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Soybean seed production: Decisions and their relationship to seed quality

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Soybean seed production: Decisions and their relationship to seed quality

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CHAPTER 1. GENERAL INTRODUCTION

Phomopsis longicolla is a devastating seed borne pathogen that aggressively infects soybean seed thereby reducing seed viability, vigor, and yield. This pathogen is part of the Diaporthe-Phomopsis disease complex and is endemic throughout the United States making it a viable concern for all soybean producers. Although previous research aimed at reducing the incidence of this pathogen exists very little information is available in regards to the new management systems currently employed by soybean producers and how they impact *P. longicolla* infection. This thesis is part of a larger, multi-state project funded by the United Soybean Board evaluating the impact of current management systems and inputs on soybean seed yield and seed composition. The goal of this project was to determine if inputs composing the management systems tested in this study impact *P. longicolla* infection and ultimately impact seed viability and vigor. Chapter two is a literature review of *P. longicolla* history, epidemiology, and the various control measures previously researched. The research conducted is reported in chapter three.

The third chapter examines the impact of seven different inputs, arranged into nine management systems, on seed viability, seed vigor, *P. longicolla* infection, seed yield, and seed weight. The inputs studied were a combination seed treatment, foliar fungicide application at R3, a combination foliar fungicide application at R3 and R5, seed inoculation, additional soil fertilizer, application of a foliar fertilizer, wide vs. narrow row spacing, and a low seeding rate of 357,100 seeds ha^{-1} vs. a high seeding rate of 604,000 seeds ha^{-1}. Many of these inputs have had little investigation as to how they impact seed quality (viability and vigor), *P. longicolla* infection, seed yield, and seed weight especially over such a large geography.

The materials presented in this thesis should serve commercial soybean producers, soybean seed producers, and researchers alike. It will provide insight as to how the different inputs and

management systems impact the variables listed above and will determine if there are any benefits in incorporating additional inputs into the management systems currently utilized by soybean producers. It is my hope that this research can be used by the parties listed above to further improve upon the production systems currently in place. I also hope this provides further information for continued research into how this pathogen can be better controlled.

CHAPTER 2. LITERATURE REVIEW

Diaporthe/Phomopsis complex

The Diaporthe-Phomopsis disease complex of soybean is composed of three distinct pathogens; *Diaporthe phaseolorum* (Cke. & Ell.) var. *sojae* Wehm. (anamorph *Phomopsis sojae* Leh.) (pod and stem blight), *Diaporthe phaseolorum* (Cke. & Ell.) var. *caulivora* Athow (stem canker), and *Phomopsis longicolla* Hobbs. (seed decay) (Hartman et al., 1999). The members of this complex are endemic throughout the United States as well as many other countries around the world (Sinclair, 1993; Wrather et al., 1997; Hartman et al., 1999). Where this disease complex exists, much attention has been given to it in order to reduce its negative impact on seed quality (Sinclair, 1993) as well as yield and economics (Wrather et al., 1997; Hartman et al., 1999). High moisture conditions, such as high relative humidity or rain, and temperatures greater than15°C tend to be the primary environmental factors that control infection rate and level of *P. longicolla* (Lehman, 1922; Jordan et al., 1986; Wyllie and Scott, 1988; Sinclair, 1993).

Diaporthe phaseolorum* var. *sojae. Pod and stem blight is caused by *D. phaseolorum* var. *sojae*. *Diaporthe phaseolorum* var. *sojae* was first reported in 1922 by S.C. Lehman in North Carolina (Lehman, 1922). Disease symptoms were noted on soybean plants during the rainy portion of the growing season. It was reported that the diseased plants were shorter than the healthy plants and brown lesions on the stem and pods were present with rows of pycnidia (imperfect fruiting body associated with asexual reproduction) forming in the infected area. *Diaporthe phaseolorum* var. *sojae* caused considerable damage to the infected plants but also to the seed quality (Lehman, 1922). In 1960, work by Wallen and Cuddy verified the negative effect of *D. phaseolorum* var. *sojae* on seedling germination (Wallen and Cuddy, 1960). The negative correlation between *D. phaseolorum* var. *sojae* and seedling germination was also later confirmed by many other

researchers (Kulik and Schoen, 1981; TeKrony et al., 1985; Sinclair, 1993; Wrather et al., 1996; Wrather et al., 2004). *Diaporthe phaseolorum* var. *sojae* is known to infest the pod, stem, and leaf portions of the plant by producing black speckled fruiting bodies, known as pycnidia (Lehman, 1922). Infection of the pod leads to the spreading of mycelium throughout the pod which eventually leads to pycnidia formation on the outside of the pod as well as infection of the pod cavity and eventually the seed (Lehman, 1922). Stem infection close to the soil is usually the first to occur and can lead to further plant infection when the environment is favorable (Lehman, 1922). Leaf tissue infection is not as prevalent as stem infections; leaf infections occur first at the tip or margin of the leaf and extend inward towards the midrib (Lehman, 1922). The color of the leaf will turn from green to white and will produce black pycnidia as the infection spreads. If seed infection occurs then seed quality, germination, vigor, and field emergence can be negatively impacted; especially if warm and high moisture weather conditions persist (Kmetz et al., 1978; Kulik and Shoen, 1981).

***Diaporthe phaseolorum* var. *caulivora*.** Stem canker, caused by *Diaporthe phaseolorum* var. *caulivora,* is known to cause stem girdling which ultimately leads to plant death (Athow and Caldwell, 1954). *Diaporthe phaseolorum* var. *caulivora* was first considered to be a symptom of *D. phaseolorum* var. *sojae*, but was later classified as being a different fungal pathogen (Welch and Gilman, 1948). The new pathogen was classified as a strain of *D. phaseolorum* var. *batatatis* which was later proven to be incorrect since it was a separate variety to which they finally named *D. phaseolorum* var. *caulivora* (Athow and Caldwell, 1954). Since its classification as being a separate fungal pathogen, *D. phaseolorum* var. *caulivora* has proven to be a very economically important disease throughout the United States (Athow and Caldwell, 1954; Wrather et al., 1997). *Diaporthe phaseolorum* var. *caulivora* was first found to be damaging to soybean in the north central United States (Athow and Caldwell, 1954) in the 1940's and 1950's, but later became an important disease

in the southern soybean producing states (Athow and Caldwell, 1954; Welch and Gilman, 1948; Krausz and Fortnum, 1983; Rothrock et al., 1985). The soybean varieties Hawkeye and Blackhawk were highly susceptible to *D. phaseolorum* var. *caulivora* and were widely planted in the northern United States during the 1940's and 1950's (Wyllie and Scott, 1988). Control of *D. phaseolorum* var. *caulivora* in the northern United States was achieved by selecting cultivars that were not as susceptible to the disease (Weaver et al., 1984). *Diaporthe phaseolorum* var. *caulivora* has proven to be more severe in the southern United States, compared to the northern United States, due to its ability to attack a wider range of cultivars and form multiple infection sites per plant (Krausz and Fortnum, 1983; Weaver et al., 1984; Wyllie and Scott, 1988). The southern strain of *D. phaseolorum* var. *caulivora* is symptomatically similar to the northern strain, but has been noted to be different in pathogenicity, etiology, and symptom expression (Wyllie and Scott, 1988). The southern strain of *D. phaseolorum* var. *caulivora* infects a wide range of cultivars which makes it tougher to manage when compared to the northern strain (Wyllie and Scott, 1988).

Diaporthe phaseolorum var. *caulivora* will first appear as a reddish-brown discoloration on the stem (Athow and Caldwell, 1954). A lesion will form from the first point of infection and eventually grow into a canker (necrotic tissue) which will frequently be a couple centimeters long and maybe encircling the stem – this will often kill the plant (Athow and Caldwell, 1954). Plants infected with stem canker are often noticeable after senescence because dead leaves will remain on infected plants after uninfected plants have lost their leaves (Athow and Caldwell, 1954).

***Phomopsis longicolla*.** Seed decay and damping off of soybean is caused by *P. longicolla* (Wyllie and Scott, 1988). This disease is regarded by many researchers to be the most economically important disease of the Diaporthe-Phomopsis disease complex of soybean, as well as one of the most significant diseases affecting soybean production (Wyllie and Scott, 1988). *Phomopsis*

longicolla is endemic throughout the United States and is found in many of the soybean producing regions of the world making it a viable concern to any soybean producer (Wrather et al., 1997).

Phomopsis longicolla was first noted in 1974, but at the time was termed *Phomopsis sp.* (Kmetz et al., 1974). *Phomopsis sp.* would decay 85% of seeds within 3 days making it more aggressive than *D. phaseolorum* var. *sojae* or *D. phaseolorum* var. *caulivora* (Kmetz et al., 1974). This new isolate was morphologically different from *D. phaseolorum* var. *sojae* and *D. phaseolorum* var. *caulivora* because it only produced pycnidia whereas *D. phaseolorum* var. *sojae* produced pycnidia and perithecia and *D. phaseolorum* var. *caulivora* produced only perithecia (Kmetz et al., 1974). In 1985, research showed that *Phomopsis sp*. was indeed morphologically different from any other *diaporthe* or *phomopsis* anamorphs and was officially termed *P. longicolla* (Hobbs et al., 1985).

Phomopsis longicolla infects seed and causes them to become shriveled, oblong, have a whitish chalky appearance, and have cracked seed coats (Sinclair, 1993). *Phomopsis longicolla*, when cultured in a laboratory, will often appear as a white coating of mycelia surrounding the seed (Hobbs et al., 1985; Wyllie and Scott, 1988; Hartman et al., 1999). Seeds infected with this pathogen will often experience reduced germination, reduced flour and oil quality, increased fatty acid content, alterations of the free-fatty acid content, and reduced yields (Sinclair, 1993). The negative effect of this pathogen coupled with the many environments it inhabits makes it an economically important pathogen to soybean producers throughout the United States (Sinclair, 1993; Wyllie and Scott, 1988) and the world (Wrather et al., 1997).

Phomopsis longicolla **epidemiology**

Inoculum. Crop residues, especially previously infected soybean straw, infected soybean seed, and soil serve as viable sources of inoculum of *P. longicolla* (Kmetz et al., 1978; Kmetz et al.,

1979; Garzonio and McGee, 1983; Hartman, 1999). Crop residue and soil have been found to serve as the main source of inoculum for localized infections (Kmetz et al., 1978; Kmetz et al., 1979), but it is the seed that is the likely source of long-range dissemination of the pathogen (Kmetz et al., 1979; Garzonio and McGee, 1983; Hartman, 1999). Seed as an inoculum source was tested when infected soybean seed was introduced to a 10 year continuous corn field planted to soybean and a significant amount of *P. longicolla* infection occurred which was attributed to the infected soybean seed (Garzonio and McGee, 1983).

The main mode of pathogen dispersal is by environmental conditions such as wind or rain which can move the conidia from the inoculum source to the plant (Lehman, 1922; Kmetz et al., 1979; Garzonio and McGee, 1983; Sinclair, 1993). Rain drop splashing is primarily responsible for the dispersal of conidia to uninfected soybean plants, but wind may also play a minor role (Lehman, 1922; Kmetz et al., 1979; TeKrony et al., 1983; Balducchi and McGee, 1987). Physical movement via field equipment has not been officially researched, but it is hypothesized that it could serve as vector for the pathogen by carrying the infected residue or conidia to other fields.

Plant infection. Overwintering mycelia will produce fruiting bodies known as pycnidia which will eventually produce and release conidia for dispersal to soybean plants (Lehman, 1922; Kmetz et al., 1979; Sinclair, 1993). Once conidia reach the plant they will either grow further infecting the plant or they will remain latent on the plant. Environment, plant growth stage, and plant resistance to the pathogen are the determining factors that will regulate *P. longicolla* growth and infection once the pathogen is on the plant (Lehman, 1922; Kmetz et al., 1978; Sinclair, 1991).

For seed infection of *P. longicolla* to occur the pod must first be infected by the pathogen, but pod infection is independent from stem infection (Kmetz et al., 1979; Sinclair, 1993). Once the

pod is infected, *P. longicolla* mycelia will penetrate the soybean seed via seed coat pores and/or cracks in the seed coat (Kunwar et al., 1985; Sinclair, 1993). The micropyle and hilar regions of the soybean tend to be the most conducive to *P. longicolla* infection (Singh and Sinclair, 1986; Sinclair, 1993). Once hyphae of *P. longicolla* penetrate and colonize the seed coat they will continue to grow into the seed where they will invade the embryo and endosperm causing tissue to break down and the seed to lose quality (Singh and Sinclair, 1986; Sinclair, 1993). Further colonization of the pathogen will cause the seed to take on a shriveled, chalky appearance that not only is aesthetically unappealing, but causes loss of functionality of the seed (Lehman, 1922; Kulik and Schoen, 1981; Singh and Sinclair, 1986; Sinclair, 1993).

Growth stage of soybean. Growth stage is a primary factor that can determine whether *P. longicolla*, upon infection, will remain latent or grow on the host (Hill et al., 1981; Sinclair, 1991). *Phomopsis longicolla* when introduced to an immature soybean plant will remain latent, within the epidermis of the stem or pod, causing no disease symptoms (Kmetz et al., 1979). Once the plant becomes stressed or starts to senesce infection will increase dramatically as will the spread of this pathogen's mycelia (Kmetz et al., 1978; Hill et al., 1981). Little *P. longicolla* infection of seeds occur prior to physiological maturity (R7; Fehr and Caviness, 1977), but this pathogen has been isolated from immature plants, mature seeds, and immature seeds proving that it is capable of infecting the plant and seed early on (Kmetz et al., 1978; McGee, 1986).

Environment. Warm and wet environmental conditions have been positively associated with *P. longicolla* infection since the disease was first recognized in 1920 (Lehman, 1922). It was noted that during rainy portions of the summer the disease would spread more readily and infect more plants and conversely during dry periods there would be less infection and spread of the disease (Lehman, 1922). This observation has been the focus of much research which has in turn

provided scientific evidence to this hypothesis. Greater deterioration of seed occurs in the earlier maturing cultivars compared to the later maturing cultivars which is attributed to the higher temperature and humidity during plant maturation (Wilcox et al., 1974). Relative humidity is recognized as the most important variable regarding incidence and severity of *P. longicolla* (Lehman, 1922; Wilcox et al., 1974; Shortt et al., 1981; Spilker et al., 1981; TeKrony et al., 1983; Balducchi and McGee, 1987). Effects of temperature and humidity on *P. longicolla* infection have been studied and a strong positive correlation between *P. longicolla* infection and high relative humidity and a negative correlation with *P. longicolla* infection and seed germination has been noted (Spilker et al., 1981; TeKrony et al., 1983). Balducchi and McGee (1987) agreed with previous findings related to humidity and *P. longicolla* seed infection, but they also determined that alternating short periods (24 hour alternations) of high relative humidity (100% RH) and lower relative humidity (40-60% RH) did not produce significant seed infection. In order for there to be significant seed infection (>90%) of *P. longicolla* the soybean plant must be at yellow pod stage or maturity and exposed to high relative humidity (100% RH) for a duration of 3 days at 25°C; the longer the duration in a low humidity environment the longer the seed will need to spend in a 100% RH environment to obtain 90% seed infection (Balducchi and McGee, 1987).

Temperature is positively correlated with *P. longicolla* incidence, but it is a minor factor in determining infection levels. Temperature was found to affect the rate of *P. longicolla* infection more so than the incidence of *P. longicolla* infection of soybean seed (Balducchi and McGee, 1987). Balducchi and McGee (1987) reported that temperatures of 15°C or less reduces the rate and severity of infection even in high relative humidity environments whereas temperatures of 25°C significantly increase *P. longicolla* infection rate and severity. A combination of high relative humidity (100%) and high temperature (25°C or greater) produces the best environment for *P.*

longicolla infection and low relative humidity (40-60% RH) and low temperature produces a poor environment for *P. longicolla* infection (Spilker et al., 1981; Balducchi and McGee, 1987).

Precipitation is also responsible for increasing the incidence and severity of *P. longicolla* infection of soybean (Lehman, 1922; Ross, 1975; Shortt et al., 1981; Tekrony et al., 1983). Irrigation, which acts as supplemental rainfall, has also been found to increase *P. longicolla* infection at the pod fill and/or crop maturity stage (Ross, 1975). Precipitation events, such as rain or fog, aid in *P. longicolla* infection by not only dispersing inoculum to the soybean plant, but by also maintaining high relative humidity conditions near the ground/plant level (Balducchi and McGee, 1987). The correlation between precipitation and relative humidity is the primary explanation for increased *P. longicolla* levels in high precipitation areas (Shortt et al., 1981; TeKrony et al., 1983; Balducchi and McGee, 1987). The duration of the precipitation event is more important in maintaining high relative humidity than is the amount of precipitation received (Balducchi and McGee, 1987).

Disease management of *Phomopsis longicolla*

Planting date and maturity group. Management of soybean maturation by altering the planting date and/or cultivar maturity has improved yields in the southern soybean producing regions of the United States (Bowers, 1995; Heatherly and Spurlock, 1999). The Early Soybean Planting System or ESPS is the practice adopted in the southern United States in which soybean producers plant soybean earlier and select earlier maturing cultivars (relative to adapted maturity groups) for production (Bowers, 1995; Heartherly and Spurlock, 1999). By utilizing this practice the soybean will undergo reproduction in cooler conditions which has had a positive impact on soybean yield (Bowers, 1995). Although higher yields have been obtained through this practice a decrease in seed quality has also been observed due to optimal infection conditions for *P. longicolla* (Green et

al., 1965; Wilcox et al., 1974; TeKrony et al., 1983; Wrather et al., 1996). The increase in *P. longicolla* infection occurs because the plant reaches growth stage R7 during late July (Shortt et al., 1981; Spilker et al. 1981; TeKrony et al., 1983; Balducchi and McGee, 1987; Wrather et al., 1996). This part of the growing season is when high relative humidity, higher temperatures, and greater amounts/durations of precipitation are occurring which are the primary drivers of *P. longicolla* infection (Shortt et al., 1981; Spilker et al. 1981; TeKrony et al., 1983; Balducchi and McGee, 1987; Wrather et al., 1996). *Phomopsis longicolla* can be reduced by a later planting date and/or selecting later maturing cultivars (Green et al., 1965, TeKrony et al., 1983; Wrather et al., 1996; Wrather et al., 2004). This will move the critical time of *P. longicolla* infection (R6 to R7) to the drier portion of the growing season thereby reducing the chance of *P. longicolla* infection (Green et al., 1965, TeKrony et al., 1983; Wrather et al., 1996; Wrather et al., 2004). Yields have been shown to decrease when planting date is delayed which may restrict this practice to seed producers or others concerned with quality (TeKrony et al., 1983).

Genetics. Genetic resistance tends to be the best form of protection against a pathogen as well as the most economical, but few cultivars exhibit resistance to *P. longicolla*. Resistance has been reported for *D. phaseolorum* var. *caulivora*, *D. phaseolorum* var. *sojae*, as well as *P. longicolla* (Keeling, 1982; Weaver et al., 1984; Brown et al., 1987; Wrather et al., 2004). Brown et al. (1987) conducted a study evaluating soybean germplasm for *P. longicolla* resistance and found PI 417479 to exhibit the best resistance (Brown et al., 1987). PI 360841 also exhibited similar resistance to *P. longicolla*, but it was hypothesized that it was from the same line as PI 417479 (Brown et al., 1987). Crosses between PI 417479 and newer varieties have produced cultivars, such as MO/PSD-0259, which have higher yield capabilities and resistance to *P. longicolla* (Brown et al., 1987; Wrather et al., 2004). MO/PSD-0259 was registered in 1993 by Brown et al. (1993) and was a cross between PI

417479 and Merschman “Dallas”; this cultivar improved yields while maintaining *P. longicolla* resistance and has been used to cross with other lines (Brown et al., 1993; Wrather et al., 2004). Current work at the University of Arkansas has found and mapped areas of resistance to *P. longicolla* in resistant cultivar lines (Jackson et al., 2009).

Weed management. Weed control has been found to strongly influence *P. longicolla* infection as well as the whole *Diaporthe/Phomopsis* complex in soybean production areas (Onkar and da Silva, 1978; Rothrock et al., 1985; Bowman et al., 1986). A higher incidence of *D. phaseolorum* var. *sojae* and *Phomopsis sp*. was reported in uncontrolled weed plots versus controlled plots (Onkar and da Silva, 1978; Bowman et al., 1986). Weeds have been found to serve as host material to seed borne pathogens such as *P. longicolla* which helps to explain the effect weed control has on *P. longicolla* infection (Hepperly et al., 1980; Roy et al., 1997). Velvetleaf (*Abutilon theophrasti*), giant ragweed (*Ambrosia trifida L.*), cocklebur (*Xanthium strumarium*), spotted spurge (*Euphorbia maculata*), and curly dock (*Rumex crispus*) are all weeds that have been found to serve as a host for *P. longicolla* (Hepperly et al., 1980; Roy et al., 1997). Tillage and herbicides are two primary management tools used to control weeds (Onkar and da Silva, 1978; Rothrock et al., 1985; Bowman et al., 1986; Bradley et al., 2002).

Current herbicides have no significant impact on seed components and are effective at controlling weeds therefore reducing host plant material for *P. longicolla* (Onkar and da Silva, 1978; Bowman et al., 1986; Bradley et al., 2002). Tillage treatments of no-tillage, reduced tillage, and conventional tillage were reported to not have an impact on *Phomopsis sp*. recovery (Bowman et al., 1986). Conversely, it has been reported that conventional tillage reduced the incidence of *D. phaseolorum* var. *caulivora* infection by 20% (Rothrock et al., 1985). Conventional tillage buries the infected residue, leaving very little above ground, thereby reducing the chance for ascospores or

conidia to contact the plant making the idea of using conventional tillage to reduce *D. phaseolorum* var. *caulivora* infection plausible (Rothrock et al., 1985). Tillage may not have a primary effect on *P. longicolla* incidence like herbicide application does, but it does have a secondary effect regarding weed control.

Foliar fungicide treatment. Foliar fungicides have been found to reduce *P. longicolla* and increase germination as well as yield in soybean (Prasartsee et al., 1975; Ross, 1975; Ellis and Sinclair, 1975; McGee and Brandt, 1979; Jeffers et al., 1982; TeKrony et al., 1985; Wrather et al., 2004). Benomyl has been regarded as the most effective and most widely studied fungicide in reducing *P. longicolla* infection of seed (Ellis and Sinclair, 1975; Prasartsee et al., 1975; McGee and Brandt, 1979; Jeffers et al., 1982; TeKrony et al., 1985; Wrather et al., 2004).

Benomyl has been found to reduce internally borne fungi such as the *P. longicolla* seed decay pathogen (Ellis and Sinclair, 1975; Prasartsee et al., 1975; Ross, 1975; McGee and Brandt, 1979; Jeffers et al., 1982; TeKrony et al., 1985; Wrather et al., 2004). Late harvest and irrigation will increase *P. longicolla* infection, but the application of benomyl has been found to significantly reduce *P. longicolla* infection (Wilcox et al., 1974; Ellis and Sinclair, 1975; Prasartsee et al., 1975; Ross, 1975; McGee and Brandt, 1979; Jeffers et al., 1982; TeKrony et al., 1985; Wrather et al., 2004). The presence of methyl 2-benzimidazolecarbamate (MBC) in the seed coat of benomyl treated plants has been found in large enough concentrations to inhibit *D. phaseolorum* var. *sojae* growth and control seed borne fungi for up to 30 days post maturity (Ellis and Sinclair, 1975). An application of 1.12 kg/ha of benomyl prior to seed infection (around growth stage R6) provides the best protection against *P. longicolla* infection (McGee and Brandt, 1979; TeKrony et al., 1985). Benomyl is no longer registered for use on soybean which makes any recommendations using benomyl useless for commercial production of soybean. The fungicide azoxystrobin (Methyl-(E)-2-{2-[6-(2-

cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, Syngenta Crop Protection, Inc., Greensboro, NC) was found to increase the amount of *P. longicolla* infection; the reason for this remains unknown (Wrather et. al., 2004). Currently, there is little research supporting the use of any foliar fungicide for the control of *P. longicolla*.

Fungicide seed treatment. Low germination is commonly associated with *P. longicolla* infected seed and may result in a reduced stand if planted. Fungicide seed treatments have been found to improve germination, stand, and yield (Hepperly and Sinclair, 1978; Wall et al., 1982; Ferriss et al., 1987). Fungicide seed treatments of captan {(N-trichloromethyl)thio]-4-cyclohexene-1,2 dicarboximide} (Captan 30DD) and carboxin-thiram (5,6-dihydro-2-methyl-1, 4-oxathiin-3-carboxanilide, 17%; tetramethylthiuram disulfide, 17%) (Vitavax 200FF) have been shown to reduce *P. longicolla* infection and increase germination (Hepperly and Sinclair, 1978; Wall et al., 1982). Metalaxyl (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester (Apron 2E) seed fungicide failed to improve the emergence of *P. longicolla*-infected seed (Wall et al., 1982). This is not surprising considering metalaxyl is primarily effective against oomycete type fungi. Captan and carboxin-thiram seed treatment were found to improve the germination of 15% or greater *P. longicolla* infected seed lots. Fungicide seed treatments are recommended for low quality seed (seed with lower than 80% germination and/or seedlots exhibiting diseased seedlings) as well as wet and cold soil conditions (Hepperly and Sinclair, 1978; Wall et al., 1982; Ferriss et al., 1987).

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CHAPTER 3. EVALUATION OF ALTERNATIVE PRODUCTION PRACTICES ON *PHOMOPSIS LONGICOLLA* INFECTION IN SOYBEAN

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Abstract

New management practices and inputs are always being investigated in order to increase soybean [*Glycines max* L. (Merr.)] yields. Little information is available regarding the effects of these management practices and inputs on *Phomopsis longicolla* infection and seed quality. The objective of this study was to evaluate seven commonly used inputs for their impact on *P. longicolla* infection, seed quality, and yield across the Corn Belt in the United States. A field study was conducted at 17 site-years in Minnesota, Michigan, Iowa, Kentucky, Arkansas, and in Louisiana during 2009 and 2010. The seven inputs were arranged in a non-factorial manner to create nine management systems. The seven inputs were: 1) fungicide/insecticide seed treatment 2) foliar fungicide application at R3 growth stage 3) foliar fungicide application at both R3 and R5 growth stages 4) *Bradyrrhizobia* seed inoculant 5) soil fertilizer application 6) row spacing (< 76 cm vs. ≥ 76 cm) and 7) seeding rate (357,100 vs. 604,000 seeds ha^{-1}). Overall, no management system influenced the warm germination or the accelerated aging tests. An application of pyraclostrobin at growth stage R3 reduced *P. longicolla* infection by 1.9%. Soybean seed yield was increased by 0.32 Mg ha^{-1} when planted in narrow rows (< 76 cm) and by 0.26 Mg ha^{-1} when a foliar fungicide application was applied at R3. These results indicate that *P. longicolla* can be reduced and seed yield increased with an application of fungicide at R3. Additionally seed yield can be increased through the use of row spacing less than

76 cm. Our data also suggests that northern states produce higher quality seed and lower *P. longicolla* infection than do southern states.

Introduction

In a competitive market situation an increase in revenue is usually followed by an increase in production costs (Duffy, 2010). In the past three years, United States soybean producers have realized an average cash price of \$0.38 kg^{-1} which is \$0.14 kg^{-1} higher than the 20-year average (National Agricultural Statistics Service, 2011b). This increase in price has generated increased revenue as well as increased production costs for the United States soybean producer (National Agricultural Statistics Service, 2011b). Variable costs such as seed have been largely responsible for the increase in production costs (Duffy, 2010). Seed cost per hectare in the United States has increased by 41% since 2005 (Duffy, 2010; National Agricultural Statistics Service, 2011a). Since increased seed prices mitigate profit potential producers are looking for ways to increase seed yield and thereby increase profit per acre.

As soybean seed prices increase there is an increasing importance on producing high quality seed that retains the ability to germinate and grow vigorously under a wide variety of conditions. High quality soybean seed for planting is critical in order to obtain optimal and uniform plant stands early in the growing season to maximize yield and ultimately profitability (Basra, 1995). The production of high quality soybean seed is affected by many biotic and abiotic stresses experienced during soybean growth, but it is mainly the stresses imposed between physiological maturity and harvest maturity that affect the viability and vigor of soybean seed (Basra, 1995). Environmental stress such as hot, humid, and alternating wet/dry weather contribute to seed

weathering as well as foster an environment conducive to fungal pathogen growth which can significantly reduce seedling viability and vigor (Basra, 1995; Wilcox et al., 1974).

Seed quality characteristics such as viability and vigor are affected by many fungal pathogens, but it is the seed borne pathogens that cause the most damage (Sinclair, 1993). Among the seed borne fungi *P. longicolla* T.W. Hobbs is regarded as the most devastating to soybean seed quality accounting for not only losses in viability and vigor, but in soybean composition (Sinclair, 1993; Wrather et al., 2003). *Phomopsis longicolla* is the primary causal agent of seed decay in soybean [*Glycines max* (L.) Merr.] (Hartman et al., 1999; Kmetz et al., 1974). *Phomopsis longicolla* is a part of the *Diaporthe-Phomopsis* disease complex of soybean which is also comprised of *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehmeyer, *Diaporthe phaseolorum* var. *caulivora* Athow and Caldwell, and *Diaporthe phaseolorum* var. *meridionalis* Fernandez (Hartman et al., 1999). These fungal pathogens are responsible for causing pod and stem blight, northern stem canker, and southern stem canker, respectively. Collectively, the *Diaporthe-Phomopsis* complex is responsible for more soybean yield loss than any other fungal pathogen, but *P. longicolla* is noted for being the most aggressive fungal pathogen on soybean seed and is isolated more often than any other disease from the *Diaporthe-Phomopsis* complex (Kmetz et al., 1974; Kunwar et al., 1985).

Phomopsis longicolla is endemic throughout the soybean growing regions of the United States, but the incidence and severity of infection is dependent on environmental conditions which vary between years and regions (Wrather and Koenning, 2006; Sinclair, 1993). *Phomopsis longicolla* infection of soybean seed favors environments that experience temperatures greater than 25°C, precipitation, and relative humidity levels of 100% (Balduchhi and McGee, 1987; Spilker et al., 1981; TeKrony et al., 1983). Soybean seed infection however, will not occur until the R7 growth stage (Fehr and Caviness, 1977), but plant infection can occur early in the growing season and remain

latent within the plant (Kmetz et al., 1978; Pedersen and Grau, 2010; Sinclair, 1991). Soybean seed infected with *P. longicolla* can also harbor the pathogen in a latent form in the seed coat exhibiting no visual symptoms, but if the seed is severely infected it will often be shriveled and appear white and chalky (Sinclair, 1991; Hartman et al., 1999). Infection of this pathogen can lead to reduced germination, stand, and vigor of seedlings and in severe cases cause a loss in yield (Hartman et al., 1999; Kmetz et al., 1974; Sinclair, 1993; Wrather et al., 2003). Altered composition of palmitic, oleic, linoleic, and linolenic acids has also been documented in infected seeds as well as reduced flour and oil quality causing quality concerns among soybean processors (Hepperly and Sinclair, 1978; Sinclair, 1993; Wrather et al., 2003).

Foliar application of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, Benlate 50 WP, E. I. DuPont de Nemours and Co., Wilmington, Delaware] has been documented to decrease the level of *phomopsis* and increase germination (Ross, 1975; Prasartsee et al., 1975; Wrather et al., 2004). Benomyl is no longer registered for use on soybean making this practice obsolete (Wrather et al., 2004). Wrather et al., (2004) reported an increase in *Phomopsis spp.* with an application of azoxystrobin (Methyl-(E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate, Syngenta Crop Protection, Inc., Greensboro, NC) compared to the control. Fungicide soybean seed treatments of captan [(N-trichloromethyl)thio-4-cyclohexene-1, 2 dicarboximide] (Captan 30DD) and carboxin-thiram (5, 6-dihydro-2-methyl1-1, 4-oxathiin-3-carboxanilide, 17%; tetramethylthiuram disulfide, 17%) (Vitavax 200FF) have been effective in increasing the germination and emergence of soybean seed in greater than 15% *Phomopsis* infected seed lots (Wall et al., 1982). Cultural practices have shown to be effective at reducing the incidence and severity of *P. longicolla* infections. Planting date and cultivar maturity selections to avoid soybean maturation during the hot and wet portion of the growing season in the south has been

effective at increasing seed quality in the southern United States (Wrather et al., 1996). The use of *P. longicolla* resistant lines, such as PI 417479, has also proven to be effective at reducing the infection of this pathogen, but unfortunately few commercial cultivars contain the necessary resistance genes (Brown et al., 1987; Wrather et al., 2003).

Soybean seed production is conducted using similar management practices and strategies that commercial soybean producers use although strategies and management practices can vary by company. Producing high quality soybean seed usually results in a premium payment from the seed company. Optimal management practices of soybean seed production fields to maximize seed quality are not clearly defined.

Research is needed to evaluate the effect of alternative management strategies utilized to maximize yield for their impact on *P. longicolla* infection. The primary objective of this multi-state research project is to determine whether or not certain management practices and/or inputs reduce the incidence of *P. longicolla* infection and consequently increase the viability and vigor of soybean seed. The secondary objective is to identify what states in the United States have a higher risk for *P. longicolla* infection as well as determine if effects of inputs on seed quality differ among states.

Materials and Methods

Field studies were conducted at 17 site-years in 6 different states in 2009 and 2010. Locations within each state were selected by the state's collaborator to represent the soybean growing areas of that particular state. The previous crop and soil information varied between the locations and years (Tables 1-3). The experimental design was a randomized complete block design with four replications. Seven common agronomic inputs were selected to compose fourteen management systems arranged in a non-factorial manner. Due to limited seed testing resources

nine of the fourteen treatments were selected for this study (Table 4). Treatments were selected based on the likelihood they would have an impact on seed quality and *P. longicolla* infection.

The first input was a combination fungicide + insecticide seed treatment. In 2009, a fungicide + insecticide seed treatment product was used which contained 0.65 g a.i. kg seed^{-1} of imidacloprid (1-[(6-Chloro-3-pyridinyl)methyl]N-nitro-2-imidizolidinimine) + 0.04 g a.i. kg seed^{-1} of metalaxyl (N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester) + 0.05 g a.i. kg seed^{-1} of trifloxystrobin ((E,E)-alpha-(methoxyimino)-2-[[[[1-[3-(trifluoromethyl)phenyl]ethylidene]amino]oxy]methyl]-, methylester) (Trilex 6000; Bayer CropScience, Research Triangle Park, NC). For 2010, a fungicide + insecticide seed treatment which contains 0.50 g a.i. kg seed^{-1} thiamethoxam (3-[(2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-*N*-nitro-*4H*-1,3,5-oxadiazin-4imine) + 0.04 g a.i. kg seed^{-1} metalaxyl-M (methyl *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-D-alaninate) + 0.03 g a.i. kg seed^{-1} fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile) was used (CruiserMaxx; Syngenta Crop Protection, Inc., Greensboro, NC). The second input was an application of foliar fungicide at R3 growth stage. For this treatment an application of 110 g a.i. ha^{-1} of pyraclostrobin (methyl N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}(N-methoxy)carbamate) (Headline, BASF Florham Park, NJ). For treatment nine pyraclostrobin was applied at R3 growth stage followed by a second application at the R5 growth stage of Quilt which contains 91 g a.i. ha^{-1} azoxystrobin (Methyl-(E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate) + 54 g a.i. ha^{-1} propiconazole ((2*RS*,4*RS*;2*RS*,4*SR*)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole) (Syngenta Crop Protection, Inc., Greensboro, NC). The third input was a seed application of the seed inoculant Vault LVL applied at a rate of 1.25 g a.i. kg seed^{-1} (Becker Underwood, Ames, IA). The fourth input was the application of additional phosphorous at 84 kg ha^{-1}, potassium at 56 kg ha^{-1},

sulfur at 22 kg ha^{-1}, boron at 0.6 kg ha^{-1}, manganese at 2.2 kg ha^{-1}, and zinc at 0.6 kg ha^{-1}. The fifth input was a foliar fertilizer application of the product Task Force 2 (Crop Production Services, Galesburg, IL) at a rate of 5.81 kg ha^{-1} (total nitrogen – 11%, P_2O_5 – 8%, K_2O – 5%, Zn – 0.05%, Mn – 0.05%, B – 0.02%, Cu – 0.05%, Fe – 0.10%, Mo – 0.0005%, and Co – 0.0005%,) at the R1 growth stage. The sixth input was narrow row spacing (< 76 cm) vs. wide row spacing (≥ 76 cm). This input variable was dependent upon each state's equipment availability. The seventh input was a base seeding rate of 357,100 seeds ha^{-1} vs. a higher seeding rate of 604,000 seeds ha^{-1}.

Plot dimensions ranged from 1.5 to 6.1 meters wide to 7.6 to 15.2 meters long depending on each state's planter capabilities. Locally adapted soybean cultivars were selected for each location and seeds were planted at a 4 cm depth into either conventional tillage or no-tillage field based upon each collaborators preference. Prior to plot establishment soil samples were collected to determine soil P, K, OM, and pH levels (Tables 1-3). Weed management was achieved through the use of a pre-emergence application of herbicide followed by subsequent applications of glyphosate [N-(phosphonomethyl)glycine] as needed based on local university recommendations. Insects were managed according to each state's university thresholds and recommendations. The center four, three, or two rows were harvested by a self-propelled combine based on row width for each plot. Plot weight and moisture were collected at harvest and yields were adjusted to 130 g kg^{-1} grain moisture. A 0.5 kg seed sample was collected from all plots and shipped to Iowa State University for viability, vigor, and *Phomopsis longicolla* infection testing.

The (AOSA) warm germination test was used to evaluate the viability of the seed samples (AOSA, 2009a). One hundred seeds were randomly selected and placed onto two sheets of 12-ply (K-24) Versa-Pak™ (Interstate Products Inc., Sarasota, FL) wetted with 840 mL of water inside a germination tray. Trays were placed into a germination cart were incubated for seven days at 25°C

with supplemental lighting. Seeds were then evaluated according to AOSA guidelines for percent germination (AOSA, 2009a).

To evaluate seedling vigor the accelerated aging test, according to AOSA guidelines, was used (AOSA, 2009b). Forty-two grams of randomly selected soybean seed were placed in a single layer onto an accelerated aging screen. The accelerated aging screen with soybeans was then placed into an accelerated aging box with 40 mL of distilled water in the bottom. The boxes were then placed into a water jacketed aging chamber at 41°C for 72 hours. One hundred seeds were randomly selected and planted on two sheets of 12-ply (K-24) Versa-Pak™ wetted with 840 mL of water inside a germination tray. Seeds were then covered with moist sand and then placed inside a germination cart and were incubated for 7 days at 25°C. Seedlings were evaluated for percent germinated according to AOSA guidelines (AOSA, 2009b).

Percentage of seed infected with *P. longicolla* was determined by using the culture plate test from the International Seed Testing Association (Walcott, 2003).*Phomopsis longicolla* infection was determined by arbitrarily selecting 100 seeds out of the plot sample and surface disinfesting them by soaking them in 0.5% sodium hypochlorite solution for 1 minute. Seeds were then air dried in a fume hood and then ten seeds were placed onto each petri dish of acidified (pH 4.5) Difco potato dextrose agar. Seeds were incubated for 7 days at 25°C. Seed borne fungi were then evaluated based on morphological characteristics for percent infection. Five representative samples were selected from each location in 2009 and 2010 and subjected to PCR testing to confirm fungal identification. For this procedure a *P. longicolla* specific probe was used to infer the true identification of pure fungal cultures isolated from the soybean seed samples.

All data were subjected to an analysis of variance using the PROC MIXED procedure (Littell et al., 1996) of SAS (SAS Institute, 2005) at $P \leq 0.05$. All effects except locations and replications were considered fixed in determining the expected mean squares and appropriate *F* tests. Mean comparisons were made using Fisher's protected LSD test ($P \leq 0.05$). The arcsin square root transformation was used to achieve normality for the warm germination, accelerated aging, and *P. longicolla* infection data.

Results and Discussion

Growing conditions varied greatly between years and locations. In general, 2009 was wetter and cooler than 2010 (table 5). Each location within a state was chosen to be representative of what is typical for soybean producers in that particular state. Soil texture ranged from poorly drained clay based soils to well drained sandy soils (Tables 1-3). The states and locations within the states were very different from one another and therefore represent well the diversity of soybean production that growers' experience in the United States.

Warm Germination

A year by state interaction was observed for warm germination (Table 6). In Kentucky and Louisiana germination scores were 58.2% and 56% lower in 2009 when compared to 2010, respectively (Table 7). This difference may be attributed to higher rainfall in the fall of 2009 that resulted in a later harvest than in 2010. The higher amounts of precipitation experienced during harvest in 2009 led to delayed seed harvest at many of the locations thereby further exposing seeds to further in field 'weathering' (Table 5). No differences were observed in warm germination among the other states. Previous research has demonstrated that delaying harvest further exposes seeds to

often time sub-optimal environments (seed weathering) and pathogens which reduce seed viability (Basra, 1995; Wilcox et al., 1974).

Overall, the warm germination test scores in 2009 averaged 68% compared to 85.2% germination in 2010. The highest warm germination score (96.5%) came from Minnesota and was closely followed by Iowa (94.0%) (Table 6). The lowest warm germination score came from Louisiana which averaged 40.3% germination. No differences in warm germination were observed among management systems across states and years (Table 6). Seed germination is impacted by a variety of abiotic and biotic stresses imposed on the mother plant and seed prior to harvest (Basra, 1995). Any treatment applied that is capable of controlling such stresses may positively impact seed germination. Our data are not consistent with previous research that demonstrated that foliar fungicide applications of benomyl can increase seed germination by controlling fungal pathogens such as *P. longicolla* (Ross, 1975; Prasartsee et al., 1975; TeKrony et al., 1985; Wrather et al., 2004). Activity of benomyl on *P. longicolla* compared to pyraclostrobin, azoxystrobin, and propiconazole may explain the results in this study.

Accelerated Aging

A year by state interaction was observed for accelerated aging (Table 6). Accelerated aging scores were lower in 2009 in Kentucky and Louisiana when compared to 2010 (Table 7). In 2009, accelerated aging scores in Kentucky and Louisiana were 37.2% and 32.4% lower compared to 2010, respectively. Similar to seed viability, seed vigor declines as harvest is delayed beyond harvest maturity and does so at a faster rate than seed viability (Basra, 1985; TeKrony et al., 1980). The higher amounts of precipitation experienced in 2009 likely played a role in reducing the seed vigor scores. Precipitation not only delays harvest due to poor field conditions for operating harvest

machinery, but causes an increase in relative humidity which also reduces seed vigor (Basra, 1995; TeKrony et al., 1980; TeKrony et al., 1983).

Overall, the accelerated aging score in 2009 was 62.2% which is a 12.7% lower germ than was recorded for 2010(Table 6). The reduced accelerated aging score in 2009 was likely driven by the higher rainfall received during the growing season (TeKrony et al., 1983). Differences did exist among states (Table 6) with the accelerated aging scores ranging from as low as 24.1% in Louisiana to as high as 95.3% in Minnesota. Management systems did not influence accelerated aging scores across states and years (Table 6). Our data showed similar trends between the warm germination scores and accelerated aging scores. Similar trends between the warm germination and accelerated aging tests are expected as they measure two qualities (viability and vigor) which are often closely related (Basra, 1995).

Phomopsis longicolla **Infection**

Phomopsis longicolla infection varied by year and state (Table 6). Arkansas and Kentucky had a higher *P. longicolla* infection % in 2009 compared to 2010 (Table 7) whereas no differences were observed in *P. longicolla* infection in Iowa, Louisiana, Michigan, and Minnesota between the two years (Table 7). *Phomopsis longicolla* infection is favored by warm and moist conditions during soybean seed development and maturation (Spilker et al., 1981; TeKrony et al., 1983). Precipitation was higher during harvest for many of the states and locations in 2009 which created moist conditions and delayed harvest thereby contributing to the higher levels of seed infection.

The infection level of *P. longicolla* was greater in 2009 compared to 2010 averaging 19.1% and 3.4%, respectively (Table 6). This was expected because of the higher amount of rainfall in 2009 (Balducchi and McGee, 1987; Spilker et al., 1981; TeKrony et al., 1983). Differences did exist among

states across years. Kentucky had the highest *P. longicolla* infection at 25.5% and Minnesota had the lowest infection at 1.3% infection.

Differences in *P. longicolla* infection were observed among management systems (Table 6). The highest level of *P. longicolla* infection was observed for management system 1 (low input system in row spacing ≥ 76 cm) and the lowest level of *P. longicolla* infection was observed for management system 9 (high input system with a higher seeding rate and an extra application of fungicide at R5). Management system 6 (all inputs minus foliar fungicide application at R3 and a low seeding rate) and management system 3 (all inputs planted in row spacing ≥ 76 cm with a low seeding rate) were also different from management system 9 (high input system with higher seeding rate and extra application of fungicide at R5), but no one input could be singled out. By comparing management systems 3 to 8 to management system 2 single inputs can be isolated to determine if they had an effect. Management system 2 was different from management system 6 which indicates the foliar fungicide application at R3 reduced *P. longicolla* infection. Previous research has shown foliar fungicides can effectively reduce *P. longicolla* when applied prior to the R7 growth stage (Ross, 1975; Prasartsee et al., 1975; Wrather et al., 2004). An application of benomyl at R6 has proven to be the most effective (Ross, 1975; Prasartsee et al., 1975; TeKrony et al., 1985) whereas foliar applied azoxystrobin actually increased *P. longicolla* infection (Wrather et al., 2004). Timing of foliar fungicide application is an important aspect to take into consideration as well. Previous studies indicate that applications be made prior to the R7 growth stage in order to control this pathogen prior to seed infection which occurs after R7 (TeKrony et al., 1985).

Seed Yield

A year by state interaction was observed for seed yield (Table 6). Yields in Louisiana were higher in 2010 than in 2009 (Table 7). The 1.32 Mg ha^{-1} increase in seed yield from 2009 to 2010 in Louisiana may be due to a large amount of precipitation during the 2009 harvest causing delays and field losses to occur (Table 5). No differences were observed in seed yield between 2009 and 2010 for Arkansas, Iowa, Kentucky, Michigan, and Minnesota (Table 7).

Overall, seed yield varied between years (Table 6). Highest seed yield was observed in 2010 averaging 4.00 Mg ha^{-1} compared to 3.86 Mg ha^{-1} in 2009. The lower yield in 2009 may be associated with the higher incidence of *P. longicolla*, lower accelerated aging score, and lower warm germination score. Seed yield, across years, was greatest in Minnesota averaging 4.73 Mg ha^{-1} compared to Michigan that experienced the lowest yield at 3.06 Mg ha^{-1} (Table 6).

Management system influenced seed yield (Table 6). Management systems behaved similarly among years and locations as indicated by the lack of a management system by year and management system by location interaction (Table 6). Management system 8 (all inputs plus a higher seeding rate) had the highest seed yield while management systems 1 and 3 (the only two management systems in wide rows) had the lowest seed yield. Seed yield loss was observed when management systems withheld a foliar fungicide application at R3 (management system 2 vs. management system 6 and management system 1) and used wide row spacing (management system 2 vs. management system 3 and management system 1). The seed yield advantage for narrow row spacing in soybean has been well documented and our results are in agreement with previous work (Ablett et al., 1991; DeBruin and Pedersen, 2008; Oplinger and Philbrook, 1992). Overall, a 0.32 Mg ha^{-1} increase in seed yield from rows < 76 cm compared to rows ≥ 76 cm was

observed. This is similar to research conducted by DeBruin and Pedersen (2008) who found a 0.25 Mg ha^{-1} increase in seed yield for narrow rows (38 cm) when compared to wide rows (76 cm) in Iowa. Although advantages to narrow rows have been shown in Iowa, narrow row production (< 76 cm) is estimated to only account for 31% of the acres whereas wide row (76 cm) production accounts for 50% of the acres (National Agriculture Statistics Service, 2007). The foliar fungicide application at the R3 growth stage increased soybean seed yield by 0.26 Mg ha^{-1}. These results do not support previous work done by Swoboda and Pedersen (2009) in which they reported no yield gains from the application of pyraclostrobin at R1, R3, and R5 growth stages in the absence of foliar disease. Foliar disease symptoms were noted in this study and were higher than the data reported by Swoboda and Pedersen (2009) which may offer a partial explanation of the difference between the two studies (data not shown). The 0.47 Mg ha^{-1} increase in seed yield for management system 2 (all inputs planted in narrow rows) compared to management system 1 (no inputs planted in wide rows) indicates that the addition of inputs to a management system can increase soybean seed yield significantly. Although a yield increase was noted producers should consider input cost and the return they will get to determine if the addition of inputs is economically feasible.

Seed Weight

A year by state interaction was observed (Table 6). Seed weight was higher in 2009 for Arkansas and Kentucky than in 2010 (Table 7). Significant differences between management systems occurred in 2009 and 2010, but no single input could be isolated (Table 8). A higher seed weight was observed in 2009 than was observed in 2010 and maybe be attributed to the higher rainfall in 2009. Average seed weight in 2009 was 17.3 g 100 $seed^{-1}$ whereas 2010 averaged 14.0 g 100 $seed^{-1}$ (Table 6). Management system differences were observed across years and states (Table 6). The application of foliar fungicide at the R3 increased seed weight by 0.4 g 100 $seeds^{-1}$(management

system 2 compared to management system 6 (Table 6)). This supports research conducted by Swoboda and Pedersen (2009) who documented increases in seed weight with a foliar fungicide application at the R3 growth stage. Increasing the seeding rate by 246,900 seeds ha^{-1} (357,100 to 604,000 seeds ha^{-1}) increased seed weight as well(management system 2 compared to management system 8 (Table 6)). These results are in agreement with DeBruin and Pedersen (2008) and Elmore (1991) who both documented increases in seed weight as populations were increased. Other differences among treatments existed for seed weight, but no single input could be attributed to the difference.

Conclusion

To our knowledge this is the first attempt at evaluating management systems and their effect on *P. longicolla* infection across the Corn Belt in the United States. With increasing commercial soybean prices and recent spikes in soybean seed prices there has been a renewed interest in managing soybean not only for high yield, but also for better quality. Our data did not show any quality differences among the nine management systems tested regarding seed viability or seed vigor. However, differences among the management systems were observed for *P. longicolla* infection, seed yield, and seed weight. The highest level of *P. longicolla* infection was observed when no inputs were used. We were able to determine that an application of fungicide at R3 growth stage does reduce *P. longicolla*, increase seed yield, and seed weight. Planting soybean in narrow rows (< 76 cm) did also increase seed yield.

Although yields were higher and *P. longicolla* infection lower with the application of fungicide at R3, previous research indicates that there is little relationship between *P. longicolla* infection of soybean seed and seed yield (TeKrony et al., 1985). However, *P. longicolla* infection has

been noted to be highly correlated with reduced seed quality (TeKrony et al., 1985; Sinclair, 1993). *Phomopsis longicolla* infection of soybean seed does not occur till after R7 which is past much of the yield determining phases of the soybean plant thereby offering partial explanation as to why *P. longicolla* infection is not associated with a reduction in seed yield (Kmetz et al., 1978; Pedersen and Grau, 2010; Sinclair, 1993).

Our data suggest that seed producers should consider an application of foliar fungicide at R3 growth stage to reduce *P. longicolla* infection and increase seed yield. Narrow row spacing (< 76 cm) should also be implemented to increase seed yield. Based on our data it is suggested that soybean seed production should be done in the northern part of the Midwestern United States to obtain the greatest seed quality.

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Table 1. Field characteristics for the locations in Michigan and Minnesota where the study was conducted during 2009 and 2010.

	Michigan			Minnesota		
	Branch	East Lansing	Tuscola	Becker	St. Paul	Waseca
Soil Series	Fox sandy loam	Capac loam	Tappan-lando loam	Hubbard Coarse Loam	Waukegan silt loam	Webster clay loam
Soil Family	fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic typic hapludalfs	Fine-loamy, mixed, active, mesic aquic glossudalfs	Fine-loamy, mixed, active, calcareous, mesic typic endoaquolls; Fine-silty, mixed, superactive, mesic oxyaquic calcixerolls	Sandy, mixed, frigid entic hapludolls	Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic typic hapludolls	Fine-loamy, mixed, superactive, mesic typic endoaquolls
Soil Fertility						
pH	6.4	6.2	7.2	5.8-6.1	6.2	5.9-7.1
P mg kg^{-1}	71	36	56	30-49	168-81	57-32
K mg kg^{-1}	135	164	214	101-126	241-147	196-184
OM g kg^{-1}	18	22	-	25-19	37-31	53-63
Previous crop						
2009	Corn	Corn	Corn	Rye	Corn	Corn
2010	Corn	Corn	Corn	Rye	Corn	Corn
Planting date						
2009	23 May	1 June	19 May	4 May	6 May	12 May
2010	6 May	24 May	6 May	27 April	1 June	4 May
Harvest date						
2009	22 October	27 October	23 November	18 October	16 October	21 October
2010	30 September	8 October	27 September	12 October	14 October	4 October

Table 2. Field characteristics for the locations in Iowa and Kentucky where the study was conducted during 2009 and 2010.

	Iowa			Kentucky		
	Creston	Hudson	Story City	Hopkinsville	Lexington	New Haven
Soil Series	Macksburg silty clay loam	Nevin silty clay[1]/Dinsmore silty clay loam[2]	Kossuth silty clay loam[1]/Nicollet loam[2]	Pembroke silt loam	Mercer Silt Loam	Lindside silt loam
Soil Family	Fine, smectitic, mesic Aquertic Arguidolls	Fine-silty, mixed, superactive, mesic aquic pachic argiudolls[1]/Fine-silty, mixed, superactive, mesic typic hapludolls[2]	Fine-loamy, mixed, superactive, mesic typic endoaquolls[1]/ Fine-loamy, mixed, superactive, mesic Aquic Hapludolls[2]	Fine-silty, mixed, active, mesic mollic paleudalfs	Fine-silty, mixed, semiactive, mesic oxyaquic fraguidalfs	Fine-silty, mixed, active, mesic fluvaquentic eutrudepts
Soil Fertility						
pH	6.4-6.8	7.0	7.3-6.9	5.98	4.76	5.02
P mg kg^{-1}	27-22	33-28	21-41	-	-	32
K mg kg^{-1}	296-195	297-245	259-232	-	-	174
OM g kg^{-1}	35	31	40	-	-	-
Previous crop						
2009	Corn	Corn	Corn	Corn	Corn	Corn
2010	Corn	Corn	Corn	Corn	Corn	Corn
Planting date						
2009	20 May	11 May	19 May	20 May	18 May	19 May
2010	3 May	6 May	10 May	26 May	2 June	8 June
Harvest date						
2009	20 October	13 October	11 October	26 October	12 November	5 November
2010	6 October	4 October	5 October	28 September	6 October	11 October

† Year 2009 soil characteristics are noted by [1] and year 2010 soil characteristics are noted by [2].

Table 3. Field characteristics for the locations in Arkansas and Louisiana where the study was conducted during 2009 and 2010.

	Arkansas			Louisiana	
	Keiser	Pinetree	Weiner	Crowley	St. Joseph
Soil Series	Sharkey silty clay	Calloway silt loam	Henry silt loam	Crowley silt loam	Sharkey clay
Soil Family	Very-fine, smectitic, thermic chromic epiaquerts	Fine-silty, mixed, active, thermic Aquic Fraglossudalfs	Coarse-silty, mixed, active, Thermic Typic Fragiaqualfs	fine, smectitic, thermic typic albaqualf	Very-fine, smectitic, thermic chromic epiaquerts
Soil Fertility					
pH	6.8	6.3	7.3	6.2-5.7	6.1-6.6
P mg kg^{-1}	45	26-25	56-58	12.5-21	88.9-42
K mg kg^{-1}	201-199	90-100	100-102	86.7-108	508.5-358
OM g kg^{-1}	-	-	-	16	-
Previous crop					
2009	Corn	Rice	Soybean	Fallow	Grain Sorghum
2010	Corn	Soybean	Rice	Fallow	Grain Sorghum
Planting date					
2009	23 June	9 June	9 June	15 April	15 May
2010	8 May	8 May	21 May	21 May	17 June
Harvest date					
2009	21 October	21 October	21 October	30 September	1 October
2010	13 October	20 September	1 October	27 September	4 October

Table 4. Study treatment structure of the nine management systems.

Management Systems	Inputs						
	Seed treatment	Foliar fungicide application	Seed inoculant application	Soil fertilizer application	Foliar fertilizer application	Row spacing	Seeding rate (seeds ha^{-1})
1	-†	-	-	-	-	W‡	357,100
2	+	+	+	+	+	N	357,100
3	+	+	+	+	+	W	357,100
4	+	+	+	+	-	N	357,100
5	+	+	+	-	+	N	357,100
6	+	-	+	+	+	N	357,100
7	-	+	+	+	+	N	357,100
8	+	+	+	+	+	N	604,000
9	+	+§	+	+	+	N	604,000

† + indicates input was applied, - indicates input was not applied

‡ W indicates row widths ≥ 76 cm, N indicates row widths < 76 cm.

§ Fungicide application at both R3 and R5 growth stage (Fehr and Caviness, 1977).

Table 5. Monthly mean air temperature and rainfall totals for years 2009 and 2010 for locations in Arkansas, Iowa, Kentucky, Louisiana, Michigan, and Minnesota.

	August		September		October	
Location	Air temperature	Rainfall	Air temperature	Rainfall	Air temperature	Rainfall
	°C	mm	°C	mm	°C	mm
Arkansas						
Keiser	24.4/28.3†	101/17	22.2/23.3	32/5	14.4/17.2	232/18
Pinetree	24.9/28.9	37/16	22.2/23.7	220/19	14.1/16.4	312/17
Weiner	24.4/27.8	103/19	21.7/23.3	246/45	13.9/17.2	336/0
Iowa						
Creston	21.3/24.4	80/0	18.3/18.0	0/0	7.8/12.8	89/0
Hudson	20.6/23.7	181/112	17.3/17.4	57/97	7.3/1.3	55/0
Story City	20.6/23.9	99/347	17.3/17.8	38/125	7.2/12.2	192/16
Kentucky						
Hopkinsville	23.2/27.4	11/43	20.4/21.9	92/0	12.8/15.4	136/36
Lexington	23.2/26.1	98/23	20.7/22.1	183/18	12.0/15.1	160/31
New Haven	23.9/26.7	31/19	21.1/22.2	126/29	12.2/15.6	190/30
Louisiana						
Crowley	28.3/29.4	89/106	26.7/27.2	128/45	21.7/21.1	318/21
St. Joseph	25.8/29.9	79/99	23.7/24.7	86/18	16.8/17.9	343/56
Michigan						
Branch	20.2/22.8	242/36	17.0/16.4	39/87	8.8/11.3	399/95
East Lansing	20.0/23.3	164/11	16.7/16.7	18/123	8.9/11.1	104/72
Tuscola	17.8/22.9	5/36	15.9/16.3	12/72	9.6/10.0	15/17
Minnesota						
Becker	21.1/22.2	90/133	19.4/13.9	13/100	5.0/10.0	71/33
St. Paul	20.6/24.4	150/99	18.9/15.6	11/121	6.1/11.7	126/34
Waseca	19.1/22.8	85/62	17.8/14.9	38/322	5.6/10.6	179/26

† Year 2009 air temperature and rainfall are noted before the backslash whereas year 2010 air temperature and rainfall are noted after the backslash.

Table 6. Warm germination test, accelerated aging test, *P. longicolla* infection, seed yield and seed weight by year, state, and management system.

Main Effect	Warm germination	Accelerated aging	*P. longicolla* infection	Seed Yield	Seed Weight
Year (Y)	——% Germinated ——		%	Mg ha^{-1}	g 100 seed^{-1}
2009	68.0b	62.2b	19.1a	3.86b	17.3a
2010	85.2a	74.9a	3.4b	4.00a	14.0b
State (S)					
Arkansas	75.6bc	61.1bc	15.5ab	4.02ab	15.5a
Iowa	94.0ab	92.2a	10.6ab	4.64a	15.5a
Kentucky	52.8cd	42.8cd	25.5a	3.57ab	14.6a
Louisiana	40.3d	24.1d	11.6ab	3.37b	15.1a
Michigan	88.6ab	81.1ab	2.8bc	3.06b	16.4a
Minnesota	96.5a	95.3a	1.3c	4.73a	15.7a
Management System (M)					
1†	76.8a	68.9a	13.4a	3.58f	15.2c
2	77.0a	68.7a	10.6cd	4.05abc	15.5b
3	76.4a	69.0a	11.7abc	3.73ef	15.7ab
4	76.9a	69.1a	10.5cd	4.01abc	15.6b
5	76.2a	69.4a	10.9bcd	3.89cde	15.5b
6	76.0a	68.1a	12.5ab	3.79de	15.1c
7	77.5a	68.2a	10.3cd	3.97bcd	15.6b
8	75.7a	66.4a	10.7cd	4.20a	15.9a
9	77.0a	69.0a	10.4d	4.15ab	15.9a
Anova					
Y	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S	0.0009	0.0006	0.0160	0.0392	0.8290
Y × S	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
M	0.5680	0.6881	0.0007	<0.0001	<0.0001
Y × M	0.9321	0.8566	0.4340	0.9343	0.0267
S × M	0.9138	0.9358	0.9755	0.8754	0.3117
Y × S × M	0.9659	0.9972	0.7844	1.0000	0.4605

† Treatment 1 = row spacing of ≥ 76 cm; treatment 2 = a seed treatment, foliar fungicide application at the R3 growth stage, seed applied inoculant, soil applied fertilizer, foliar fertilizer, < 76 cm row spacing, and a seeding rate of 357,000 seeds ha^{-1}; treatment 3 = same as treatment 2 except with a row spacing of ≥ 76 cm; treatment 4 = same as 2 except but no foliar fertilizer application; treatment 5 = same as treatment 2 except with no soil fertilizer application; treatment 6 = same as 2 except with no foliar fungicide application; treatment 7 = same as 2 except with no seed treatment; treatment 8 = same as 2 except with a seeding rate of 604,000 seeds ha^{-1}; treatment 9 = same as 8 with an additional foliar fungicide application at the R5 growth stage.

Table 7. Warm germination, accelerated aging, *P. longicolla* infection, seed yield, and seed weight means for the interaction of state and year.

State	Year	Warm germination	Accelerated Aging	*P. longicolla* infection	Seed yield	Seed Size
		% Germinated		%	Mg ha^{-1}	g 100 seed^{-1}
Arkansas						
	2009	77.6a†	56.3a	29.5a	4.36a	19.6a
	2010	73.7a	66.0a	1.3b	3.66a	11.2b
Iowa						
	2009	93.4a	91.3a	13.0a	4.38a	16.3a
	2010	94.5a	93.1a	8.2a	4.90a	14.7a
Kentucky						
	2009	23.8b	24.3b	50.6a	4.01a	20.0a
	2010	82.0a	61.5a	0.2b	3.13a	12.6b
Louisiana						
	2009	12.3b	7.9b	13.4a	2.70b	15.9a
	2010	68.3a	40.3a	9.8a	4.02a	14.3a
Michigan						
	2009	87.9a	82.7a	4.8a	2.68a	16.6a
	2010	89.3a	79.5a	0.8a	3.43a	16.1a
Minnesota						
	2009	95.4a	93.4a	0.7a	4.61a	16.2a
	2010	97.6a	97.2a	2.0a	4.86a	15.3a

† Values followed by the same letter not different at $P \leq 0.05$ in each column.

Table 8. Effect of management system on seed weight during 2009 and 2010.

Management system	2009	2010
	g 100 seed^{-1}	
1‡	17.2abc†	13.5b
2	17.3abc	14.0ab
3	17.4abc	14.2ab
4	17.3abc	14.1ab
5	17.1bc	14.1ab
6	16.8c	13.5b
7	17.2abc	14.1ab
8	17.9a	14.3a
9	17.8ab	14.3a

† Values followed by the same letter not different at $P \leq 0.05$ in each column.

‡ Treatment 1 = row spacing of ≥ 76 cm; treatment 2 = a seed treatment, foliar fungicide application at the R3 growth stage, seed applied inoculant, soil applied fertilizer, foliar fertilizer, < 76 cm row spacing, and a seeding rate of 357,000 seeds ha^{-1}; treatment 3 = same as treatment 2 except with a row spacing of ≥ 76 cm; treatment 4 = same as 2 except but no foliar fertilizer application; treatment 5 = same as treatment 2 except with no soil fertilizer application; treatment 6 = same as 2 except with no foliar fungicide application; treatment 7 = same as 2 except with no seed treatment; treatment 8 = same as 2 except with a seeding rate of 604,000 seeds ha^{-1}; treatment 9 = same as 8 with an additional foliar fungicide application at the R5 growth stage.

Appendix 1. Effect of management system by state across 2009 and 2010 for warm germination, accelerated aging, *P. longicolla* infection, seed yield, and seed weight.

Management System	Warm germination	Accelerated aging	*P. longicolla* infection	Seed yield	Seed weight
	% Germinated		%	Mg ha^{-1}	g 100 seed^{-1}
		Arkansas			
1‡	75.5a†	61.2a	17.3a	3.53c	15.2b
2	77.2a	58.1a	15.2a	4.06abc	15.4ab
3	77.3a	65.1a	14.1a	3.67c	15.3ab
4	75.5a	64.2a	15.6a	4.11abc	15.5ab
5	74.0a	61.5a	15.8a	3.97abc	15.3ab
6	72.7a	60.9a	18.8a	3.97abc	15.1b
7	77.6a	59.4a	13.8a	3.88bc	15.8a
8	75.7a	57.8a	13.3a	4.35ab	15.8a
9	75.0a	61.5a	15.7a	4.65a	15.8a
		Iowa			
1	93.8a	92.2a	12.0a	4.27b	14.9d
2	94.5a	92.8a	9.7a	4.78a	15.4c
3	94.0a	91.0a	12.1a	4.52ab	15.7bc
4	94.4a	90.3a	10.7a	4.84a	15.6c
5	94.7a	95.2a	9.8a	4.60ab	15.3c
6	94.4a	92.9a	10.0a	4.52ab	14.9d
7	95.0a	92.7a	9.9a	4.71a	15.3cd
8	91.8a	89.8a	10.9a	4.79a	16.1ab
9	93.0a	92.5a	10.2a	4.69a	16.2a
		Kentucky			
1	51.3a	41.0a	30.1a	3.11b	14.4bc
2	52.0a	42.7a	26.3a	3.86ab	14.6abc
3	51.8a	44.0a	27.1a	3.25b	14.7abc
4	52.2a	42.6a	22.0a	3.54ab	14.5bc
5	52.9a	42.5a	25.0a	3.39ab	14.9ab
6	52.8a	40.4a	25.9a	3.21b	13.7d
7	53.9a	42.4a	24.3a	3.72ab	14.3c
8	52.4a	43.9a	24.4a	4.27a	15.1a
9	55.8a	45.8a	24.5a	3.77ab	14.7abc
		Louisiana			
1	42.4a	23.8a	17.6a	3.14a	15.5a
2	39.2a	25.5a	9.4a	3.31a	14.9a
3	39.5a	23.5a	11.8a	3.28a	15.3a
4	41.7a	26.5a	10.3a	3.36a	15.2a
5	41.5a	24.5a	9.4a	3.36a	15.0a
6	37.1a	23.9a	16.0a	3.28a	14.9a
7	41.4a	22.6a	10.3a	3.35a	14.7aa
8	38.7a	24.8a	11.1a	3.52a	14.9a
9	40.9a	21.9a	8.5a	3.72a	14.9a

		Michigan			
1	89.4a	84.2a	3.2a	2.87bc	15.9a
2	88.6a	81.3a	2.7a	3.15abc	16.4a
3	87.3a	80.6a	3.4a	2.83c	16.8a
4	89.6a	80.9a	3.3a	3.19ab	16.4a
5	86.5a	82.9a	3.0a	2.94abc	16.2a
6	90.9a	80.3a	3.0a	2.97abc	16.0a
7	88.1a	80.7a	2.1a	3.14abc	16.3a
8	88.0a	77.7a	2.6a	3.23a	16.7a
9	89.1a	81.3a	1.8a	3.18ab	16.5a
		Minnesota			
1	97.0a	96.2a	1.4a	4.42b	15.3c
2	96.5a	95.7a	0.8a	4.88a	15.6bc
3	96.3a	94.9a	1.7a	4.67ab	15.7bc
4	96.4a	95.6a	1.1a	4.77ab	15.9ab
5	97.1a	95.7a	1.4a	4.88a	15.8ab
6	95.7a	96.5a	2.0a	4.57ab	15.3c
7	96.9a	96.3a	1.4a	4.81ab	15.9ab
8	96.4a	91.8a	1.3a	4.81ab	16.0ab
9	96.2a	95.2a	1.0a	4.77ab	16.2a

† Values followed by the same letter not different at $P \leq 0.05$ in each column.

‡ Treatment 1 = row spacing of ≥ 76 cm; treatment 2 = a seed treatment, foliar fungicide application at the R3 growth stage, seed applied inoculant, soil applied fertilizer, foliar fertilizer, < 76 cm row spacing, and a seeding rate of 357,000 seeds ha^{-1}; treatment 3 = same as treatment 2 except with a row spacing of ≥ 76 cm; treatment 4 = same as 2 except but no foliar fertilizer application; treatment 5 = same as treatment 2 except with no soil fertilizer application; treatment 6 = same as 2 except with no foliar fungicide application; treatment 7 = same as 2 except with no seed treatment; treatment 8 = same as 2 except with a seeding rate of 604,000 seeds ha^{-1}; treatment 9 = same as 8 with an additional foliar fungicide application at the R5 growth stage.

CHAPTER 4. GENERAL CONCLUSION

General discussion.

Phomopsis longicolla has been reported to be endemic throughout the United States (Sinclair, 1993; Wyllie and Scott, 1988). *Phomopsis longicolla* was observed in all states involved in this project although levels of seed infection varied. Our data suggest that *P. longicolla* levels are higher in the southern United States than in the northern United States. This is likely due to the higher levels of precipitation, higher humidity, and warmer climate that is typical of the southern United States. High humidity, precipitation, and warmer weather are climatic conditions that are conducive to infection of *P. longicolla* which help to explain our results (TeKrony et al., 1983; Spilker et al., 1981; Sinclair, 1993).

Lower levels of *P. longicolla* were achieved by the application of a foliar fungicide at the R3 growth stage. Previous studies have shown foliar applications of fungicide to be beneficial in reducing the infection of the pathogen (Ross, 1975; Prasartsee et al., 1975; Wrather et al., 2004). Although our data showed a reduction in *P. longicolla* infection with the application of a fungicide at R3 we did not see any effect of management system on seed viability or seed vigor. Seed yield however increased with the foliar fungicide application at R3 and by using narrow row spacing (> 76 cm).

Based on our data we suggest an application of foliar fungicide at R3 in order to reduced *P. longicolla* infection. We also support the use of a foliar fungicide application at the R3 growth stage and the use of narrow row spacing (< 76 cm) to increase soybean seed yield. Soybean producers should take into consideration market price and input cost before adding a new input to their management system.

Recommendations for future research

In this study we tested two different fungicides with three main active ingredients. We suggest for future research to test a wider array of foliar fungicides with different active ingredients in order to determine which ones would be most effective for *P. longicolla* control. Also future research should focus on applying foliar fungicides at different growth stages in order to determine which timing of application would be most effective at reducing *P. longicolla* infection.

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