UNDERSTANDING VARIABILITY ASSOCIATED WITH CORN GRAIN YIELD RESPONSE TO FOLIAR FUNGICIDE APPLICATION

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THESIS

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ABSTRACT

Quinone outside inhibitor (Q_oI) fungicide (e.g., pyraclostrobin, azoxystrobin, and trifloxystrobin) fungicide application is becoming a common practice in corn production systems. The strobilurin class of fungicides inhibits mitochondrial respiration in fungal cells, and preventing plant pathogen infection. In addition, these compounds have been shown to decrease ethylene biosynthesis and reduce oxidative stress in plants. Yield response to fungicide application tends to be highly variable as a result of varying disease pressure, but may also be affected by environmental factors and management practices that influence corn yield potential. The focus of this research was to determine if there is a differential yield response to strobilurin fungicide application under standard and intensive management systems. The standard management system in this study consisted of crop input levels that are consistent with current university recommendations (i.e., adequate soil fertility, 180 lb N, and a plant density of 32,000 plants per acre). In contrast, the intensive management system consisted of extra P fertility, extra side-dress N, a plant density of 45,000 plants per acre, and application of a strobilurin fungicide during the tassel stage. Treatments were designed to assess the yield difference associated with adding a fungicide treatment to the standard and intensive management system. A combined analysis of 30 site-years (2009 to 2013) indicated that adding a fungicide application to the basic system did not result in a significant yield increase (+3.5 bushels per acre; $P = 0.17$), while the addition of fungicide in the intensive system led to a 8.8 bushel per acre increase in yield ($P < 0.01$). Individual data sets contributing to the overall analysis were divided into three groups based on their mean yields (150 to 175, 175 to 200, and greater than 200 bushels per acre) to investigate the influence of yield potential on fungicide response. In general, response to fungicide increased as the yield potential of an environment increased, especially for the

intensive management system. The results of this research could provide corn producers a valuable guide to determine if strobilurin fungicide application is warranted based on the estimated yield potential of their crop and other contributing factors like management system.

TABLE OF CONTENTS

CHAPTER 1

Literature Review

Corn (*Zea mays* L.) has evolved from a local feed source to one of the United States' leading exports during the last one hundred years with yields increasing from around 1.6 Mg ha^{-1} to over 9.9 Mg ha^{-1} (USDA National Agricultural Statistics Service, 2014). Future predictions show increasing demands for grain in the next twenty years (Alexandratos, 2011). As the demand for higher corn yields increases, farmers and researchers alike are looking for ways to increase grain production per unit area, instead of bringing more land into production. In the last decade, producers have experimented with fungicides to increase productivity. Over time these producers have seen that yield response to fungicide application tends to be highly variable as a result of varying disease pressure, but may also be affected by environmental factors and management practices that influence corn yield potential.

The introduction of the Highly Erodible Land Act in the 1985 Farm Bill provided incentives to farmers to remove wetlands and highly erodible lands from production, or use production practices such as no-till and conservation tillage systems to limit extensive erosion (Glaser, 2012). Conservation tillage requires that greater than 30% of crop residue remain on the soil surface, thereby reducing erosion (Simmons and Nafziger, 2009). Pathogens will infect live plant tissue during the cropping season and can overwinter in the remaining residue. This overwintering strategy provides inocula that can affect the same crop in the next growing season. The resulting crop residue increases the opportunity for saprophytic fungi to colonize the residue and provide primary inoculum to infect future crops. Since the widespread adoption of conservation tillage, gray leaf spot, caused by *Cercospora zeae-maydis*, incidence has increased considerably because *C. zeae-maydis* overwinters in the residue and produces primary inocula that infect subsequent corn crops (Ward et al.,1999). It only takes a few days of warm humid weather to cause *C. zeae-maydis* conidia to germinate and begin infecting leaf tissue (Lipps, 1998). As a result of increased disease pressure, corn producers expressed interest in fungicides for control of foliar diseases. In the mid-2000's, the first strobilurin fungicides, azoxystrobin and pyraclostrobin, were labeled and sold for use on corn, but were historically only used on specialty corn fields such as seed corn production due to the high cost of fungicides and their application (Wise and Mueller, 2011). In 2007, 4.5 to 5.6 million hectares of a total of 30.8 million hectares of corn in the United States were treated with fungicides (Munkvold et al., 2008).

Strobilurins are one of the most popular fungicide options currently available for corn. These compounds are members of the quinone outside inhibitor (QoI) fungicide class. Strobilurin fungicides were developed from β-methoxyacrylic acid, isolated from *Strobiluris tenacellus*, a wood rotting fungus (Bartlett et al., 2002; Vincelli, 2012). Studies of β-methoxyacrylates have shown that they have a wide range of antifungal, insecticidal, and antiviral properties in diverse biological systems (Wood et al., 1996). Starting with natural isolates, chemists altered the natural chemical structure to create a synthetic compound that was more stable when exposed to ultra-violet light (Vincelli, 2012). Fungicides that include strobilurin chemistries inhibit fungal proliferation by inhibiting mitochondrial respiration. More specifically, these compounds bind in the quinol outer binding site of the cytochrome bc_1 complex to block electron transport, thereby halting ATP production (Bartlett et al., 2002; Vincelli, 2012). Strobilurins are effective on germinating spores as well as mycelium; however, germinating spores are more sensitive to QoI chemistries (Vincelli, 2012). Because of differential sensitivity between mycelium and germinating spores, applications of strobilurins are more efficacious when applied before plants have infected leaf tissue. Certain strobilurins such as pyraclostrobin and azoxystrobin exhibit limited translaminar movement, allowing the active ingredient to be absorbed into the leaf cuticle (Vincelli, 2012). These factors confer excellent preventative and curative properties, by preventing disease development and slowing the spread of disease lesions (Bertelsen et al., 2001; Bartlett et al., 2002; Byamukama, 2013).

Since their introduction in the mid-1990s, strobilurins have become widely adopted in corn production systems due to their versatility and efficacy. Strobilurins control a wide range of pathogens (e.g., ascomycetes, basidomycetes, deuteromycetes, and oomycetes) on a wide range of plant species including pome fruits, grain, fruit, and vegetable crops (Grossmann and Retzlaff, 1997). Many producers include fungicides when utilizing aerial applications for insecticides around tasseling to help protect their corn crop during grain-fill, thereby spreading the application costs over two products (Blandino et al., 2012). Owing to the higher grain prices in the last few years, the yield increase needed to pay for the fungicide application has been lowered (Bradley and Ames, 2010). The hope of greater profits has encouraged farmers to incorporate strobilurin fungicides into their production systems.

Crop production systems have always aimed to maximize interception of solar radiation and convert that light energy into usable plant biomass, and thereby grain, as efficiently as possible (Koehle et al., 2002). As crop production practices have evolved, new management decisions increase efficiency, sustainability, and profitability, but may also increase the probability of disease pressure. Specific high-risk practices that promote disease include reduced tillage, susceptible hybrids with high yield potential, and frequent monoculture (Wise and Mueller, 2011). For example, large fungicide responses are usually observed when a hybrid of moderate disease susceptibility is planted in a reduced tillage system following a corn crop in the preceding year (Jeschke, 2008). The benefits of conservation tillage come with the risk of creating a large bank of primary inoculum on the soil's surface. Research on the interaction between tillage and disease incidence shows that no-till causes disease lesions to develop earlier and more severely in comparison to conventional tillage systems. Conservation tillage systems increase the primary inoculum load in a field, hastening disease development (Payne and Adkins, 1987). Several economically important pathogens including *C. zeae-maydis*, *Exserohilum turcicum* (causal agent of northern corn leaf blight), and *Kabatiella zeae* (causal agent of eyespot), overwinter in corn residue remaining on the soil surface (Jeschke, 2008). Producing corn in a conservation tillage system requires a more proactive approach to disease management, often resulting in increased fungicide use (Wise and Mueller, 2011). Conservation tillage has positive environmental and financial benefits that cannot be overlooked, but it also increases the need for intensive management because of the increased incidence of disease.

Before the widespread use of fungicides, producers had to rely on crop rotation, tillage, and hybrid disease resistance to manage disease effects on yield. This allowed hybrids to yield well in the presence of disease. As fungicide use has become a standard practice, producers have placed more emphasis on yield potential and less on the disease resistance when selecting hybrids (Wise and Mueller, 2011). Hybrids with moderate disease susceptibility ratings tend to be more responsive to foliar fungicide applications than hybrids with low disease susceptibility ratings (Paul et al., 2011). Furthermore, a yield response could result from controlling latent pathogen infection on the moderately resistant hybrids (Blandino et al., 2012). Producers struggle to identify environments which would result in profitable fungicide responses due to a lack of economic disease thresholds (Munkvold et al., 2001). A disease severity forecasting system might allow producers to accurately identify environments that would realize a return on the investment of a fungicide application.

In the last five years, producers have received higher prices for their grain. From 1972 to 2005, corn prices averaged $2.00 per bushel (Wise and Mueller, 2011). In contrast, the last four years (2009-2013) of corn prices have averaged above $5.50, which has been driven by federal mandates for increased alternative fuel production and expanding global exports (USDA National Agricultural Services, 2014; Wise and Mueller, 2011). Increased grain prices have encouraged a number of producers to forgo the typical corn-soybean rotation in favor of continuous corn. Even though producers may make more gross profit from continuous corn systems, they must invest more in disease management (Bailey, 1996). Continuous corn increases the level of corn residue left on the soil surface even in intensive tillage systems, which means there are large levels of primary inoculum. As a result, producers tend to rely more heavily on foliar fungicide applications to manage disease; possibly making multiple fungicide applications to keep disease levels low (Wise and Mueller, 2011).

Aside from the disease control aspects of fungicides, researchers and producers have noticed that corn treated with strobilurins has a tendency to stay green longer. “Stay-green”, or “greening effect”, can be defined as a reduction in the rate at which chlorophyll is degraded, (Thomas and Howarth, 2000). The crop protection industry has capitalized on the strobilurin greening effect by claiming that if the crop canopy stays greener, for longer, the period of grain fill may be extended and kernel abortion can be reduced while kernel weight is increased (Ward et al., 1999; Byamukama et al., 2013). Some researchers have reported yield increases following foliar applications of pyraclostrobin that have been too great to attribute to only disease control (Koehle et al., 2002). Promising results in field trials have lead researchers to study the physiological effects of the strobilurin stay-green effect in plants and have found connections between strobilurins and several biochemical processes including increased antioxidant concentration, decreased ethylene biosynthesis, increased leaf protein concentration, and increased nitrate reductase activity (Bartlett et al., 2002).

Plant senescence is the process of aging causing metabolic processes to slow and stop, leading to cell death. Oxidative stress is a large contributing factor to senescence rates since reactive oxygen species react with subcellular structures to change membrane permeability and degrade proteins and organelles. Reactive oxygen species result from abiotic stresses such as extreme heat or cold exposure, drought, intense light, and herbicide damage. These oxygen species are typically found in plant cells but their effect in the cell is usually mitigated by antioxidants such as superoxidase dismutase, catalase, and peroxidase (Prochazkova et al., 2001). As plants age, antioxidant functions in the cell decline and fail to capture and eliminate reactive oxygen, which increases the amount of cellular damage leading to cell death. Research on the effects of strobilurins on oxidative stress has shown that antioxidant levels increase, correlating to a decrease in reactive oxygen. By reducing oxidative stress, leaf protein concentration increases because of reduced oxidative damage (Wu and von Tiedemann, 2001; Wu and von Tiedemann, 2002). Reduction in oxidative stress helps prevent early senescence due to ethylene biosynthesis, stopping the ethylene overreaction to abiotic stresses, thereby decreasing senescence rate (Grossmann and Retzlaff, 1997; Jabs et al., 2004).

Ethylene is a plant growth regulator responsible for seed germination, seedling growth, fruit ripening, and plant senescence. Studies in wheat have demonstrated that strobilurins reduce the activity of ACC synthase, which decreases ethylene production (Grossman et al., 1999). Cytokinins are known to reduce leaf senescence, and reduction of cytokinin levels in plants, caused by ethylene perception, will promote senescence. The reduction of ACC synthase, in conjunction with decreased oxidative stress, could delay ethylene biosynthesis, allowing leaves to stay green longer. By reducing ethylene production directly through reduced ACC synthase activity, oxidative stress is reduced and photosynthesis rate remains high in corn plants.

Foliar strobilurin applications can also have positive benefits on nitrogen assimilation. Studies in wheat and barley have demonstrated that strobilurin applications improve grain nitrogen concentration, all while helping to maintain leaf area duration (Ruske et al., 2003). Leaf area duration is the integration of the ratio of green leaf laminae to land area over time (Peltonen-Sainio et al., 1997). Nitrate reductase is also the first and rate-limiting step of the assimilation of nitrate (Koehle et al., 2002; Ruske et al., 2003). Leaf disks treated with kresoxim-methyl, an early strobilurin from BASF, demonstrated increased levels of nitrate reductase activity and reduced degradation of nitrate reductase (Glaab and Kaiser, 1999). The increase in nitrate reductase activity seen in laboratory experiments has also been observed in field studies. Strobilurin treated corn demonstrated increased grain nitrogen concentration, due to more rapid nitrogen accumulation and increased nitrogen harvest index (Ruske et al., 2003). Soil tests demonstrated decreased soil nitrogen in strobilurin treated plots, showing that strobilurin applications may increase nitrogen assimilation by activating nitrate reductase. In wheat, application of strobilurins increased nitrate reductase activity, causing increased nitrogen uptake and metabolism, resulting in improved nitrogen harvest index and increased nitrogen concentration in the grain as the yields increased (Jabs et al., 2004).

By keeping disease from spreading, leaves have longer leaf area duration which increases the amount of photoassimilates that can be produced and converted into biomass. The yield response to fungicide application is a function of several factors including host growth stage when sprayed, disease severity, fungicide effective period, and fungicide efficacy (Ward et al., 1997). A strobilurin greening effect could maximize photosynthetic capacity of the crop canopy by extending leaf area duration through grain fill (Bartlett et al., 2002). Corn leaf canopies treated with strobilurins have demonstrated longer leaf area duration, which could increase grain fill duration (Byamukama et al., 2013). Foliar diseases decrease photosynthetically active leaf area of a plant, so a longer fungicide effective period would delay disease development and resulting defoliation. The increase in leaf area duration hinges on the fungicide's effective period which is defined as the time period after application in which disease infection is inhibited. As photosynthetic capacity diminishes, the plant has a limited amount of photoassimilates available for grain production (Ward et al., 1997). The response to defoliation from disease tends to have a negative linear effect on yield (Adee et al., 2005). This decrease is a result of fungal infections reducing the crop canopy's ability to intercept solar radiation, reducing grain yield and total biomass (Koehle et al., 2002). The upper leaves of the canopy (i.e., the eight to ten leaves above the ear) are responsible for supporting approximately 88% of the photoassimilates contributing to grain yield (Adee et al., 2005). By protecting the leaves of the upper canopy and increasing leaf area duration, yield is protected from loss due to disease defoliation and final kernel weight is increased.

Kernel weight is the yield component established during the grain fill period. It works in combination with the number of ears per acre and the number of kernels per ear to form overall grain yield. The number of ears formed is a direct result of plant population, whose upper limit is established at planting. Potential kernel number is a function of row number and ovules per row. Row number is influenced by early season growth around V5, while ovules per row are determined as early as V12 but at least by V15 (Nielsen, 2007). Final kernel weight is formed during the grain fill period and is sensitive to changes in photosynthetic capacity (Gambin et al., 2008). Strobilurin applications protect the upper canopy and maintain the photosynthetic capacity during the grain fill period to provide adequate photoassimilates for kernel growth. There is a positive correlation between kernel weight and plant growth rate during grain fill (Gambin et al., 2008). The effect of a fungicide application on kernel weight in past research studies has been inconsistent. Some studies suggest that foliar applications of strobilurins, applied at VT increase kernel weight while others state there is no change (Blandino et al., 2012; Byamukama et al., 2013). This discrepancy may be attributed to different growing conditions following pollination. In each case, increased grain moisture was reported in response to a strobilurin fungicide application, suggesting delayed senescence (Byamukama et al., 2013). Final kernel weight is a highly variable parameter that is responsive to environmental changes as well as management factors.

Another possible benefit of foliar strobilurin application is stalk integrity and reduction of lodging. Foliar disease can decimate leaf area in the upper canopy, causing the corn plant to remobilize assimilates from the stalk to the grain (Ward et al., 1999). This increases the probability of stalk rots and lodging (Byamukama et al., 2013; Ward et al., 1999). Currently pyraclostrobin is labeled for plant health benefits, including increased growth rate and decreased stalk lodging, and has been shown to reduce the incidence of stalk rots (Byamukama et al., 2013; BASF, 2008). Lodging is a major concern of producers because of harvest losses from ears lying on the ground. Lodged stalks are harder to pick up with a combine, and the yield loss from lodged corn can be substantial.

The frequency of fungicide application is increasing, making it important to address resistance management issues associated with using all fungicides. The increased use of strobilurins places increased selection pressure on fungal genotypes that are less susceptible or entirely resistant to these compounds (Bradley and Pedersen, 2011). Quantitative resistance (i.e., a strain is less sensitive than the wild type, but still controlled by the labeled rate) and qualitative resistance (control of the pathogen does not occur at the labeled application rate of fungicide) are serious concerns because of the broad spectrum use of strobilurins. Strobilurins are site specific fungicides because they only block electron transport at the cytochrome bc_1 complex, and the occurrence of a single mutation would render this fungicide ineffective (Vincelli, 2012). The Fungicide Resistance Action Committee (FRAC) has classified QoI fungicides as high risk for developing resistance (FRAC, 2013). Losing the efficacy of strobilurin chemistry because of fungal resistance would be highly detrimental due to the wide range of labeled crops and broad spectrum of disease control it provides. Fortunately, there are several cultural practices that can be implemented to reduce the risk of developing resistance fungal strains. These include limiting the number of strobilurin applications in a growing season, using multiple modes of action, and early application (Vincelli, 2012). Limiting the number of consecutive applications of strobilurin and using multiple modes of action in a foliar treatment will help reduce the probability of developing resistance by lessening selection pressure. Several products on the market come formulated with strobilurins and demethylation inhibitors (DMI) such as triazoles (Wise and Mueller, 2011). DMI fungicides work by inhibiting ergosterol production, a component needed for the formation of fungal plasma membranes. Since mutations are random, spraying early to control a smaller population might decrease the probability of there being a natural and random mutation for resistance in the population. These tactics can be used in combination with the proven practices of tillage, crop rotation, and selection of disease resistant hybrids to improve resistance management. Timely planting is also important to allow the crop to develop earlier than the disease so that when the disease becomes a problem the crop is almost mature, limiting the exposure to yield loss from defoliation (Shah and Dillard, 2010).

As producers work to increase corn grain yield, fungicides will become an integral factor in mainstream crop production. Cultural practices such as reduced tillage have made farming more sustainable and reduced depletion of natural resources, but have also promoted increased disease pressure. These changes make disease management even more crucial to successful crop production. The role of strobilurins and their use needs to be carefully considered to ensure their longevity, while maintaining high levels of productivity.

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CHAPTER 2

Introduction

The increase in corn (*Zea mays* L.) grain yield over the last hundred years, from around 1.6 Mg ha^{-1} to over 9.9 Mg ha^{-1}, has been a result of genetic improvement and intensification of crop management systems (USDA National Agricultural Statistics Service, 2014). In the next 15 years, demand for cereal crops is expected to total 2.8 billion megagrams; an approximate 50 % increase in production relative to 2000 (Lobell et. al., 2009). Crop producers are looking for innovative ways to improve the efficiency of their production systems. As researchers, it is appropriate to understand how a single management factor affects yield when other management factors are non-limiting, thereby defining that management factor's contribution to yield potential. When all management factors are non-limiting, Doberman et al. (2003) defines this situation as potential yield (Ymax), or the maximum yield of a certain crop species in a given environment. Ymax, in the absence of water or nutrient limitations, is determined by genetic characteristics, photosynthetic efficiency, temperature, and CO_2 concentrations (van Ittersum et al., 2003). Ymax also can be limited by defoliation due to reduced leaf area to efficiently capture photosynthetically active radiation. Adee et al. (2005) demonstrated that the upper canopy is responsible for 88% of the photoassimilates that contribute to grain yield. In order to protect the upper canopy, producers have turned to strobilurin fungicides to reduce disease pressure and protect the photosynthetic capacity of the upper canopy.

Since being labeled for use in corn in the mid-2000's, strobilurin fungicides have gained popularity because higher grain prices have increased the probability of a profitable yield response, even in the absence of severe disease pressure. In effect, this has enticed producers to increase the frequency of fungicide applications in their management systems (Bradley and Ames, 2010). Additionally, fungicides have been labeled for their 'plant health' benefits such as increased stalk strength, decreased harvest losses, increased tolerance to abiotic stresses such as water limitation, and greater kernel weight (BASF, 2008). By applying fungicides, producers are reducing the risk of yield loss as a result of disease, while potentially protecting the crop from losses that increase the gap between realized yield and Ymax (Byamukama et al., 2013).

Many plant pathologists investigating fungicide grain yield response have achieved mixed results. For example, Paul et al. (2011) completed a meta-analysis on the grain yield response of corn to foliar fungicides. This meta-analysis included data reported in *Plant Disease Management Reports* (PDMR) and *Fungicide & Nematacide Tests* (F&N), and determined that while fungicides may increase grain yield in some environments, the result is not statistically significant nor does it come with a high probability of occurrence. A meta-analysis approach can underestimate the importance of fungicides in different subsets of environments such as environments with high disease pressure or production systems that promote high yield potentials, where even a small level of foliar protection can translate into greater yield increases. As plant density increases, inter-plant competition for light increases because of increased shading in the crop canopy. The application of foliar fungicides, to protect photosynthetically active leaf area could provide a means to counteract the shading effect of increased plant density. A cursory search of PDMR and peer-reviewed journal articles revealed that many fungicide evaluations were conducted over a relatively narrow range of planting densities: 74,000 to 86,500 plants ha^{-1} (Schriver and Robertson, 2009a; Schriver and Robertson, 2009b; Grybauskas and Reed, 2011; Jackson and Behn, 2010; Hagan and Akridge, 2011; Schleicher and Jackson, 2011,). While these populations are representative of current university recommendations, these recommendations focus on maximizing net income, not maximizing biological potential or Ymax (Nafziger, 2008; Elmore and Abendroth, 2009). In order to double average grain yields in the United States to 16 Mg ha^{-1}, plant densities closer to 111,200 plants ha^{-1} may be necessary (Edgerton, 2009; Haegele et al., 2014).

Several studies have demonstrated that modern hybrids have a greater tolerance to high plant densities, allowing for rapid crop canopy development and greater interception of photosynthetically active radiation (Tokatlidis and Koutroubas, 2004; Hammer et al, 2009). For example, Sangoi et al. (2002) demonstrated that at low densities (2.5, 5, 7.5, and 10 plants m^{-2}) modern hybrids were out performed by hybrids developed decades earlier. In contrast, modern hybrids excelled at greater planting densities. Testing effects of management practices on modern hybrids (e.g., response to fungicide application, at sub-optimal or current optimum plant densities) may result in grain yields that are a function of density limitations, thereby obscuring response to other management inputs.

In addition to the increased tolerance to plant density of modern hybrids, Bender et al. (2013a) demonstrated that total nutrient uptake by modern insect-protected transgenic hybrids has nearly doubled in comparison to uptake totals provided by Hanway (1962a). Similarly, Bender et al. (2013b) demonstrated increased nutrient uptake in transgenic hybrids (expressing *Bacillus thuringiensis* (Bt) toxin to control corn rootworm) compared to their non-Bt isoline (refuge) hybrid counterparts. Uptake of macronutrients such as N, P, and K increased by 31, 24, and 38 percent, respectively, as a result of transgenic insect protection. The combination of increased plant density tolerance and increased fertility use efficiency of modern hybrids suggest that intensified production systems may have increased fertility requirements. Monitoring and managing macronutrients (N, P, and K) and nutrients with high harvest indices (P, N, S, and Zn) are essential elements for increased grain production (Bender et al., 2013). Management of nutrients with a high harvest index (the percentage of total nutrient uptake removed with the grain) is critical to ensure that the nutrient content in the soil is not depleted and Ymax is not nutrient limited.

In order to assess the grain yield response of foliar fungicides to Ymax, yield-limiting factors need to be removed by appropriate management practices. The Crop Physiology Laboratory at the University of Illinois has developed a unique resource in the corn omission plot design (Ruffo et al., manuscript in preparation). Utilizing a 30 site-year data set derived from these corn omission plot studies (2009-2013), one objective of this research was to investigate how strobilurin fungicide applications at tassel emergence impact corn grain yield in 'standard' and 'intensive' management systems. The hypotheses were that i) response to fungicide application will be greater as the yield potential of the environment increases, and ii) that response to fungicide application will be enhanced by an intensive management system that removes additional yield limitations such as planting density or inadequate soil fertility. The results of this research could provide corn producers a valuable guide to determine if strobilurin fungicide application may increase grain yield based on the estimated yield potential of their crop and other management inputs.

Treatment Structure and Experimental Design

This experiment was designed to test the contribution of a foliar fungicide application at tassel emergence (VT) in standard and intensive management systems. The standard management system included a plant density of 79,000 plants ha^{-1}, N applied at or before planting at a rate of 202 kg N ha^{-1}, and no additional P fertility. In contrast, the intensive system included a higher plant density (111,195 plants ha^{-1}), 202 kg N ha^{-1} pre-plant, an additional 112 kg N ha^{-1} side-dressed at V6, and 112 kg ha^{-1} P_2O_5. The nitrogen side-dressed at V6 was applied as Super U urea (46-0-0). Super U is a product produced by Koch Fertilizer which contains a nitrification and urease inhibitor to decrease nitrogen loss by volatilization, leaching, and denitrificaiton. The additional 112 kg ha^{-1} P_2O_5 was applied as Mosaic's Microessentials SZ (12-40-0-10[S]-1[Zn]). Three commercially available fungicides as well as one experimental compound, applied at the maximum labeled rates, were used in this study: Headline (pyraclostrobin), Headline AMP (pyraclostrobin + metoconazole), Quilt Xcel (azoxystrobin + propiconazole) and an experimental DuPont fungicide, containing Q_oI and DMI chemistries (Table 2.1). Fungicide treatments were applied using a CO_2 pressurized backpack sprayer, with an output of 140 L ha^{-1}, at VT. The boom was approximately 1.5 m in width with nozzles spaced 0.5 m apart. Flat fan nozzles (TeeJet XR11002) with a 110° spray pattern were used. Hybrids were not consistent throughout this study; different hybrids were used at the various locations and in different years and hybrid and fungicide combinations listed by trial are shown in Table 2.1.

Treatments were arranged in a randomized block design with six replications. Each experimental unit consisted of four 11.43 m rows. All trials had soybeans (*Glycine max*) as the previous crop in a corn-soybean rotation. Row spacing was generally 0.76 m; however, several trials also included twin rows on 0.76 m or 0.51 m rows. Plots were planted with an ALMACO Seed Pro 360 research planter with variable seeding rate technology. Soil preparation consisted of a fall chisel plow pass followed by two field cultivations in the spring. Tefluthrin (Force 3G, Syngenta), a soil insecticide, was applied at planting at a rate of 0.11 kg a.i. ha^{-1} for control of seedling insect pests and corn rootworm larvae. Weeds were controlled using a pre-emergence application of S-metoachlor + atrazine + mesotrione at a rate of 10.94 kg a.i. ha^{-1}, 4.09 kg a.i. ha^{-1}, and 1.09 kg a.i. ha^{-1}, respectively. A post-emergence application of glyphosate, 4.09 kg a.e. ha^{-1}, was applied to control late emerging weeds when necessary.

The center two rows of each experimental unit were harvested to measure grain yield and moisture. Grain yields are expressed as bushels $acre^{-1}$ at 15.5 % moisture. Additionally, grain yield is also expressed as Mg ha^{-1} at 0% moisture. A grain sample was collected from each plot to determine individual kernel weight based on a representative sub-sample of 300 kernels. Kernel number on a per-area basis was calculated algebraically by dividing total grain weight by the estimate of individual kernel weight.

Statistical Analysis

Raw means, of the grain yield, from each trial were used to segregate trials into yield potential categories of 150 to 175, 175 to 200, and greater than 200 bushels per acre. This segregation was used to assess the grain yield response to foliar fungicides in different yield potential categories. An analysis of variance (ANOVA) was then completed using PROC MIXED in SAS 9.3 (SAS Institute). Management system, fungicide application, and yield potential were treated as fixed effects. Trial, replication nested within trial, hybrid, and the trial × treatment interaction were included as random effects. In order to assess the value of fungicide to standard and intensive management systems, pairwise comparisons between treatment means (Intensive vs. Standard, Intensive vs. +Fungicide, and Standard vs. +Fungicide) by yield potential category were conducted with an experiment-wise error rate of $\alpha_e = 0.10$. A Bonferroni adjustment, accounting for 6 simultaneous comparisons, was utilized to maintain experiment-wise error rate at $\alpha_e = 0.10$. The Bonferroni adjustments resulted in a comparison-wise error rate of $\alpha_c = 0.01667$. Each treatment comparison, within a yield potential category, was tested at $\alpha_c = 0.01667$ to control the probability of a type I error.

Results and Discussion

The ANOVA for grain yield showed significant main effects of treatment ($P < 0.0001$), yield potential ($P < 0.0001$), as well as the interaction between treatment and yield potential ($P < 0.0006$) (Table 2.2). The ANOVA for kernel number demonstrated a significant main effect of yield potential ($P < 0.0001$), while also revealing a significant main effect of treatment ($P < 0.0013$) and the two-way interaction ($P < 0.0001$) (Table 2.2). The ANOVA included treatments other than the addition of fungicide to the standard and intensive management system. As a result, the significant treatment effects, as well as the treatment × yield potential interaction, does not necessarily imply that the fungicide was significant or the fungicide × yield potential interaction was significant.

When averaged across levels of yield potential, there was a significant grain yield difference ($P < 0.10$) of 4.3 bushels per acre between the standard and intensive management systems (Table 2.3). The 4.3 bushel increase appeared to result from a significant increase ($P<0.0001$) in the number of kernels per unit area, despite a significant decrease ($P < 0.0001$) in kernel weight (Table 2.3). At the yield potential of 150-175 bushels per acre, grain yield decreased 4 bushels per acre, due to a significant decrease in kernel weight and decreased kernel number. Decreased kernel weight was consistent across all yield potential classes when the intensive management system was compared to the standard management system (Table 2.3). At the intermediate (175-200 bushels per acre) and highest (>200 bushels per acre) levels of yield potential, intensive management increased grain yield by 6.6 and 10.1 bushels per acre, respectively (Table 2.3). The yield increases in the intermediate and high yield potential categories were a result of significant increases in kernel number in the intensive system, which compensated for the significant decrease in kernel weight (Table 2.3). As yield potential increased, the yield advantage of the intensive management system also increased.

The yield response to the intensive management system demonstrated that standard production practices may limit Ymax because of insufficient plant density, nitrogen, or phosphorus fertility (Doberman et al., 2003). Increased plant density did result in decreased kernel weight, but that decrease was overcome by increased kernel number associated with greater plant density. This finding is supported by the work of Borras et al. (2003) who demonstrated that increased plant density decreases both kernel weight and kernel number on a per-plant basis. In the low yield potential category (150-175 bushels per acre), there was a decrease in grain yield as a result of decreased kernel number and decreased kernel weight coinciding with higher plant densities, suggesting that environmental conditions could not support the elevated planting density. Andrade et al. (1999) attributed reduced kernel number to increased kernel abortion as a result of weaker sink strength and less source material partitioned to reproductive structures. As yield potential increased in this study, greater kernel number on a per-area basis was able to offset the negative effects of plant density on kernel weight.

Adding a fungicide to the standard management system increased grain yield by 3.6 bushels per acre when averaged over all levels of yield potential (Table 2.4). At the lowest level of yield potential, the application of a foliar fungicide resulted in a 1.2 bushels per acre yield increase. The magnitude of the fungicide yield response increased in the intermediate and high yield potential categories to 5.1 bushels per acre and 4.3 bushels per acre, respectively (Table 2.4). Overall and across all three levels of yield potential, there were no significant differences detected for either kernel weight or kernel number; however, foliar fungicide applications tended to result in greater kernel weight (Table 2.4). The trend of increased kernel weight in response to foliar fungicide application may be a result of increased photoassimilate availability per kernel, because final kernel weight is increased when plant growth rate is increased during flowering and grain-filling periods (Gambin et al., 2008).

The application of fungicide to the intensive management system resulted in greater numerical yield responses compared to the standard system when averaged across trials as well as by level of yield potential. The overall fungicide response in the intensive management system was 8.8 bushels per acre ($P<0.001$), which was a result of significant increases ($P<0.01$) in both kernel weight and kernel number (Table 2.5). In the low yield potential category, the response to fungicide was 6 bushels per acre. Similarly, the intermediate yield potential category responded with increased yield of 8.3 bushels per acre. The greatest measured yield response to fungicide occurred in the high yield potential category with intensive management; under these conditions, fungicide application resulted in an increase of 12 bushels per acre (Table 2.5) The yield response of foliar fungicide in the intensive management system resulted from the non-significant trend of increased kernel number and kernel weight in all three yield potential classes (Table 2.5). In the intensive management system, the grain yield response to fungicide increased in magnitude as yield potential became greater (Table 2.5).

The greater grain yield responses to foliar fungicides in the intensive management systems may be attributed to the physiological benefits of strobilurin fungicides in addition to their fungicidal activity. Other researchers have demonstrated that foliar strobilurin applications sometimes increase grain yield compared to the untreated control (Nelson, 2011; Byamukama, 2013). These reports agree with the findings of this study as the application of strobilurins tended to increase yield in every yield potential. Increased kernel weight suggests that the foliar application of strobilurins may have increased plant growth rate per kernel (Gambin et al. 2008), or extended the duration of the grain filling period. The yield benefits associated with fungicide application in the intensive management system were primarily driven by increased kernel weight, although this effect was likely enhanced by the greater number of kernels resulting from greater planting density. The increased population density tolerance of modern hybrids, and the ability to respond to management inputs when planting density is increased are likely a function of greater interception of photosynthetically active radiation interception by the canopy, as well as enhanced plant growth rate during flowering (Tokatlidis and Koutroubas, 2004; Gambin et al., 2008; Hammer et al., 2009).

Paul et al. (2011) reported that grain yield response to strobilurin containing foliar fungicide applications decreased as yield potential increased, which is opposite of the fungicide responses measured in this study. As yield potential increased, the response to foliar fungicide applications increased in both management systems. Responses in the intensive management system were greater than those in the standard management system, both across site-years as well as in each yield potential category. An additional 5.3 bushels per acre was produced as a result of foliar fungicide applications to the intensive management system versus the standard management system with a foliar fungicide application, over all 30 site-years (Table 2.4 and 2.5).

Conclusions

The results of this study demonstrate that the value of a strobilurin fungicide application increases with increased management system intensity and yield potential. Strobilurin fungicides have increased value as agricultural management systems are intensified in environments that can support high yield potentials. Further research of strobilurin fungicides and their interactive effects with plant density, plant growth rate during flowering and grain-fill, fertility management, and modern hybrids can help identify management practices that increase the value of these compounds. A better understanding of these interactions could provide producers with management guidelines to maximize the grain yield benefits of strobilurin fungicides.

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Tables

Table 2.1. Treatment descriptions by year and location, listing average trial yield, hybrids, fungicide and fungicide rate, from 30 site-years at six locations across Illinois from 2009-2013.

Trial Number	Year	Location	Average Trial Yield	Standard Hybrid	Intensive Hybrid	Fungicide	Product rate	
			bushels ac^{-1}				L ha^{-1}	fl oz acre^{-1}
1	2009	Champaign	234.8	DKC 61-22 RR2	DKC 61-19 VT3	Headline	0.88	12
2	2009	Dixon Springs	209.9	DKC 61-22 RR2	DKC 61-19 VT3	Headline	0.88	12
3	2010	Champaign	207.8	DKC 61-22 RR2	DKC 61-19 VT3	Headline	0.88	12
4	2010	Dixon Springs	198.0	DKC 61-22 RR2	DKC 61-19 VT3	Headline	0.88	12
5	2011	Harrisburg	189.3	DKC 62-63 VT3P	DKC 63-84 VT3	Headline AMP	1.05	14.4
6	2011	Champaign	176.5	DKC 62-63 VT3P	DKC 63-84 VT3	Headline AMP	1.05	14.4
7	2011	Champaign	186.7	DKC 61-72 RR2	DKC 61-69 VT3	Headline AMP	1.05	14.4
8	2011	Champaign	175.7	DKC 63-84 VT3	DKC 62-63 VT3P	Headline AMP	1.05	14.4
9	2011	Champaign	173.4	DKC 62-63 VT3P	DKC 63-84 VT3P	Headline AMP	1.05	14.4
10	2011	Champaign	170.2	P1018HR	P1184XR	Experimental	—	—
11	2011	Dekalb	195.7	DKC 61-22 RR2	DKC 61-19 VT3	Headline AMP	1.05	14.4
12	2011	Champaign	176.6	DKC 61-22 RR2	DKC 61-19 VT3	Headline AMP	1.05	14.4
13	2011	Champaign	210.7	N72A-3111	N74R-3000GT	Quilt Xcel	1.02	14
14	2011	Rushville	176.2	DKC 59-35 VT3	DKC 65-63 VT3	Headline AMP	1.05	14.4
15	2011	Rushville	165.3	P1018HR	P1184XR	Experimental	—	—
16	2011	Pleasant Plains	146.1	DKC 59-35 VT3	DKC 65-63 VT3	Headline AMP	1.05	14.4
17	2011	Pleasant Plains	153.4	N72A-3000 GT	N74R-3000 GT	Quilt Xcel	1.02	14
18	2012	Champaign	172.1	DKC 62-97 VT3P	DKC 62-97 VT3P	Headline AMP	1.05	14.4
19	2012	Champaign	194.2	P1184XR	P1018HR	Experimental	—	—
20	2012	Dekalb	231.5	85V88-3000GT	N63R-3000GT	Quilt Xcel	1.02	14
21	2012	Champaign	163.2	N72A-3111	83R38-3000GT	Quilt Xcel	1.02	14
22	2012	Champaign	162.7	CG 7505 VT3P	CG 7505VT3P	Headline AMP	1.05	14.4

Table 2.1 Continued.

Trial Number	Year	Location	Average Trial Yield	Standard Hybrid	Intensive Hybrid	Fungicide	Product rate	
			bushels ac^{-1}				L ha^{-1}	fl oz acre^{-1}
23	2012	Rushville	164.6	CG 7505 VT3P	CG 7505VT3P	Headline AMP	1.05	14.4
24	2012	Champaign	158.5	N68A-3000GT	N68A-3000GT	Quilt Xcel	1.02	14
25	2013	Champaign	192.2	Stone 6358 RIB	Stone 6358 RIB	Headline AMP	1.05	14.4
				Stone 6258 RIB	Stone 6258 RIB	Headline AMP	1.05	14.4
26	2013	Harrisburg	238.8	DKC 62-08 RIB	Stone 6358 RIB	Headline AMP	1.05	14.4
				Stone 6358 RIB	DKC 62-08 RIB	Headline AMP	1.05	14.4
27	2013	Champaign	200.8	H-9341 3000GT	H-9011 4011	Quilt Xcel	1.02	14
				H-9011 4011	H-9341 3000GT	Quilt Xcel	1.02	14
28	2013	Dekalb	250.9	N61P-3000GT	N63R-3000GT	Quilt Xcel	1.02	14
				N63R-3000GT	N61P-3000GT	Quilt Xcel	1.02	14
29	2013	Champaign	182.1	W7477 RIB	W6487 VT3P	Headline AMP	1.05	14.4
				W6487 VT3P	W7477 RIB	Headline AMP	1.05	14.4
30	2013	Champaign	192.5	CG 6640 VT3P	CG 6265 SS	Headline AMP	1.05	14.4
				CG 6265 SS	CG 6640 VT3P	Headline AMP	1.05	14.4

Table 2.2. Analysis of variance for the effects of treatment and yield potential on grain yield and yield components measured in 30 trials conducted from 2009 to 2013 at six geographical locations in Illinois.

Sources of Variation	Degrees of Freedom	Yield	Kernel Weight	Kernel Number
Treatment (T)	11	<0.0001	0.34	0.0013
Yield Potential (Y)	2	<0.0001	<0.0001	<0.0001
T x Y	22	0.0006	0.097	<0.0001

Significance declared for main effects and the interaction at $P < 0.05$

Table 2.3. Grain yield and yield components for the standard and intensive management systems, by category of yield potential. Data represent 30 trials conducted between 2009 and 2013. The number of trials in each category of yield potential is indicated in parentheses. Grain yield is represented as bushels acre^{-1} at 15.5% moisture and Mg ha^{-1} at 0% moisture. Kernel weight is expressed as milligrams per kernel at 0% moisture.

Yield Potential	Standard		Intensive	
Grain Yield	bushels acre^{-1}	Mg ha^{-1}	bushels acre^{-1}	Mg ha^{-1}
Overall (n = 30)	186.7	9.58	190.9 ††	10.14 ††
150-175 bushels acre^{-1} (n = 10)	165.3	8.79	161.3	8.57
175-200 bushels acre^{-1} (n = 12)	180.5	9.59	187.2	9.95
>200 bushels acre^{-1} (n = 8)	214.1	11.38	224.2	11.92
Kernel Weight	mg kernel^{-1}		mg kernel^{-1}	
Overall (n = 29)	276		260 †	
150-175 bushels acre^{-1} (n = 9)	261		252 *	
175-200 bushels acre^{-1} (n = 12)	279		260 *	
>200 bushels acre^{-1} (n = 8)	288		269 *	
Kernel Number	kernels m^{-2}		kernels m^{-2}	
Overall (n = 29)	3471		3780 †	
150-175 bushels acre^{-1} (n = 9)	3347		3398	
175-200 bushels acre^{-1} (n = 12)	3254		3701 *	
>200 bushels acre^{-1} (n = 8)	3811		4241 *	

† Significant difference between Standard and Intensive management system ($P < 0.001$).
†† Significant difference between Standard and Intensive management system ($P < 0.10$).
* Significant difference between Standard and Intensive Management system within a yield potential using a Bonferroni adjustment ($P < 0.10$).

Table 2.4. Grain yield and yield components of the standard management system and the addition of foliar fungicide, by category of yield potential. Data represent 30 trials conducted between 2009 and 2013. The number of trials in each category of yield potential is indicated in parentheses. Grain yield is represented as bushels acre^{-1} at 15.5% moisture and Mg ha^{-1} at 0% moisture. Kernel weight is expressed as milligrams per kernel at 0% moisture.

Yield Potential	Standard		Standard + Fungicide	
Grain Yield	bushels acre^{-1}	Mg ha^{-1}	bushels acre^{-1}	Mg ha^{-1}
Overall (n = 30)	186.7	9.58	190.2	10.11
150-175 bushels acre^{-1} (n = 10)	165.3	8.79	166.6	8.85
175-200 bushels acre^{-1} (n = 12)	180.5	9.59	185.6	9.87
>200 bushels acre^{-1} (n = 8)	214.1	11.38	218.4	11.61
Kernel Weight	mg kernel^{-1}		mg kernel^{-1}	
Overall (n = 30)	276		278	
150-175 bushels acre^{-1} (n = 9)	261		264	
175-200 bushels acre^{-1} (n = 12)	279		280	
>200 bushels acre^{-1} (n = 8)	288		291	
Kernel Number	kernels m^{-2}		kernels m^{-2}	
Overall (n = 30)	3471		3500	
150-175 bushels acre^{-1} (n = 9)	3347		3336	
175-200 bushels acre^{-1} (n = 12)	3254		3335	
>200 bushels acre^{-1} (n = 8)	3811		3830	

Table 2.5. Grain yield and yield components of the intensive management system and the addition of foliar fungicide by category of yield potential. Data represent 30 trials conducted between 2009 and 2013. The number of trials in each category of yield potential is indicated in parentheses. Grain yield is represented as bushels acre^{-1} at 15.5% moisture and Mg ha^{-1} at 0% moisture. Kernel weight is expressed as milligrams per kernel at 0% moisture.

Yield Potential	Intensive		Intensive + Fungicide	
Grain Yield	bushels acre^{-1}	Mg ha^{-1}	bushels acre^{-1}	Mg ha^{-1}
Overall (n = 30)	190.9	10.14	199.7 †	10.61 †
150-175 bushels acre^{-1} (n = 10)	161.3	8.57	167.3	8.89
175-200 bushels acre^{-1} (n = 12)	187.2	9.95	195.5	10.39
>200 bushels acre^{-1} (n = 8)	224.2	11.92	236.2 *****	12.56 *****
Kernel Weight	mg kernel^{-1}		mg kernel^{-1}	
Overall (n = 30)	260		265 ††	
150-175 bushels acre^{-1} (n = 9)	252		256	
175-200 bushels acre^{-1} (n = 12)	260		265	
>200 bushels acre^{-1} (n = 8)	269		273	
Kernel Number	kernels m^{-2}		kernels m^{-2}	
Overall (n=30)	3780		3888 ††	
150-175 bushels acre^{-1} (n = 9)	3398		3479	
175-200 bushels acre^{-1} (n = 12)	3701		3801	
>200 bushels acre^{-1} (n = 8)	4241		4383	

† Significant fungicide response to Intensive management system ($P < 0.001$).
†† Significant fungicide response to Intensive management system ($P < 0.01$).
***** Significant fungicide response to Intensive management system with in a yield potential using a Bonnferroni adjustment ($P < 0.10$).