fungicide treatment interactions indicated that the timing of fungicide application may affect ear and kernel weight and the number of kernels per ear and row,
and that the effects of Domark or Headline on these responses depended on when they were applied and the hybrid to which they were applied. Ear weight, kernels per row, and kernels per ear tended to be higher (numerically and sometime statistically) in fungicide treated plots than in the untreated check of the two hybrids with high yield potential (SC1085 and SC10RR33). Ear weight, kernels per row, kernels per ear, and rows of kernels per ear were generally higher for the Headline treatments than the Domark treatments on SC10RR33 but not on the other hybrids in both years. This may be due in part to the known physiological effects of QoI fungicides (1, 3), but it is unclear why the response was restricted to one hybrid. Among the Domark treatments on SC10RR33, the responses were consistently higher and lower for the R2 and R3 applications, respectively, than for the other applications. Among the Headline treatments, the R2 applications were most consistent in terms of its effects on ear weight.

In the cropping sequence x fungicide timing study, contrary to what was observed in the hybrid x fungicide study, fungicide treatment had a significant effect on 100-kernel weight at Western 2009 and Wooster 2010, with the treated plots generally having higher mean 100-kernel weights than the untreated checks. The effect of fungicide application on kernel weight in this study and the effects of fungicide interaction with hybrids on other yield components may suggest that fungicide applications between R1 and R3 contribute to these responses by reducing stresses due to GLS and other foliar diseases during pollination, grain-fill, and kernel development. Stresses at these developmental stages are known to affect the size of the corn kernels (14). However, the best treatments in terms of disease control were not always the treatments with the greatest effect on yield components. In addition, the effects of fungicide treatment on the yield components
of certain hybrids did not necessarily translate into an overall effect on yield in terms of grain production per unit area. As mentioned above, the apparent disparity among disease, yield, and yield component responses to treatments and hybrids may be due in part to the low overall levels of disease observed in this investigation. Other explanations may be differences in tolerance among the hybrids, with some being able to support a certain level of disease without suffering reductions in yield or yield components, or differential ability of the hybrids to compensate for the reduction in one yield component by increasing another. This latter explanation is supported by our findings, which clearly showed that neither fungicide nor hybrid affected all yield components equally. Hence, since final grain yield is a function of several yield components, if one of these component increases to compensate for the reduction in another, the overall yield may remain unchanged, even when there is a clear effect of hybrid and fungicide on yield components as was the case in this investigation.

The results from this investigation clearly emphasize the complexity of yield response to fungicides and serve to reiterate the fact that such a response is highly variable and unreliable. Fungicide effects on yield and yield components, especially at low levels of disease, are more than likely confounded by other factors with stronger effects, such as hybrid-specific reactions and edaphoclimatic conditions. Although there was some evidence of disease and yield component responses to application timing of the QoI, DMI, and combination fungicides evaluated in this study, the responses were highly variable and not sufficient to result in a significant increase in grain yield. Preliminary results from a meta-analysis of the pooled data from the five experiments (with hybrid and previous crop treated as separate studies) showed that there was an overall numerical
yield increase to fungicide over the untreated check, with the magnitude of the response being greater for the R1 application than for the R2 and R3 applications (Paul unpublished). However, the yield increases were small and not statistically significant, further emphasizing that fungicide application may not be profitable when disease levels are low.
Table 2.1. Summary statistics from linear mixed model analyses of the effects of corn hybrid (HYB) and fungicide treatment (TRT) on disease severity, yield, and yield components showing $F$ statistics and probability values (significance levels) for trials conducted in Wooster, Ohio in 2009 and 2010.

<table>
<thead>
<tr>
<th>Response$^a$</th>
<th>Test Statistic</th>
<th>2009</th>
<th>2010</th>
<th>2010</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HYB$^b$</td>
<td>TRT$^c$</td>
<td>HYB*TRT</td>
<td>HYB$^b$</td>
</tr>
<tr>
<td>Disease Severity Ear leaf</td>
<td>F value</td>
<td>8.27</td>
<td>15.55</td>
<td>1.47</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.006</td>
<td>&lt;0.001</td>
<td>0.126</td>
<td>0.241</td>
</tr>
<tr>
<td>Disease Severity_Whole</td>
<td>F value</td>
<td>8.43</td>
<td>4.53</td>
<td>0.79</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.01</td>
<td>&lt;0.001</td>
<td>0.71</td>
<td>0.124</td>
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<tr>
<td>Yield</td>
<td>F value</td>
<td>25.47</td>
<td>1.40</td>
<td>0.93</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&lt;0.001</td>
<td>0.225</td>
<td>0.544</td>
<td>0.176</td>
</tr>
<tr>
<td>Test Weight</td>
<td>F value</td>
<td>19.95</td>
<td>0.48</td>
<td>0.95</td>
<td>12.74</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&lt;0.001</td>
<td>0.824</td>
<td>0.528</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$^a$Disease Severity Ear Leaf and Disease Severity_Whole = arcsine-transformed mean gray leaf spot severity rated on the ear leaf and the whole-plant, respectively. Yield = grain yield in MT/ha. Test Weight = kg/m$^3$. Ear weight = weight of husk-free ear in grams at physiological maturity. 100-Kernel weight = weight of 100 kernels in grams. Number of Kernels = obtained by multiplying mean number of kernels per row by mean number of rows per ear. Kernels per Row = obtained by averaging counts of two arbitrarily selected rows of kernels per ear. Rows per Ear = obtained by counting the number of rows of kernels per ear.

$^b$HYB = Seed Consultant hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 - GLS susceptible, high-yielding, and late-maturing (cumulative relative maturity [CRM] of 108 days); SC1103 - GLS resistant, low-yielding and late-maturing (CRM of 110 days); SC1006 - GLS moderately resistant, moderate-yielding and early-maturing (CRM of 100 days), and SC10RR33 - GLS resistant, high-yielding and early-maturing (CRM of 103 days).

$^c$TRT = Treatments were Domark (20.5% tetraconazole, Valent, Walnut Creek, CA), Headline (23.6% pyraclostrobin, BASF, Research Triangle Park, NC), and untreated check. Applications were either made at growth stage R1, R2, or R3. Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce (Helena Chemical Company, Collierville, TN), was added to each treatment at a rate of 0.125%, vol/vol.
Table 2.1 continued

<table>
<thead>
<tr>
<th>Response^a</th>
<th>Test Statistic</th>
<th>2009</th>
<th>2010</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>HYB^b</td>
<td>TRT^c</td>
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<tr>
<td>Ear weight</td>
<td>F value</td>
<td>1.79</td>
<td>1.80</td>
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<td>P-value</td>
<td>0.220</td>
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<td>100-Kernel weight</td>
<td>F value</td>
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<td>P-value</td>
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<td>Number of Kernels</td>
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<td>13.92</td>
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<td>P-value</td>
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<td>0.061</td>
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<td>Kernels per Row</td>
<td>F value</td>
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<td>2.32</td>
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<td></td>
<td>P-value</td>
<td>0.041</td>
<td>0.042</td>
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<tr>
<td>Rows per Ear</td>
<td>F value</td>
<td>143.65</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&lt;0.001</td>
<td>0.714</td>
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Table 2.2. Summary statistics from linear mixed model analyses of the effects of previous crop (PC) and fungicide treatment (TRT) on disease severity, yield and yield components showing F statistics and probability values (significance levels) for trial conducted in Wooster and South Charleston (Western), Ohio in 2009 and 2010.

<table>
<thead>
<tr>
<th>Response</th>
<th>Test Statistic</th>
<th>2009 Western</th>
<th>2009 Wooster</th>
<th>2010 Wooster</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>PC</td>
<td>TRT</td>
<td>PC*TRT</td>
</tr>
<tr>
<td>Disease Severity Ear leaf</td>
<td>F value</td>
<td>4.60</td>
<td>1.52</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.129</td>
<td>0.245</td>
<td>0.109</td>
</tr>
<tr>
<td>Disease Severity_Whole</td>
<td>F value</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Yield</td>
<td>F value</td>
<td>0.07</td>
<td>3.62</td>
<td>7.44</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.805</td>
<td>0.033</td>
<td>0.002</td>
</tr>
<tr>
<td>Test Weight</td>
<td>F value</td>
<td>5.51</td>
<td>0.35</td>
<td>3.93</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.101</td>
<td>0.791</td>
<td>0.025</td>
</tr>
<tr>
<td>Ear weight</td>
<td>F value</td>
<td>0.55</td>
<td>0.49</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.512</td>
<td>0.693</td>
<td>0.687</td>
</tr>
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</table>

\[a\] Disease Severity_Ear Leaf and Disease Severity_Whole = arcsine-transformed mean disease severity rated on the ear leaf and the whole-plant, respectively. Yield = grain yield in MT/ha. Test Weight = kg/m³. Ear weight = weight of husk-free ear in grams at physiological maturity. 100-Kernel weight = weight of 100 kernels in grams. Number of Kernels = obtained by multiplying mean number of kernels per row by mean number of rows per ear. Kernels per Row = obtained by averaging counts of two arbitrarily selected rows of kernels per ear. Rows per Ear = obtained by counting the number of rows of kernels per ear.

\[b\] PC = Previous crop in which either corn or soybean was planted the growing season before establishing the experiment.

\[c\] TRT = Fungicide treatments; Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin; Bayer CropScience, Research Triangle Park, NC) at growth stages, R1, R2 or R3, and an untreated check. Stratego YLD was applied at a rate of 365 ml/ha, with a nonionic surfactant, Induce (Helena Chemical Company, Collierville, TN) added to each treatment at a rate of 0.125%, vol/vol.

continued
Table 2.2 continued

<table>
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<tr>
<th>Response</th>
<th>Test Statistic</th>
<th>2009 Western</th>
<th>2009 Wooster</th>
<th>2010 Wooster</th>
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<td></td>
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<td>PC</td>
<td>TRT</td>
<td>PC*TRT</td>
</tr>
<tr>
<td>100-Kernel weight</td>
<td>F value</td>
<td>0.97</td>
<td>6.29</td>
<td>1.67</td>
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<td></td>
<td>P-value</td>
<td>0.398</td>
<td>0.004</td>
<td>0.208</td>
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<tr>
<td>Number of Kernels</td>
<td>F value</td>
<td>0.00</td>
<td>2.10</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>0.976</td>
<td>0.136</td>
<td>0.534</td>
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<tr>
<td>Kernels per Row</td>
<td>F value</td>
<td>0.20</td>
<td>0.57</td>
<td>0.31</td>
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<td></td>
<td>P-value</td>
<td>0.683</td>
<td>0.641</td>
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<td>Rows per Ear</td>
<td>F value</td>
<td>5.92</td>
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<td></td>
<td>P-value</td>
<td>0.093</td>
<td>0.275</td>
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</tbody>
</table>
Fig. 2.1. Mean arcsine-transformed disease severity (averaged across four replicates) for the ear leaf and whole-plant rated on a 1 to 7 scale where 1 = 0% severity, 2 = <1%, 3 = 2-5%, 4 = 6-20%, 5 = 21-50%, 6 = 51-95%, and 7 = 100% disease severity, for each treatment in 2009 and 2010. Prior to data analysis, disease severity classes were converted to percent severity by using the midpoint of the range of severity values for each level on the 1-7 scale. Treatments were Domark (20.5% tetraconazole), Headline (23.6% pyraclostrobin) and an untreated check (UTC). Applications were either made at growth stage R1, R2, or R3. Domark (white hatched bars) was applied at a rate of 366 ml/ha and Headline (black hatched bars) at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol. In each year, treatments with the same letters are not significantly different at the 5% probability level.
**Fig. 2.2.** Mean grain yield (MT/ha) (averaged across four replicates) in 2009 and 2010 for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 - GLS susceptible, high-yielding, and late-maturing (cumulative relative maturity [CRM] of 108 days); SC1103 - GLS resistant, low-yielding and late-maturing (CRM of 110 days); SC1006 - GLS moderately resistant, moderate-yielding and early-maturing (CRM of 100 days), and SC10RR33 - GLS resistant, high-yielding and early-maturing (CRM of 103 days). In 2009, hybrids with the same letters are not significantly different at the 5% probability level.
Fig. 2.3. Mean grain yield (MT/ha) (averaged across four replicates) in 2009 and 2010 for seven treatments: Domark (20.5% tetraconazole), Headline (23.6% pyraclostrobin), and an untreated check (UTC). Applications were either made at growth stage R1, R2, or R3. Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.4. Mean test weight (kg/m³) (averaged across four replicates) in 2009 and 2010 for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 - GLS susceptible, high-yielding, and late-maturing (cumulative relative maturity [CRM] of 108 days); SC1103 - GLS resistant, low-yielding and late-maturing (CRM of 110 days); SC1006 - GLS moderately resistant, moderate-yielding and early-maturing (CRM of 100 days), and SC10RR33 - GLS resistant, high-yielding and early-maturing (CRM of 103 days). In each year, hybrids with the same letters are not significantly different at the 5% probability level.
Fig. 2.5. Mean ear weight (g) (averaged across four replicates) four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 (GLS susceptible, high-yielding, and late-maturing [cumulative relative maturity, CRM of 108 days]); SC1103 (GLS resistant, low-yielding and late-maturing [CRM of 110 days]); SC1006 (GLS moderately resistant, moderate-yielding and early-maturing [CRM of 100 days]), and SC10RR33 (GLS resistant, high-yielding and early-maturing [CRM of 103 days]) for seven treatments. Treatments were Domark (20.5% tetraconazole, Valent), (white hatched bars), Headline (23.6% pyraclostrobin) (black hatched bars), or the untreated check (solid black bars). Applications were either made at growth stage R1 (horizontal stripes), R2 (upward left-to-right stripes), or R3 (downward left-to-right stripes). Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.6. Mean weight of 100 kernels (g) (averaged across four replicates) for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 (GLS susceptible, high-yielding, and late-maturing [cumulative relative maturity, CRM of 108 days]); SC1103 (GLS resistant, low-yielding and late-maturing [CRM of 110 days]); SC1006 (GLS moderately resistant, moderate-yielding and early-maturing [CRM of 100 days]), and SC10RR33 (GLS resistant, high-yielding and early-maturing [CRM of 103 days]) for seven treatments. Treatments were Domark (20.5% tetraconazole, Valent), (white hatched bars), Headline (23.6% pyraclostrobin) (black hatched bars), or the untreated check (solid black bars). Applications were either made at growth stage R1 (horizontal stripes), R2 (upward left-to-right stripes), or R3 (downward left-to-right stripes). Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol. Letters are for mean comparison among hybrids averaged across treatments; hybrids with the same letters are not significantly different at the 5% probability level.
Fig. 2.7. Mean number of kernels per ear (averaged across four replicates) for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 (GLS susceptible, high-yielding, and late-maturing [cumulative relative maturity, CRM of 108 days]); SC1103 (GLS resistant, low-yielding and late-maturing [CRM of 110 days]); SC1006 (GLS moderately resistant, moderate-yielding and early-maturing [CRM of 100 days]), and SC10RR33 (GLS resistant, high-yielding and early-maturing [CRM of 103 days]) for seven treatments. Treatments were Domark (20.5% tetraconazole), (white hatched bars), Headline (23.6% pyraclostrobin) (black hatched bars), or the untreated check (solid black bars). Applications were either made at growth stage R1 (horizontal stripes), R2 (upward left-to-right stripes), or R3 (downward left-to-right stripes). Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol. Letters are for mean comparison among hybrids averaged across treatments; hybrids with the same letters are not significantly different at the 5% probability level.
Fig. 2.8. Mean number of kernels per row base on the mean of two arbitrarily selected rows of kernels (averaged across four replicates) for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 (GLS susceptible, high-yielding, and late-maturing [cumulative relative maturity, CRM of 108 days]); SC1103 (GLS resistant, low-yielding and late-maturing [CRM of 110 days]); SC1006 (GLS moderately resistant, moderate-yielding and early-maturing [CRM of 100 days]), and SC10RR33 (GLS resistant, high-yielding and early-maturing [CRM of 103 days]) for seven treatments. Treatments were Domark (20.5% tetraconazole), (white hatched bars), Headline (23.6% pyraclostrobin) (black hatched bars), or the untreated check (solid black bars). Applications were either made at growth stage R1 (horizontal stripes), R2 (upward left-to-right stripes), or R3 (downward left-to-right stripes). Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol. Letters are for mean comparison among hybrids averaged across treatments; hybrids with the same letters are not significantly different at the 5% probability level.
Fig. 2.9. Mean number of rows of kernels per ear (averaged across four replicates) for four Seed Consultant corn hybrids with different GLS resistance, yield potential, and maturity profiles: SC1085 (GLS susceptible, high-yielding, and late-maturing [cumulative relative maturity, CRM of 108 days]); SC1103 (GLS resistant, low-yielding and late-maturing [CRM of 110 days]); SC1006 (GLS moderately resistant, moderate-yielding and early-maturing [CRM of 100 days]), and SC10RR33 (GLS resistant, high-yielding and early-maturing [CRM of 103 days]) for seven treatments. Treatments were Domark (20.5% tetraconazole), (white hatched bars), Headline (23.6% pyraclostrobin) (black hatched bars), or the untreated check (solid black bars). Applications were either made at growth stage R1 (horizontal stripes), R2 (upward left-to-right stripes), or R3 (downward left-to-right stripes). Domark was applied at a rate of 366 ml/ha and Headline at 437 ml/ha. The nonionic surfactant, Induce, was added to each treatment at a rate of 0.125%, vol/vol. Letters are of mean comparison among hybrids averaged across treatments; hybrids with the same letters are not significantly different at the 5% probability level.
**Fig. 2.10.** Mean arcsine-transformed disease severity assessed on the ear leaf and whole-plant (averaged across four replicates) converted from a 1 to 7 scale where 1 = 0% severity, 2 = <1%, 3 = 2-5%, 4 = 6-20%, 5 = 21-50%, 6 = 51-95%, and 7 = 100% disease severity, for trials conducted at Wooster and South Charleston (Western), OH, in 2009 and 2010. Prior to data analysis, disease severity classes were converted to percent severity by using the midpoint of the range of severity values for each level on the 1-7 scale. Plots planted following corn or soybean as previous crop were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin) at Western 2009 and Wooster 2010 or Stratego (11.4% propiconazole + 11.4% trifloxystrobin) at Wooster 2009 either at growth stage R1, R2 or R3, or left untreated (UTC). Stratego YLD was applied at a rate of 365 ml/ha and Stratego at 730 ml/ha, with a nonionic surfactant, Induce, added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.11. Mean grain yield (averaged across four replicates) for trials conducted at Wooster and South Charleston (Western), OH, in 2009 and 2010. Plots planted following corn or soybean as previous crop were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin) at Western 2009 and Wooster 2010 or Stratego (11.4% propiconazole + 11.4% trifloxystrobin) at Wooster 2009 either at growth stage R1, R2 or R3, or left untreated (UTC). Stratego YLD was applied at a rate of 365 ml/ha and Stratego at 730 ml/ha, with a nonionic surfactant, Induce, added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.12. Mean test weight (averaged across four replicates) for trials conducted at Wooster and South Charleston (Western), OH, in 2009 and 2010. Plots planted following corn or soybean as previous crop were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin) at Western 2009 and Wooster 2010 or Stratego (11.4% propiconazole + 11.4% trifloxystrobin) at Wooster 2009 either at growth stage R1, R2 or R3, or left untreated (UTC). Stratego YLD was applied at a rate of 365 ml/ha and Stratego at 730 ml/ha, with a nonionic surfactant, Induce, added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.13. Mean weight of 100 kernels (averaged across four replicates) for trials conducted at Wooster and South Charleston (Western), OH, in 2009 and 2010. Plots planted following corn or soybean as previous crop were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin) at Western 2009 and Wooster 2010 or Stratego (11.4% propiconazole + 11.4% trifloxystrobin) at Wooster 2009 either at growth stage R1, R2 or R3, or left untreated (UTC). Stratego YLD was applied at a rate of 365 ml/ha and Stratego at 730 ml/ha with a nonionic surfactant, Induce, added to each treatment at a rate of 0.125%, vol/vol.
Fig. 2.14. Mean corn ear weight, number of kernels per ear, and number of kernels per row (based on the mean of two arbitrarily select rows of kernels for each ear evaluated) for corn plots planted following corn and soybean at Western 2009 (WEST_09), Wooster 2009 (WOO_09), and Wooster 2010 (WOO_10). Each bar represents the average across four replicates and four fungicide treatments.
Fig. 2.15. Mean number of rows of kernels per ear (averaged across four replicates) for trials conducted at Wooster and South Charleston (Western), OH, in 2009 and 2010. Plots planted following corn or soybean as previous crop were treated with Stratego YLD (10.8% prothioconazole + 32.3% trifloxystrobin) at Western 2009 and Wooster 2010 or Stratego (11.4% propiconazole + 11.4% trifloxystrobin) at Wooster 2009 either at growth stage R1, R2 or R3, or left untreated (UTC). Stratego YLD was applied at a rate of 365 ml/ha and Stratego at 730 ml/ha with a nonionic surfactant, Induce, added to each treatment at a rate of 0.125%, vol/vol.
References


## Appendix A: Trial Summary

<table>
<thead>
<tr>
<th>Trial</th>
<th>Location</th>
<th>Year</th>
<th>Planting Date</th>
<th>Inoculation date/Growth stage</th>
<th>Fungicide application date/Growth stage</th>
<th>Disease assessment date/Growth stage Ear Leaves</th>
<th>Disease assessment date/Growth stage Whole Plant</th>
<th>Harvest date</th>
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<tbody>
<tr>
<td>IPM-2</td>
<td>Wooster</td>
<td>2009</td>
<td>6-May</td>
<td>21 Jul @ V8, 28 Jul @ V10, and 3 Aug @ VT</td>
<td>2&amp;5 on 6 Aug @ R1, 3&amp;6 on 13 Aug @ R2, and 4&amp;7 on 24 Aug @ R3</td>
<td>18 Aug @ R2, 27 Aug @ R3, 2 Sept @ R4</td>
<td>3 Sept @ R4, 10 Sept @ R4</td>
<td>16-Nov</td>
</tr>
<tr>
<td>IPM-2</td>
<td>Wooster</td>
<td>2010</td>
<td>4-Apr</td>
<td>11 Jun @ V6, 19 Jun @ V8, 25 Jun @ V10, and 2 Jul @ V12</td>
<td>2&amp;5 on 13 Jul @ R1, 3&amp;6 on 27 Jul @ R2, and 4&amp;7 on 2 Aug @ R3</td>
<td>20 Jul @ R1, 29 Jul @ R2, 16 Aug @ R4</td>
<td>20 Jul @ R1, 29 Jul @ R2, 16 Aug @ R4</td>
<td>14-Oct</td>
</tr>
<tr>
<td>IPM-3</td>
<td>Wooster</td>
<td>2009</td>
<td>30-May</td>
<td>N/A</td>
<td>2 on 15 Jul @ R1, 3 on 27 Jul @ R2, and 4 on 6 Aug @ R3</td>
<td>17 Jul @ R1, 25 Jul @ R1/2, 1 Aug @ R2, 8 Aug @ R3, 15 Aug @ R4, 21 Aug @ R4/5, 29 Aug @ R5, 2 Sept @ R5</td>
<td>30 Aug @ R5, 11 Sept @ R5</td>
<td>30-Oct</td>
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<td>IPM-3</td>
<td>Wooster</td>
<td>2010</td>
<td>19-Apr</td>
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<tr>
<td>IPM-3</td>
<td>Western</td>
<td>2009</td>
<td>11-May</td>
<td>N/A</td>
<td>2 on 17 Jul @ R1, 3 on 24 Jul @ R2, and 4 on 3 Aug @ R3</td>
<td>19 Aug @ R4, 25 Aug @ R5, 1 Sept @ R5</td>
<td>N/A</td>
<td>6-Nov</td>
</tr>
</tbody>
</table>