Soybean Yield Response in High and Low Input Production Systems

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

By

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2015

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Abstract

Since 2000, soybean [*Glycine max* (L.) Merr] grain commodity price has increased by almost 300% generating interest in agricultural inputs to maximize soybean yield. The objective of this study was to evaluate the effect of common inputs on soybean grain yield in enhanced (high-input) and traditional (low-input) production systems. The inputs evaluated included: *Rhizobia* inoculant, gypsum, pyraclostrobin fungicide, lambda-cyhalothrin insecticide, and manganese (Mn) foliar fertilizer. A sixteen site-year trial was established in Ohio during 2013 and 2014. *Rhizobia* inoculant was seed applied before planting, gypsum was applied at the VC growth stage (unrolled unifoliate leaves), and fungicide, insecticide, and Mn foliar fertilizer were applied at the R3 growth stage (initial pod development). Measurements of percent leaf area affected by foliar disease and insect defoliation and Mn and sulfur (S) concentration in leaves were collected at six site-years. The omission of pyraclostrobin from the enhanced production system significantly reduced yield in five of sixteen site-years by 0.21 to 0.79 Mg ha^{-1}, but its addition to a traditional system increased yield significantly at only one of sixteen site-years by 0.47 Mg ha^{-1}. Fields with high disease and above average yield (>3.5 Mg ha^{-1}) that received over 25 cm of precipitation in June and July tended to be responsive to the fungicide application. During 2013 and 2014, with established corn/soybean rotations, no

S or Mn deficiencies, and minimal insect pressure, there were limited effects of inoculant, gypsum, insecticide, and Mn foliar fertilizer on grain yield. Knowledge of potential yield limiting factors is useful in identifying inputs that will increase soybean yield on a field by field basis.

Dedicated to my parents, Jim and Sally, and my fiancée, Wayde Looker.

Acknowledgments

I am very thankful for the opportunity to have attended graduate school and appreciate beyond words all the love and support I have received along this chapter in my life. I thank my parents, Jim and Sally, for instilling in me my passion for agriculture, showing me the rewards of hard work through their examples, and all their celebrations of success with me. Thank you Mom and Dad for all the love, cake, and your amazing support. I also thank my older brothers, Josh and Abe, for their support of me, for introducing me to pop freezes that would prove priceless in reviving me after a hot day of research, and showing me that I can accomplish anything with just the right amount of determination. I also thank my loving fiancée, Wayde Looker. He has been here for me through every step of the process, even from a thousand miles away, offering me lots of laughs and amazing encouragement.

I would also like to thank my friends and faculty from Wilmington College for pushing me to do my very best. I also thank my friends and faculty at the Ohio State University. A special thank you is extended to my graduate advisor, Dr. Laura Lindsey. Laura is the best advisor I could have asked for. She was genuinely happy and excited for my successes, taught me professional skills that will prove valuable in my career, and became a friend. Also, thank you to my committee members Dr. Anne Dorrance and Dr. James Metzger for the push to be one step better and helping me accomplish my dream of being an agronomist. I am also very thankful for my lab group (Matthew Hankinson, Stephanie Verhoff, Aaron Brooker, and Joe Ng) for all of their help, especially in the field.

Lastly, I would like to thank you. If you are taking the time to read any bit of this thesis, then you deserve a pat on the back.

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1.1 OHIO SOYBEAN PRODUCTION

Soybean [*Glycine max* (L.) Merr.] is one of the world's most important crops, supplying approximately half of the world demand for vegetable oil and protein (Oerke, 2006). The United States is among the world's top soybean producers, producing approximately 32% of the world's soybeans at over 89 million MT (FAO, 2015). Soybean is the most widely planted crop in Ohio, accounting for over 1.8 million hectares of cropland, and the Ohio soybean industry is valued at over 2.7 billion dollars (NASS, 2014). According to a 2012 census, Ohio ranks 8th in the nation for soybean acreage harvested and 6th in the nation for overall soybean production, indicating the state's high productivity (NASS, 2014).

Most of Ohio's soybean production occurs on the western half of the state where the land is flatter and better suited for crop production (Figure 1). In 2014, Ohio had a record high soybean yield with a state average of 3.53 Mg ha^{-1} (NASS, 2014). Ohio yields have been increasing at a rate of 0.02 Mg ha^{-1} yr^{-1} (Figure 2). Yield increases are attributed to a combination of genetic and agronomic effects. Genetics are estimated to account for 0.009 to 0.019 Mg ha^{-1} yr^{-1}, and agronomic practices are estimated to account for 0.060 to 0.015 Mg ha^{-1} yr^{-1} (Specht et al., 1999). Demand for increased soybean production is evidenced by an increase in U.S. soybean exports from 37.2

million Mg in 2011 to a projected 41.7 million Mg in 2014, and this demand can be met through an increase in yield (USDA, 2014).

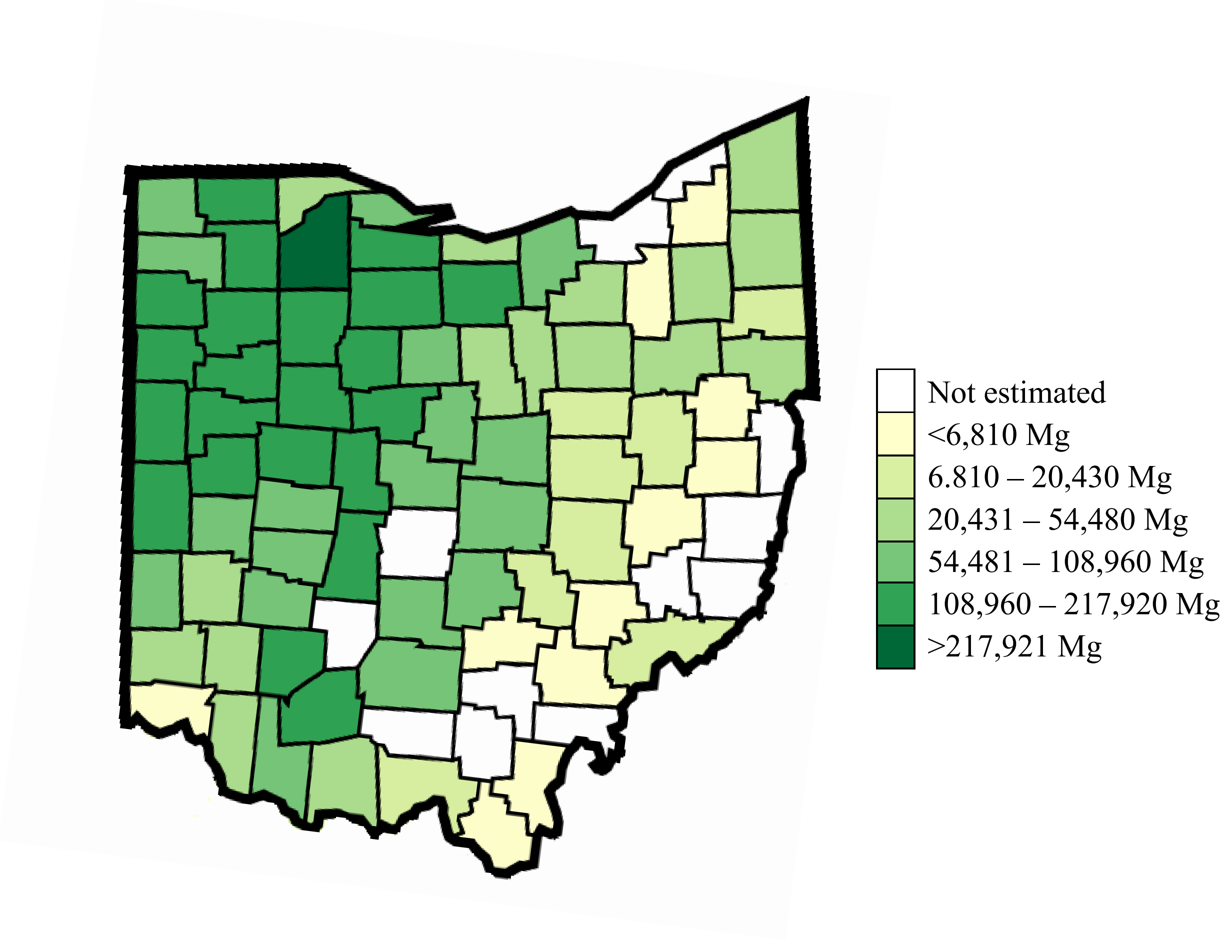

Figure 1. 2012 Ohio soybean production by county in total Mg of soybean produced (data from NASS, 2014).

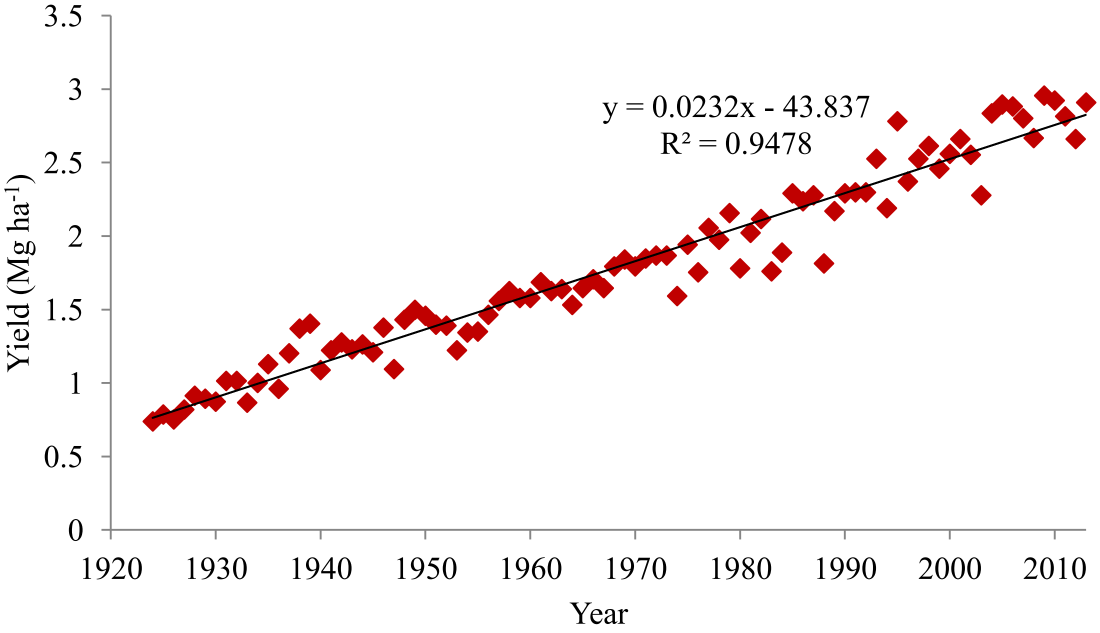

Figure 2. Ohio soybean yield in Mg ha^{-1} from 1924 to 2013 (data from NASS, 2014).

1.2 RHIZOBIA INOCULANT

Nitrogen acquisition and use is the second most important factor for plant growth, preceded only by photosynthesis (Sadowsky, 2005). Protein and oil are the principal constituents of soybean grain (Yazdi-Samadi, et al., 1977). This large amount of protein (approximately 370 to 450 g kg^{-1} protein by weight) results in a high N demand for grain development, which, in the case of legumes, is met through both residual soil N and a symbiotic relationship with soil bacteria (Beuerlein, 2009). Biological N fixation allows for yield increases without the input of additional N fertilizers (Stephens and Rask, 2000). Approximately 52% of N uptake in soybean is from biological fixation, and biological fixation rates decrease when N fertilizer is added to the crop (Salvagiotti et al., 2008). Soybean is a legume that forms a symbiotic relationship with *Bradyrhizobium japonicum* bacteria, which allows the plant to fix atmospheric nitrogen (N_2) into the plant available form, ammonia (NH_3). This process results in the creation of nodules on the soybean roots that can begin fixing N_2 as early as the V2 (second trifoliate) growth stage (Conley and Christmas, 2005).

If *Rhizobia* are not present in the soil, then they must be established to promote growth, and this can be accomplished through inoculation of seed with the bacteria (Conley and Christmas, 2005). Inoculation is the application of N-fixing bacteria to the seed or soil prior to planting. Inoculation is performed to provide the maximum amount of *Rhizobia* to the plant so it can begin nodule formation (Lupwai et al., 2000). Inoculant carriers come in different forms for application, most commonly powder in the form of peat, liquid, or granular (Stephens and Rask, 2000). Most products will make available 500,000 to 1,000,000 *Rhizobia* cells per seed when used according to label rates (Conley and Christmas, 2005). Factors affecting N fixation include soil type, nutrient availability, soil pH, soil temperature, water stress, plant genetics, agronomic practices, and environmental conditions (Brockwell and Bottomley, 1995; Pueppke, 2005; Sadowsky, 2005).

1.2.1 History of inoculation

Scientists first began to notice the relationship between nodule formation and available N in the late 1800s when soil from fields previously planted to soybean was moved to first-time soybean fields and better performance of the crop was observed (Fred et al., 1932; Miller and May, 1991; Pueppke, 2005). This led to the development of a market for soils previously planted to soybeans, referred to as “infected soils”, starting in 1904 (Pueppke, 2005). Following the symbiotic relationship discovery, the practice of artificial inoculation was launched when *Bradyrhizobium japonicum* was isolated for the first time in 1895 and quickly became common in the early 1900s (Pueppke, 2005).

Non-sterile inoculation materials were used for many years. Now in the U.S. Mid-West, inoculant is commonly used as a low-cost insurance when applied to fields in a corn-soybean rotation (Graham and Vance, 2000). Currently, advances in inoculation practices are being actively researched. Improvement trials focus on better strain selection, more viable bacteria per gram of product, increasing simplicity of product application, combining inoculant with other products such as plant growth hormones or disease control chemicals, and extending the time inoculant can be applied prior to planting (Beuerlein, 2009; Lupwayi et al., 2000; Stephens and Rask, 2000).

1.2.2 The nodulation process

The soybean nodulation process as described by Beuerlein (2009) and Sadowsky (2005) is dependent on penetration of *Bradyrhizobium japonicum* bacteria. The bacteria may infect the plant through root hairs, wounds, lesions, or cavities surrounding adventitious roots. Germinating seeds release chemical signals called flavanoids that are received by the bacteria. The bacteria respond with a return signal known as a nod factor that allows the plant to prepare for infection by curling the root hair. The curling of the root hair essentially traps the bacteria on the root surface. The development of an infection thread allows for the bacteria to grow in number until reaching the center of the root. Meanwhile, the root cells divide ultimately forming a nodule, about 6 to 18 days after initial infection. The nodule is fundamental because it is where leghemoglobin is produced, which creates the environment essential for the enzyme nitrogenase to convert N_2 to NH_3. The bacteria receive energy to obtain N_2 from sugar in the leaf that moves down to the roots. Nodulation first occurs on the crown roots and then the lateral roots (Beuerlein, 2009; Sadowsky, 2005).

Nodules reach mature size at approximately four weeks after beginning nodule formation and will continue to fix N for two or three more weeks before they begin to senesce. Soybeans begin to fix N at the V2 growth stage (second trifoliate) and reach maximum fixation rates later at approximately the pod development stages, R5 and R6. Active nodules contain a red to pink color caused by leghemoglobin (Conley and Christmas, 2005).

1.2.3 Effect of inoculant on nitrogen uptake

Intensified soybean production practices result in higher-yielding crops, thus creating a greater N demand (Salvagiotti et al., 2008). An analysis of multiple experiments determined the average worldwide N fixation by soybean on non-irrigated land is 100 and 40 kg N ha^{-1} for shoot and root N, respectively (Unkovich and Pate, 2000). Similar results have been found with an average N uptake in aboveground biomass from biological fixation of 111 kg ha^{-1} (Salvagiotti et al., 2008). In this same article, aboveground soybean biomass contained approximately 219 kg N ha^{-1}; therefore, approximately 50% of the total N in aboveground biomass was supplied from biological N fixation (Salvagiotti et al., 2008). The upper limit of biological N fixation has not been reached, as it has been proposed that fixation could reach a maximum of 360 to 450 kg N ha^{-1} (Salvagiotti et al., 2008). One factor limiting biological N fixation is that this process requires a considerable amount of plant energy, so legumes will only expend what they need for optimal growth, not what they need for excessive production (Mortier et al., 2012). Also, inoculation has not yet resulted in achieving maximum rates of biological fixation due to problems with varying responses to inoculation methods, problems guaranteeing sufficient inoculant quality, and the difficulty *Rhizobia* inoculant has competing with indigenous *Rhizobia* (Miller and May, 1991).

With advances in inoculation technology, inoculant could potentially serve as a method to increase maximum biological N fixation. In fields where soybean had not previously been planted, N fixation increased with seed-applied inoculants (Muldoon et al., 1980). A study conducted in Brazil on a field with a soybean and winter wheat rotation for the previous ten years observed that non-inoculated plots fixed 79% of their nitrogen requirement, while inoculated plots were higher, fixing 85% of their N requirement (Hungria et al.,2006).

1.2.4 Effect of inoculant on yield

Research indicates that inoculation is unnecessary when a sufficient amount of effective and active *Rhizobia* are available in the soil (Brockwell and Bottomley, 1995; Lupwayi et al., 2000). However, 60% of Ohio farmers inoculate soybean seed even though they have previously planted soybean in the field for several years, with most farmers in a corn soybean rotation (L. Lindsey, unpublished data). Inoculation is normally not recommended on fields that have had a soybean crop within the last five years (Conley and Christmas, 2005); however, results from an Illinois greenhouse study indicated that sufficient populations of *Bradyrhizobium japonicum* to survive in the soil for at least ten years (Elkins et al., 1976).

In Indiana, inoculant was found to significantly increase yield by 0.189 Mg ha^{-1} in only one of fourteen treatments using seed or soil-applied *Rhizobia* treatment factors (Nelson et al., 1978). A study conducted in Indiana, Iowa, Minnesota, Nebraska, and Wisconsin between 2000 and 2008 found a positive yield response ranging from 5% to 23% due to inoculant application at only six of 73 environments with a history of soybean cropping (De Bruin et al., 2010). A Maryland study on a *Rhizobia* populated soil found various *Rhizobium japonicum* strains and rates had no effect on soybean yield (Boonkerd et al., 1978). Similar results were found in Canada when a field that had been previously planted to soybean had no significant yield increases due to inoculant (Muldoon et al., 1980). A Minnesota study on fields that had been planted to soybean within the last two years exhibited no yield increases from inoculation (Ham et al., 1971). In California, there were no significant increases in seed yield due to inoculant in fields with soybean history (Abel and Erdman, 1964).

Occasional yield increases in fields with soybean history are observed. Sixty-four inoculation trials were carried out in Ohio over a period of eleven years on fields with previous soybean cropping history, and an average yield response of 0.13 Mg ha^{-1} was observed (Beuerlein, 2009). In Indiana, different forms of inoculant were evaluated over a ten year period, and yield increases varied from 0 to 0.161 Mg ha^{-1}, with the average response being 0.067 Mg ha^{-1} (Conley and Christmas, 2005). In Brazil, a field that had been in a soybean and winter wheat rotation for ten years saw yield increases due to inoculant ranging from 0.226 to 0.694 Mg ha^{-1}, but only in five out of twenty treatments (Hungria et al., 2006). Similarly in Michigan, out of thirteen site-years, only five had a significant yield increases from inoculant application with an average increase of 0.24 Mg ha^{-1} (Schulz and Thelen, 2008).

Yield response due to inoculation is more likely to occur on fields without soybean cropping history. A 1976 to 1977 Ontario study found seed yields were significantly higher when inoculant was applied on a new soybean field by 0.5 to 1.0 Mg ha^{-1} (Muldoon et al., 1980). In California, fields without a soybean cropping history exhibited yield increases; however, the extent to which the yields increased was not discussed (Abel and Erdman, 1964). In Michigan, yield increases were observed on fields with no previous soybean cropping history in all three site-years, with increases varying between 0.18 to 1.44 Mg ha^{-1} (Schulz and Thelen, 2008).

1.2.5 Effect of inoculant on seed quality

Inoculant has been found to have inconsistent effects on seed quality traits. A Maryland study on a field sufficiently populated with *Rhizobia* found no increase in seed protein or oil concentration with the application of inoculant (Boonkerd et al., 1978). A

Minnesota study on fields that had been planted to soybean in the previous two years found no significant protein content increases (Ham et al., 1971).

A California study on fields with and without a soybean cropping history found protein content increased and oil content decreased with inoculant application (Abel and Erdman, 1964). In Ontario, fields where soybeans had not previously been planted found significant increases in protein content by 7%, while a decrease in oil content of 3% was observed (Muldoon et al., 1980). In two out of three site-years with no previous soybean cropping history in Michigan, protein content was 8.2% and 11.8% higher in inoculated treatments, and oil content was higher by 3.3% and 7% in these treatments (Schulz and Thelen, 2008).

1.3 GYPSUM

Hydrated calcium sulfate ($CaSO_4 \cdot 2H_2O$), more commonly known as gypsum, has historically been used to improve soil conditions for crop growth (Dontsova et al., 2005). Gypsum has been applied to fields for over 250 years and is one of the oldest soil amendments used in the U.S. (Chen and Dick, 2011). Gypsum sources include mined gypsum, phosphogypsum, recycled casting gypsum, and flue gas desulfurization (FGD) gypsum. These different sources vary in their nutrient concentrations and physical characteristics. However, gypsum is approximately 23% calcium and 19% sulfur (Dontsova et al., 2005).

While gypsum application cannot be used to increase soil pH (Dontsova et al., 2005), other claims of benefits associated with gypsum application include improved plant growth, better seedling emergence, enhanced soil properties through better aggregation, lower erosion, reclamation of sodic soils, and mitigation of soil acidity (Amezketa et al., 2003; Chen and Dick, 2011; Scotter and Loveday, 1966). However, a decrease in corn emergence has been recorded following gypsum application (Borselli et al., 1996). Gypsum has relatively high solubility in water of up to 2 g L^{-1} which allows for it to readily release the essential nutrients Ca^{+2} and SO_4^{-2} into the soil solution (Dontsova et al., 2005). Although Ca is an essential nutrient for plant growth, Ca deficiency has never been documented for soybean in Ohio (L. Lindsey, unpublished data).

Gypsum is applied at various rates depending on the goal of the grower such as increasing S or Ca concentration, serving as an amendment for soil reclamation, or for improvement of soil physical properties (Chen and Dick, 2011). However, yield responses are inconsistent and more research is needed to analyze its use for Ohio soybean production (see section 1.3.3).

1.3.1 Sulfur

Sulfur is an essential macronutrient required in relatively large quantities for plant growth, and is taken up from the soil solution in the form of SO_4^{-2}. Sulfur is part of the amino acids cysteine and methoinine, and when deficient it lowers protein synthesis and photosynthetic rates (Chen et al., 2005; Hawkesford et al., 2012). Sulfur uptake is potentially influenced by available N, with higher rates of available N increasing S uptake and efficiency when both N and S are deficient (de Wit, 1992; Gutierrez Boem et al., 2007). Sulfur is not readily absorbed in soils with a pH of 5.5 or lower, and soils with greater than 1.0% organic matter normally supply sufficient S for soybean (Barker et al., 2005).

1.3.2 Sulfur deficiency in soybean

Soybean requires a large amount of S. Soybean yielding 4.03 Mg ha^{-1} will contain approximately 11.35 kg of S, 60% of which is in the soybean seed (Barker et al., 2005). Soybean removes 0.013 Mg ha^{-1} of S when yields are 4.032 Mg ha^{-1} (Chen and Dick, 2011). Sulfur deficiency in soybean can be identified by stunted plants that are pale green in color, and it is most likely to occur on sandy soils due to excessive leaching or during cool, wet conditions due to slower rates of S mineralization (Ackley et al., 2010; Culman et al, 2014; Vitosh et al., 1995). Sulfur deficiency is commonly confused with N deficiency; however, in S deficiency, unlike N deficiency, chlorosis is often more apparent on the upper leaves (Ackley et al., 2010; Culman et al., 2014). In plants experiencing S deficiency, chlorophyll content can be reduced by up to 40% and plants can have lower photosynthetic rates (Sexton et al., 1997). Deficiency can result in lower yields by stunting crop growth during the critical period and subsequently lowering the number of seeds (Gutierrez Boem et al., 2007). Sulfur deficiency could also potentially result in lower seed quality by reducing the feeding value of soybean (Chen et al., 2005; Sexton et al., 1997).

Although Ohio soils commonly supply sufficient S (Vitosh et al., 1995), S deficiency is believed to be increasing due to greater use of fertilizers with little or no S, more intensified cropping systems, larger S removal from high yielding plants, and a reduction in deposition of S from the atmosphere (Chen et al., 2005; McGrath and Zhao, 1995). In Delaware, Ohio, SO_4 deposition from rain has been steadily decreasing. In 1979, approximately 40 kg ha^{-1} of SO_4 was deposited each year from rainfall, and by 2012 this had been reduced to 10 kg ha^{-1} (Figure 3).

Soil tests are not accurate for S concentration, and so plant tissue analysis may be used to test for availability (Vitosh et al., 1995). The uppermost fully developed soybean trifoliate should be sampled just prior to R1 (early flowering) for S concentration (Vitosh et al., 1995). Sufficient S levels are 0.21 to 0.40% concentration for soybeans (Vitosh et al., 1995). Sulfur deficiency can be corrected by application of gypsum, potassium sulfate, elemental S, ammonium sulfate, or potassium sulfate magnesia (Barker et al., 2005). Typically to use gypsum as a means of increasing S concentration in soybean, approximately 0.34 Mg ha^{-1} of gypsum is needed to be applied annually (Chen and Dick, 2011).

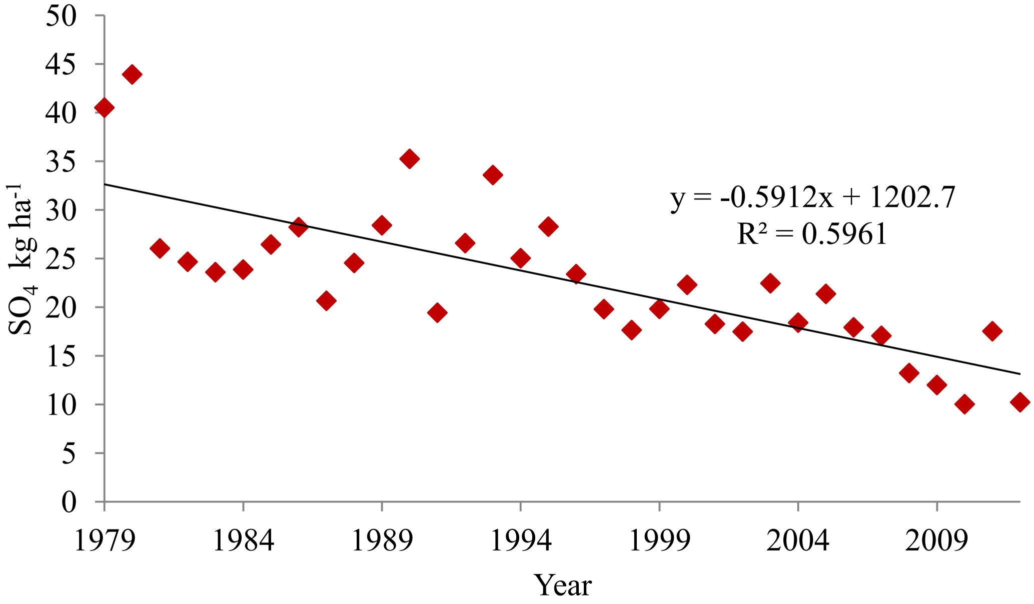

Figure 3. SO_4 atmospheric deposition in Delaware, OH from 1979 to 2012 (data from National Atmospheric Deposition Program, 2014).

1.3.3 Effect of gypsum on yield

In the U.S Midwest, gypsum will generally only increase crop yields in S deficient conditions (Rehm, 2003; Sawyer, 2003). In fields with low S concentration in India, addition of S fertilizer in the form of ammonium sulfate or elemental S resulted in yield increases of 16 to 30% when S was applied at a rate of 20 to 40 kg ha^{-1} (Agrawal and Mishra, 1994). In another experiment conducted in India on S deficient land, soybean yields were significantly higher by 0.20, 0.34, and 0.41 Mg ha^{-1} when S was applied as gypsum at rates of 180, 360, and 540 kg ha^{-1}, respectively (Saha et al., 2001). Similarly in Argentina, soybean yield has been found to increase with gypsum application at a rate of 15 kg S ha^{-1} in S deficient fields from 0.16 to 0.50 Mg ha^{-1} (Gutierrez Boem et al., 2007). On an Oxisol soil in a greenhouse experiment, gypsum rates of 0.28 to 2.28 g kg^{-1} of soil increased soybean yield by 5.54 to 6.32 g soybean $plant^{-1}$ (Fageria et al., 2014). This same study found increased pods $plant^{-1}$ of 3.5 to 7.3 and increased seed pod^{-1} of 0.4 to 0.9 seeds (Fageria et al., 2014).

In the U.S., similar results have been found for fields experiencing S deficiency. A Georgia study found surface applications of gypsum at a rate of 3.5 Mg ha^{-1} increased soybean yield in one out of two years by 0.33 Mg ha^{-1}, but this yield increase was strongly influenced by rainfall (Hammel et al., 1985). The significant soybean yield increase occurred in the year with the most rainfall, and in the previous year non-significant yield effects were associated with less uniform rainfall (Hammel et al., 1985). In Wooster, Ohio yield increases of 0.13 and 0.31 Mg ha^{-1} were observed after applications of 16 and 67 kg S ha^{-1} in the form of gypsum, respectively; however, no yield increases were detected the following year at their Clark County field site (Chen et al., 2005). The lack of yield response in Clark County was attributed to that site receiving higher S depositions from the atmosphere and the soil containing more organic matter than the Wooster site (Chen et al., 2005).

Where S deficiency is not present, soybean yield increases due to gypsum application have not commonly been observed. A Brazil study showed application rates of gypsum ranging from 3 to 9 Mg ha^{-1} had no significant effect on soybean yield (Caires et al., 2006). In Minnesota, gypsum was applied to soybean for five consecutive years at a rate of 448 kg ha^{-1}, and no significant yield increases were present in all years of the study (Vetsch and Randall, 2006). A 2012 study conducted in Iowa found no significant yield increases when S was applied as gypsum at a rate of 16.8 kg S ha^{-1} (Sawyer and Bestor, 2012). Gypsum applied at 0.34, 0.56, and 1.12 Mg ha^{-1} did not significantly increase soybean yields in Missouri (University of Missouri, 2005). Similarly, a South Dakota study found gypsum rates of 0.34 and 1.68 Mg ha^{-1} did not increase soybean yield (Gelderman et al., 2004). Iowa also did not observe yield increases across six sites when gypsum was applied at 0.07, 0.14, and 2.80 Mg ha^{-1} in both years the study was conducted (Sawyer, 2003).

1.3.4 Effect of gypsum on biomass and growth rate

Gypsum can potentially have a positive effect on biomass in S deficient conditions. In fields with low S concentration, dry matter was found to increase from 1 to 1.64 g ha^{-1} when S was applied as either ammonium sulfate, super phosphate, or elemental S at rates of 20 to 40 kg ha^{-1} (Agrawal and Mishra, 1994). Conversely, another study found aboveground biomass and growth rate of soybean plants from R2 (late flowering) to R5 (beginning seed) were not affected by gypsum application in S deficient environments; however, at R8 (full maturity) aboveground biomass has been observed to increase by 307 kg ha^{-1} (Gutierrez Boem et al., 2007).

1.3.5 Effect of gypsum on seed quality

Gypsum has not commonly been found to affect seed weight, even in S deficient fields (Gutierrez Boem et al., 2007). However, in a study conducted in India, fields deficient in S were found to increase one-thousand grain test weight from 2.3 to 12.9% depending on the S source used and rate at which it was applied, which varied from 20 to 40 kg ha^{-1} (Agrawal and Mishra, 1994). Weight of soybean seed was found to increase in a greenhouse experiment conducted with Oxisol soils by 8.0 to 18.8 g 100 seeds^{-1} (Fageria et al., 2014).

Soybean protein and oil concentration are usually not found to increase with gypsum application. However, oil content has been shown to increase by 2% when S was applied as gypsum at a rate of 540 kg ha^{-1}, but showed no significant increase at the 180 or 360 kg ha^{-1} rates (Saha et al., 2001). Conversely, a Brazil study showed no significant increases in soybean oil content at gypsum application rates of 3, 6, and 9 Mg ha^{-1}, but it was reported in one of two years of the study an increase in protein content by 4.8 to 6.6%, depending on gypsum application rate (Caires et al., 2006).

1.3.6 Effect of gypsum on soil properties

Gypsum application has not been found to affect the concentration of the essential elements P, K, Ca, Mg, S, Fe, B, Mn, Zn, Cu, Ni, and Mo in the top 30 cm of soils four months after application, except for having a 10.2 mg kg^{-1} increase on S concentration in the 15 to 30 cm soil layer in an Ohio study (Chen et al., 2005). However, a study in Brazil found gypsum increased Ca and SO_4 concentration free forms from 0 to 0.8 m in soil depth, but had no effect on K concentration, and caused a decrease in magnesium at the 0.05 to 0.2 m soil depth (Zambrosi et al., 2007). Other studies have documented gypsum may reduce exchangeable Mg in the subsoil (Caires et al., 2006; Toma et al., 1999)

Gypsum is believed to ameliorate subsoil acidity by lowering exchangeable Al up to 0.7 m deep sixteen years after application (Toma et al., 1999). When soil pH is less than 5.0, Al is soluble, taken up by plants, and has a toxic effect (Liu and Hanlon, 2012). If soil pH is maintained at the recommended 6.0 to 6.8, Al is not soluble and poses no toxic effect to plants.

Gypsum reportedly has high residual effects, as evidenced by extractions of SO_4 concentrations in soil water that have been observed 55 months after gypsum application, but this increase in SO_4 is not correlated with soybean leaf S levels (Zambrosi et al., 2007). A long-term gypsum study in Georgia found that sixteen years after gypsum was surface applied, accumulated sulfate and calcium were retained in the soil profile as compared to the control (Toma et al., 1999).

1.4 FUNGICIDE

Plant diseases can be controlled by regulatory actions, cultural practices, biological methods, or chemical control. One form of chemical control is the application of foliar fungicides, which are traditionally applied to prevent or inhibit the production of diseases in plants. Fungicides are fungitoxicants, which are substances that are toxic to fungi through either killing or inhibiting growth of fungi. Protectant fungicides kill fungal spores before they germinate or affect the germ tube to prevent penetration and infection of the plant. Curative fungicides inhibit further development of the fungus after penetration of the pathogen. Fungicides are classified depending on their chemical group and mode of action.

1.4.1 Foliar fungicide use in the U.S. Upper Midwest

Although fungicides are commonly used in the southern U.S., fungicide use on soybean in the Upper Midwest was seldom recommended until the mid-2000s. Foliar fungicide use in the Upper Midwest became more widespread when *Phakopsora pachyrhizi*, the soybean rust pathogen, was identified in Louisiana in 2004 (Kyveryga et al., 2013; Morton and Staub, 2008; Schneider et al., 2005). In a review of soybean rust collection data from 2004 to 2013, soybean rust has never been found in Ohio (IPM PIPE, 2014). However, soybean rust has been indentified in Indiana and Kentucky (IPM PIPE, 2014). An increase in fungicide use was also observed when studies were published showing presumed plant health benefits (Kyveryga et al., 2013; Glaab and Kaiser, 1999; Grossmann et al., 1999). Also, an increase in soybean disease occurrence and severity has resulted from intensified soybean production and greater use of reduced tillage practices (Dorrance et al., 2002). Reduced tillage and no-till operations create a favorable environment for pathogens because they are able to survive and over-winter on crop residue (Dorrance et al., 2002). Fungicide foliar applications have also received additional attention due in part to higher soybean commodity prices in recent years (Figure 4). These relatively higher prices have resulted in a lower yield response required to see an economic return of fungicide application (Bestor, 2011; Kyveryga et al., 2013).

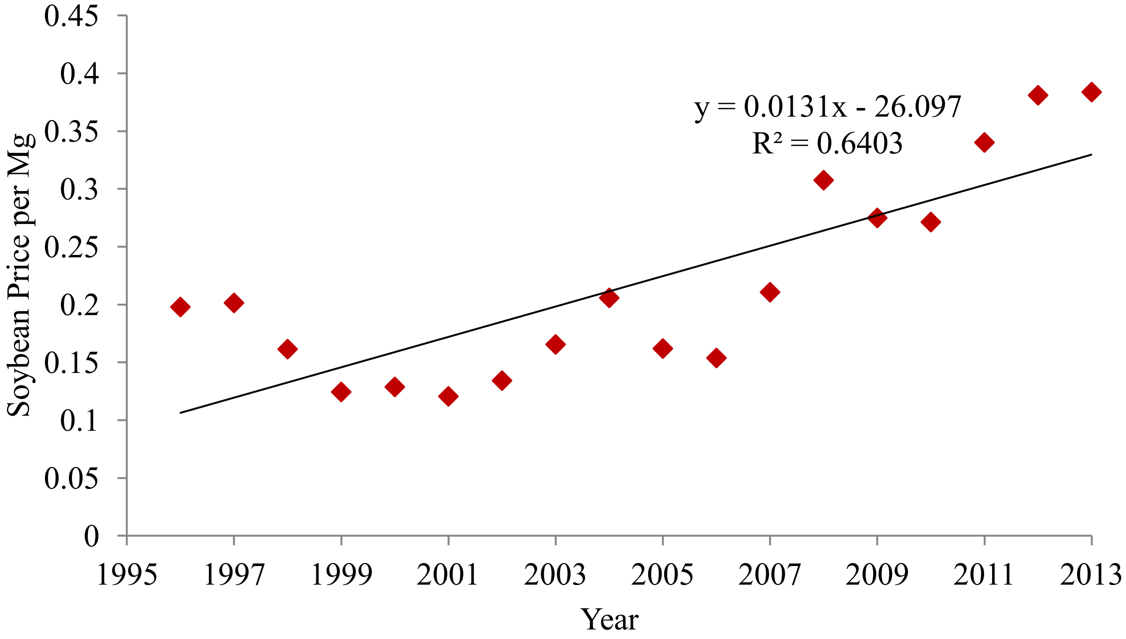

Figure 4. Average soybean prices from 1996 to 2013 (Data from NASS, 2014).

1.4.2 Strobilurin fungicides

Strobilurin fungicides, first introduced in 1996, are now the second largest chemistry group of fungicides due in large part to their common application to cereal crops and soybean (Morton and Staub, 2008). These fungicides prevent ATP production through the inhibition of mitochondrial respiration by binding on the bc_1 complex at the Q_o site (Ammermann et al., 2000). Strobilurins are well-known for their broad-spectrum activity against the four major classes of pathogenic fungi: ascomycetes, basidiomycetes, dueteromycetes, and oomycetes (Ammermann et al., 2000). In addition to their advantageous broad-spectrum action, these fungicides possess other benefits such as the ability to control pathogens resistant to other modes of action, are not highly volatile, have few signs of specific toxicity, contain minimal health risks, are rapidly degraded, and require a low dosage rate (Bartlett et al., 2002).

Strobilurins are well-known for their preventative activity, which is caused by their negative effects against spore germination and zoospore motility (Bartlett et al., 2002). Due to their preventative ability, strobilurins are the most effective when applied before infection has occurred or in the very early stages of disease development (Bartlett et al., 2002). In addition to preventative actions, strobilurins have also been found to have curative effects against disease (Bartlett et al., 2002). First sold in 1996, there are now six different types of strobilurin fungicides on the market (Table 1).

Application of strobilurins may possibly result in yield increases from a combination of effects from reduced disease pressure and stress (Kyveryga et al., 2013). Recently, strobilurin fungicides have received further attention due to claims and publications asserting the chemicals have overall plant health benefits beyond disease suppression. Yield increases by strobilurins have been observed where little evidence of differences in disease control between classes of fungicides have been present (Bartlett et al., 2002). Yield increases of 0.93 to 0.53 Mg ha^{-1} were found in wheat plants treated with kresoxim-methyl, and these increases were attributed to a combination of increased green leaf area and control of the diseases Septoria tritici (caused by the fungus *Mycosphaerella graminicola*) and powdery mildew (*Blumeria graminis*) (Bryson et al., 2000). In Ohio, a significant yield increase was observed for applications of azoxystrobin or pyraclostrobin at six out of twenty-eight locations, and three of these locations saw yield gains larger than 0.28 Mg ha^{-1} (Dorrance et al., 2010). In a Kentucky study across 51 non-replicated field trials, application of azoxystrobin with lambda-cyhalothrin, an insecticide, resulted in yield increases ranging from 0 to 0.81 Mg ha^{-1}, with an average increase of 0.31 Mg ha^{-1}; however, this yield increase was not observed when azoxystrobin or lambda-cyhalothrin were applied alone (Hershman et al., 2004).

Strobilurins may also have an effect on plant physiological processes such as carbon dioxide compensation point, chlorophyll content, photosynthesis, stomatal aperture, water consumption, and nitrate reductase activity (Bartlett et al., 2002). However, reduced fluorescence ratio (Fv Fm^{-1}), an indicator of plant health, has been observed in soybean (Nason et al., 2007). Kresoxim-methyl has been found to result in delayed leaf senescence in wheat (Bryson et al., 2000; Grossmann and Retzlaff, 1997). In a 1997-1998 study conducted on saprophyte-inoculated wheat, it was found that azoxystrobin delayed senescence of the plants; however, this did not occur in non-inoculated wheat plants (Bertelsen et al, 2001).

A study on the strobilurin kresoxim-methyl in wheat found significantly higher SPAD values in fungicide-applied plots (Bryson et al., 2000). SPAD meters read a plant's transmittance index, which serves as a measure of leaf chlorophyll content (Bryson et al., 2000). Chlorophyll levels in the leaves can signify the condition of the entire plant, with healthier plants containing more chlorophyll than less healthy plants (Spectrum Technologies, 2010).

Other physiological effects associated with strobilurins include evidence supporting lessened inactivation of nitrate reductase in spinach leaf discs treated with kresoxim-methyl (Glaab and Kaiser, 1999). Kresoxim-methyl has also been shown to reduce stomatal aperture and water consumption of wheat plants (Grossmann et al., 1999). In a study of picoxystrobin, pyraclostrobin, azoxystrobin, kresoxim-methyl, and trifloxystrobin on wheat, soybean, and barley it was determined that the fungicides reduced transpiration rates, water conductance rates through stomata, net photosynthesis, and intercellular carbon dioxide concentration of treated leaves in well-watered plants (Nason et al., 2007). Water use efficiency was weakly improved on well watered wheat plants treated with these five different strobilurins, but conversely was reduced in drought-stressed plants (Nason et al., 2007).

Claims also exist stating strobilurins can increase photosynthetic efficiency (Bartlett et al., 2002; BASF Corporation, 2009). However, presence of foliar diseases reduces photosynthetic rates of the infected areas, so application of a fungicide to lower disease pressure would account for the observed increase in photosynthesis because the infected area is being controlled (Bassanezi, et al., 2001; Debona et al., 2014). It was also found in a study of applying kresoxim-methyl to spinach discs that the fungicide had no effect on photosynthesis (Glaab and Kaiser, 1999).

In regards to seed quality, an increase of wheat grain weight of 2.2 g per one thousand seeds was observed when kresoxim-methyl was applied in combination with epixiconazole, and this has been attributed to the delayed senescence that resulted in more time for grain filling (Bryson et al., 2000).

Table 1. Strobilurin fungicides (data from Bartlett et al., 2002).

Fungicide	Company	Announced	First Sales
Azoxystrobin	Syngenta	1992	1996
Kresoxim-methyl	BASF	1992	1996
Metominostrobin	Shionogi	1993	1999
Trifloxystrobin	Bayer	1998	1999
Picoxystrobin	Syngenta	2000	2002
Pyraclostrobin	BASF	2000	2002

1.4.3 Pyraclostrobin fungicide

Pyraclostrobin was first discovered in 1993 (Ammermann et al., 2000) and released for sale in 2002 (Bartlett et al., 2002). Pyraclostrobin has protectant, curative, translaminar, and locosystemic characteristics that allows it to have a relatively wide range of application timings (Ammermann et al., 2000). Like other strobilurins, pyraclostrobin inhibits mitochondrial respiration by binding onto the bc_1-complex and has long lasting preventative disease control through its prevention of spore germination (Ammermann et al., 2000). It also has curative effects by stopping further growth of the mycelium in the leaves and successive yellowing and necrosis of leaf tissue (Stierl et al., 2000). Like other strobilurins, pyraclostrobin has been found to have favorable toxicological and ecotoxicological properties and results in no crop injury at recommended application rates (Ammermann et al., 2000).

BASF claims its pyraclostrobin product, Headline®, results in more efficient photosynthesis, allows the plant to store more carbon for growth, increases nitrate reductase activity, elevates antioxidant levels, increases defense signaling compounds, decreases the stress hormone ethylene, and decreases stress caused by other factors such as drought, hail, ozone, frost, and heat (BASF Corporation, 2009). BASF also claims that Headline® results in better seed quality and more uniform seed size for soybean (BASF Corporation, 2009).

The "greening effect" from pyraclostrobin application has been observed in many studies. In an Iowa study across 282 fields, the greening effect was observed using color-infrared images in approximately 80% of the experiments (Kyveryga et al., 2013). The greening effect is believed to be associated with delayed senescence caused by fungicide application, which could have then resulted in an extended period for grain filling and thus higher yields (Kyveryga et al., 2013). However, in another Iowa study, pyraclostrobin was found to have no effect on leaf chlorophyll meter readings with a SPAD meter (Swoboda and Pederson, 2009). Another strobilurin, azoxystrobin, was found to delay defoliation by approximately one week when applied from R3 (beginning pod) to R5 (beginning seed) in a 2003 Kentucky study across 30,000 acres (Hershman et al., 2004).

Yield responses have been observed in many studies in response to applications of pyraclostrobin. In an Iowa study spanning five years across 282 fields in the state, the average yield response to pyraclostrobin application, including fields that were non-responsive, fluctuated from 0.11 to 0.25 Mg ha^{-1} each year, with an overall average response of 0.16 Mg ha^{-1} (Kyveryga et al., 2013). In this study, years that received rainfall above the state average resulted in a greater yield increase from application by

0.03 to 0.13 Mg ha^{-1} over the other years, and foliar disease pressure was found to be low (<3%). However, these ratings were only conducted for the upper canopy and thus would not contain brown spot ratings (Kyveryga et al., 2013). In the years this study was conducted, 2005-2009, about 55% of the trials met the economic threshold for application, and most of these trials were performed in years with above normal rainfall (Kyveryga et al., 2013).

However, other studies have shown no significant yield responses to pyraclostrobin. A 2005-2006 study conducted in Iowa found no yield increase from pyraclostrobin application at R1 (beginning flower), R3 (beginning pod), or R5 (beginning seed) alone or in conjunction with tebuconazole (Swoboda and Pederson, 2009). A 2003 study over 30,000 acres of soybeans in Kentucky found no significant yield increases when pyraclostrobin was applied alone at R3 or R5 (Hershman et al., 2004). However, the authors did find an application combination of pyraclostrobin and lambda-cyhalothrin, an insecticide, increased yield when applied at R4 (late pod) by approximately 0.54 Mg ha^{-1} (Hershman et al., 2004).

Claims of improved seed quality have also been investigated. An Iowa study conducted in 2005-2006 saw no differences among seed moisture, protein content, and oil content at harvest for plants treated with pyraclostrobin alone or in conjunction with tebuconazole (Swoboda and Pederson, 2009). This study also found that plants receiving pyraclostrobin at R1 or R3 had higher seed mass, but only ranging from 0.5 to 0.7 g 100 $seed^{-1}$ (Swoboda and Pederson, 2009).

1.4.4 Fungicide resistance

In order to prevent resistance to pyraclostrobin, many factors need to be considered. The strobilurin class of fungicides has a high risk of resistance (Morton and Staub, 2008). Repeated use of a fungicide with the same mode of action results in selection pressure, and this could potentially result in the development of resistance (Kyveryga et al., 2013; Morton and Staub, 2008; Mueller et al., 2013). Resistance of strobilurins has occurred in powdery mildew of wheat, and risk of resistance can be minimized by altering between different modes of action, using mixtures, and applying at the correct dose (Bartlett et al., 2002). Resistance of fungicides that act in inhibiting binding at the Q_o site, such as strobilurins, has been detected in 35 disease species as of December, 2012, including frogeye leaf spot (*Cercospora sojina*) in soybean (Fungicide Resistance Action Committee, 2012).

1.4.4 Ohio soybean disease

The most common foliar disease for Ohio soybean is Septoria brown spot (*Septoria glycines*) (Dorrance et al., 2010). Septoria brown spot can be identified by the irregularly shaped dark brown spots that form on the leaves of up to 4.76 mm in diameter, which can then coalesce and make the infected leaf area chlorotic (Ackley et al., 2010; Culman et al., 2014). Septoria brown spot occurs on the leaves during the growing season and can lead to defoliation, and severity is strongly dependent on soybean growth stage and environmental conditions (Lim, 1980). Septoria brown spot can create yield losses of 0.20 to 0.40 Mg ha^{-1} or 2.5 to 9.5% in Ohio (Cruz 2008; Cruz et al., 2010;

Dorrance et al., 2010;). A study conducted in the late 1970's in Illinois also reported yield losses due to brown leaf spot on cultivars at that time period ranging from 0.20 to 1.14 Mg ha^{-1} (Lim, 1980). In this Illinois study, Septoria brown spot severity was rated as area under brown spot progress curves, and the greater the severity the greater the yield losses were (Lim, 1980). In Ohio, positive yield responses to strobilurin applications have been documented for soybean by 0.18 to 0.49 Mg ha^{-1}, and these responses occurred mostly where Septoria brown spot severity was decreased (Cruz et al., 2010). However, it is unknown what the threshold for action is for Septoria brown spot because the disease severity is dependent on environmental conditions and more studies must be performed (Dorrance et al., 2010).

Frogeye leaf spot (*Cercospora sojina*) is becoming a more problematic disease for Ohio, even though there are no recorded losses due to this disease prior to 2006 (Cruz and Dorrance, 2009; Dorrance et al., 2010). Yield losses of up to 35% occurred across half a million acres to frogeye leaf spot susceptible cultivars throughout Ohio in 2006 (Cruz and Dorrance, 2009). Frogeye leaf spot can be identified by its circular to irregular shaped lesions with a gray center and deep purple outer edge (Ackley et al., 2010; Culman et al., 2014). The increase in frogeye leaf spot presence has been attributed to widespread planting of susceptible cultivars, reduced tillage, and relatively warmer winter temperatures (Cruz and Dorrance 2009). It is unknown what the action threshold for frogeye leaf spot is at different stages of soybean development for Ohio because this disease is strongly dependent on environmental conditions and more studies are necessary to develop a conclusion (Dorrance et al., 2010).

1.5 INSECTICIDE

Insects contribute to yield losses by feeding on crop foliage, boring into petioles and stems, spreading diseases, and damaging seed (Hons and Saladino, 1995). Insecticides belong to a class of pesticides that are used to control pests such as insects, mites, and spiders through prevention, suppression, or eradication. Prevention is used when pest damage is predicted, suppression is the most common goal and is used to reduce pest pressure to an acceptable economic threshold, and eradication is the complete elimination of a pest species in a given area (McDonald, 1992). Insects can be categorized as nonpests, occasional pests, or perennial pests. Nonpests very rarely create economic crop losses, occasional pests are damaging only in very specific environmental conditions or when insecticides result in selection pressure, and perennial pests create economic losses regularly (Roll, 2005). Insecticides serve as a means to prevent loss of yield (Roll, 2005).

Insecticides have been used more frequently since detection of the soybean aphid (*Aphis glycines*) in North America (Johnson et al., 2009). Also, as high input production systems that result in maximum yield potential become more common, it is becoming more economically viable to apply insecticides because the yield loss potential is especially high in this management style (Oerke, 2006).

1.5.1 Insect damage to soybean

Soybean is generally considered to be resistant to yield reductions following defoliation, depending on the extent of defoliation, crop growth stage, environmental conditions, and canopy recovery (Haile et al., 1998; Ingram et al., 1981). Animal pests, including insects, account for the second highest cause of potential yield loss in crops, exceeded only by weeds (Oerke, 2006). Worldwide, the average animal pest damage to soybeans, including damage from arthropods, nematodes, rodents, birds, slugs, and snails, is 8.8 to 10.7% of yield (Oerke, 2006). Soybean is more tolerant to defoliation in the vegetative stages (Haile et al., 1998), and particularly prone to insect defoliation damage in the pod and early seed development stages, R3 to R5 (Dobrin and Hammond, 1983).

Soybean defoliation results in a lower leaf area index, causing reduced light interception, which then results in negative effects on photosynthetic ability and rate of pod growth (Ingram et al., 1981). Defoliation has varied effects on soybean yield. A 1994-1995 study found no yield reductions from defoliation despite a lower leaf area index and less light interception in 1994; however, in 1995 defoliation reduced yield for all treatments (Haile et al., 1998). A Florida study found defoliation rates of approximately 50% during the reproductive growth stages reduced soybean yields by 0.065 to 0.070 Mg ha^{-1}, and this reduction in yield was associated with slower seed growth (Ingram et al., 1981). Defoliation at the pod development stages, R3 and R4, by 33% had no effect on yield, but 66% defoliation did result in a yield decrease of 15.1% (Thomas et al., 1974). In the seed development stages, R5 and R6, 33% and 66% defoliation resulted in significant yield losses ranging from 13.8% to 24.8% (Thomas et al., 1974).

Thresholds for soybean defoliation have been examined in Ohio. The maximum level of defoliation a soybean plant can tolerate without significant yield loss depends on crop growth stage (Table 2). It is generally accepted that from seedling to bloom soybean can tolerate 40% defoliation, from bloom to pod fill the plants can tolerate 15% defoliation, and from pod fill to maturity the plants can undergo 25% defoliation (Ackley et al., 2010; Culman et al., 2014).

Table 2. Expected soybean yield loss from defoliation (data from Ackley et al., 2010).

Soybean Growth Stage	**Percent Defoliation**				
	10	25	50	75	100
	Percent Yield Loss				
V2	0	3	4	5	18
V6	0	3	5	8	26
R2	2	4	9	15	37
R4	7	13	18	36	83
R6	6	6	7	14	33
R7	0	0	0	0	0

1.5.2 Pyrethroid efficacy

Pyrethroid insecticides were introduced in 1978 and quickly gained in popularity due to their benefits that include low active ingredient rates, control of a large range of insects, and prolonged residual activity (Hammond, 1996; Watkinson, 1989). In an Ohio study, the pyrethroid insecticides permethrin and cypermethrin resulted in mortality of Mexican bean beetle adults until fourteen days after treatment at the low label recommended rate and twenty-one days at the high rate (Dobrin and Hammond, 1983). Although the insecticides no longer caused mortality after the aforementioned respective time frames, the chemicals continued to repel Mexican bean beetle adults (*Epilachna varivestis*) (Dobrin and Hammond, 1983). Lambda-cyhalothrin has long residual activity against bean leaf beetle (*Ceratoma trifurcate*), and has also been found to significantly control the beetle in Ohio, even for the second generation that occurs later in the season (Hammond, 1996).

1.5.3 Effect of pyrethroids on yield

Lambda-cyhalothrin was not found to result in significant yield increases in a Kentucky study where insect pressure was low (Hershman et al., 2004). Similarly, permethrin, a pyrethroid, was not found to significantly increase yields in Alabama or Georgia (Walker et al., 1984). In an Ohio study, lambda-cyhalothrin increased yields in only one of ten locations in 2004 by 0.269 Mg ha^{-1} (Dorrance et al., 2010). However, in 2005 yield increases varying between 0.202 and 1.021 Mg ha^{-1} were observed in ten of fourteen locations and eight of the locations with significant yield increases also had a significant reduction in aphids when the insecticide was applied (Dorrance et al., 2010).

1.5.4 Effect of pyrethroids on plant and seed quality

Permethrin, a type of pyrethroid, was found to have no effect on soybean canopy height, pods per soybean plant, or height to first pod from the ground (Walker et al., 1984). However, permethrin was found to significantly increase soybean seed weight by 0.3 and 0.9 g 100 seed^{-1} (Walker et al., 1984). Lambda-cyhalothrin results in low levels of pod injury (Hammond, 1996).

1.5.5 Insecticide resistance

Eventual resistance to pesticides used currently and those developed in the future is highly likely, despite mode of action, chemical structure, and application method (Li et al., 2007). Current pest management practices concentrate on chemical control methods for many insects, including soybean aphid (Johnson et al., 2009). However, this can result in insecticide resistance which develops with multiple applications of insecticides with the same mode of action, resulting in a selection pressure. The speed at which resistance occurs is dependent not only on chemical pressure, but also on genetics, biology, and ecology (Ramoutar et al., 2009). It is important to minimize the risk of resistance because it is becoming increasingly complicated and costly to discover and develop new insecticides (Metcalf, 1980; Watkinson, 1989). Resistance is able to be spread quickly over a large range by migrating insects (Jacobson et al., 2009). Resistance to insecticides was first discovered in 1914, and it has exponentially increased since (Metcalf, 1980). Insects can either exhibit cross resistance, which allows them to survive contact to related chemicals, or multiple resistance, which is resistance to a wide variety of insecticide classes with varying modes of action (Metcalf, 1980).

In order to prevent the development of resistance, an Integrated Pest Management (IPM) program must be implemented. IPM was introduced in the early 1970s and places an emphasis on crop scouting and economic thresholds (Roll, 2005). This reduces reliance on insecticides by focusing on other modes of control such as biological and cultural practices. In this program, it is maintained that insecticides should only be applied when the economic threshold is met. This approach also prevents the creation of an enemy-free environment, which happens when broad-spectrum pesticides are applied regardless of thresholds (Johnson et al., 2009). Beneficial insects can serve as a means for control of damaging insects through consumption of these damaging insects. When an insecticide is applied, both beneficial and damaging insects are killed, which could potentially result in an enemy-free environment for the damaging insects, making it easier for them to come back in destructive numbers.

Yield increases from insecticide application are not different when the chemicals are applied in an IPM program or as a form of crop insurance; however, the IPM approach has a higher likelihood of recovering treatment costs (Johnson et al., 2009). It is also critical to maintain a chemical rotation with various modes of actions in order to prevent resistance (Jacobson et al., 2009; Oerke, 2006). Specifically, pyrethroid efficacy is decreasing and has been found to be doing so since 2003 (Jacobson et al., 2009; Ramoutar et al., 2009).

1.6 MANGANESE

Discovered in 1922 as an essential micronutrient for plants, Mn is taken up as ions from the soil solution as Mn^{2+} (Kirkby, 2012). Manganese utilization is a microbially-driven process, and the nutrient is available to the plants after it is released as Mn^{2+} in the soil solution, where it is then transported to the roots (Hong et al., 2010). Manganese is essential for photosynthesis, especially for the oxygen-evolving complex (Broadley et al., 2012). Manganese also plays an important part in its role as a cofactor for over 35 enzymes (Broadley et al., 2012). As Mn concentration decreases chlorophyll content also decreases (Broadley et al., 2012).

1.6.1 Manganese toxicity in soybean

Manganese can become toxic when present at too high of a concentration, and soybean is particularly sensitive to Mn toxicity (Barker et al., 2005). Manganese tissue concentration greater than 300 mg kg^{-1} indicates toxicity (Hong et al., 2010). Manganese toxicity is most common on acidic and poorly drained soils (Hong et al., 2010). As pH levels decrease, Mn becomes more water soluble and converts more readily into the plant available form, Mn^{2+} (Gotoh and Patrick, 1972). As more Mn^{2+} becomes available, plants may take up an excessive amount of the nutrient creating toxicity. Toxicity is most likely to occur at pH 5 or lower, because at pH 5 most Mn will be water soluble and in the plant available form (Gotoh and Patrick, 1972).

When a toxic amount of Mn is applied, symptoms of toxicity are present within 25 days of application (Heenan and Campbell, 1980). Manganese toxicity is characterized by darkening of leaf veins, most commonly on older trifoliates, and interveinal cholorosis with leaf cupping (Hong et al., 2010). High levels of Mn can reduce plant growth, number of pods per plant, and seed weight (Heenan and Campbell, 1980). However, toxic levels have not been found to affect oil or protein concentration of the seed (Heenan and Campbell, 1980). Manganese toxicity can have negative yield impacts. An Ohio study associated soybean yield losses of 0.20 Mg ha^{-1} to Mn toxicity

(Diedrick, 2010). In Ohio, Mn toxicity is most commonly found in the eastern part of the state where the soil is more acidic (Barker et al., 2005).

1.6.2 Manganese deficiency in soybean

Although Mn deficiency is uncommon in soybean, it is the most common micronutrient deficiency (Alley et al., 1978; Randall et al., 1975). Manganese is considered sufficient at 21 to 100 mg kg^{-1} from the uppermost trifoliate just prior to R1 (Ackley et al., 2010; Culman et al., 2014; Vitosh et al., 1995). Soybean cultivars may also have varying tolerances of Mn concentration (Heenan and Campbell, 1980). Organic matter, soil type, and weather all affect Mn uptake, but the most important contributing factor to Mn deficiency is soil pH, as it becomes less soluble as pH increases (Barker et al., 2005). The pH at which Mn could potentially become deficient depends on the soil type. Silt loams and clayey soils rarely experience deficiency below pH 6.8, sandy soils high in organic matter may have deficiency at pH 6.2 or higher, and muck and peat soils could be deficient at pH levels 5.8 or higher (Barker et al., 2005). The deficiency is more likely to occur under drought conditions (Ackley et al., 2010; Culman et al., 2014). In dry soil, Mn is readily oxidized and so it is present in the soil in the plant unavailable form Mn^{4+} rather than the plant available form of Mn^{2+} (Hong et al., 2010).

Since Mn is highly immobile, deficiency symptoms are first observed on new tissue (Hong et al., 2010). Manganese deficiency in soybean is characterized by interveinal chlorosis (Ackley et al., 2010; Culman et al., 2014). The leaves will appear pale green to yellow and sometimes nearly white with prominent green veins, and in serious circumstances the plants will be stunted (Barker et al., 2005). Deficient Mn concentration results in earlier flower and pod formation, earlier sensescence, reduced pods per plant, lower seed weight, and decreased yield (Heenan and Campbell, 1980). In a Georgia study where Mn concentration was severely deficient at 4 mg kg^{-1}, grain yield was close to zero, whereas the Mn sufficient beans had yields of 3.00 Mg ha^{-1} (Wilson et al., 1982).

Deficiency may also reduce seed oil concentration, but had no effect on protein content in an Australian study (Heenan and Campbell, 1980). A Georgia study also found reduced oil content by approximately 4% with a Mn deficiency of less than 15 mg kg^{-1}, but unlike the Australian study they also found a significantly higher protein content of approximately 7% on the plants with deficient seed (Wilson et al., 1982).

Manganese deficiency in soybean can be corrected for through either banding or a foliar application (Barker et al., 2005). Banding may be used in soybean with wide-row spacing, and foliar applications are better suited for narrow rows (Barker et al., 2005).

1.6.3 Effect of manganese on yield

Manganese application has been found to increase soybean yield when Mn deficiency is present. In a 1976-1977 study, no significant yield increases were observed when Mn was sufficient, and yield increases were observed on deficient fields (Ohki et al., 1987). In a trial in Virginia, a yield increase of 1.78 Mg ha^{-1} from foliar application of Mn was only observed on one of three experimental sites, and this site had a Mn deficiency (Alley et al., 1978). Another study in Virginia found an average yield increase from foliar Mn application across three soil types ranging from 0.74 to 1.71 Mg ha^{-1} (Gettier, 1985). On Mn deficient soils in Wisconsin, a yield increase was observed from foliar application of Mn-EDTA on one of six trials in 1970 by 0.58 Mg ha^{-1}, and yield increases were observed from all foliar six foliar treatments in 1971, ranging from 0.75 to 1.03 Mg ha^{-1} (Randall et al., 1975). Yield was also increased by Mn foliar application when Mn deficiency was present in North Carolina by 0.38 to 1.14 Mg ha^{-1} (Mascagni and Cox, 1985).

In a study on Mn application to conventional and glyphosate resistant soybeans, soybean yield increased when Mn was applied by 0.27 to 0.81 Mg ha^{-1} in three of ten of the location/seed types, with two increases observed for glyphosate resistant cultivars and one increase for a conventional cultivar (Loecker et al., 2010). In an Ohio study from 2007 to 2009, yield effects due to Mn application were only observed in 2007 (Diedrick, 2010). In this year, applications of Mn-EDTA, Mn-glucoheptonate, and Mn-nitrogen all increased yield by 0.21 to 0.52 Mg ha^{-1} in one of two locations; however, the other location resulted in a significant yield decrease of 0.20 Mg ha^{-1} (Diedrick, 2010). This yield decrease was associated with Mn toxicity (Diedrick, 2010). The following two years of this study found no significant yield increases from applications of Mn-EDTA, Mn-citric acid, Mn-nitrogen, or $MnSO_4$ at all trials in both locations, and initial Mn concentrations were all within the Ohio recommended sufficiency range (Diedrick, 2010).

1.7 OMISSION TRIALS AS AN EXPERIMENTAL DESIGN

Omission trials are a relatively new type of experimental design which allows for the evaluation of multiple factors while minimizing the number of individual treatments. A factorial arrangement of treatments would result in numerous treatments, consequently requiring a large field area that may not be uniform. In an omission trial, two treatment controls are used, with one control having every input factor applied (enhanced production system) and the other control having none of the input factors applied (traditional production system). To evaluate treatment effects, a factor removed from the enhanced production system is compared to the enhanced production system control containing all factors (Below, 2011; Florence, 2012; Henninger, 2012; Ruffo, 2010). Conversely, a factor added into the traditional production system is compared to the traditional production system containing none of the treatment factors (Below, 2011; Henninger, 2012; Ruffo, 2010; Florence, 2012). Omission trials have been used in Illinois to evaluate factors affecting corn grain yield (Henninger, 2012) and in Ohio to study management practices that affect corn grain yield (Florence, 2012). However, a similar study has not been conducted for Ohio soybean production.

1.8 SUMMARY

Soybean is one of the world's most important crops (Oerke, 2006), and it is the most widely planted crop in Ohio (NASS, 2014). Although Ohio had record high soybean yields in 2014 with a state average of 3.53 Mg ha^{-1} (NASS, 2014), it is critical soybean yield continues to increase to meet the demand of our growing population. Soybean yield may be increased through a combination of genetic and agronomic practices (Specht et al., 1999). The objectives of this study were to assess if *Rhizobia* inoculant, gypsum, pyraclostrobin fungicide, lambda-cyhalothrin insecticide, and Mn foliar fertilizer applications increase soybean grain yield. Although all these factors have been evaluated in separate studies, they have not been evaluated together in one study. Farming practices are changing such as increased use of no-till or reduced tillage, earlier planting dates, heavier seeding populations, advances in application technology, and greater use of high-input production systems. It is unknown if these management practices are affected by changes in farm management style. All factors will be studied in an omission trial to see if exclusion of one factor reduces yield in a high-input system, or if addition of an input increases yield in a traditional system.

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Chapter 2: Soybean Yield Response and High and Low Input Production Systems

2.1 ABSTRACT

From 2000 to 2013 soybean [*Glycine max* (L.) Merr] grain commodity price has increased by almost 300% generating interest in agricultural inputs to maximize soybean yield. The objective of this study was to evaluate the effect of common inputs on soybean grain yield in enhanced (high-input) and traditional (low-input) production systems. The inputs evaluated included: *Rhizobia* inoculant, gypsum, pyraclostrobin fungicide, lambda-cyhalothrin insecticide, and manganese (Mn) foliar fertilizer. A sixteen site-year trial was established in Ohio during 2013 and 2014. *Rhizobia* inoculant was seed applied before planting, gypsum was applied at the VC growth stage (unrolled unifoliate leaves), and fungicide, insecticide, and Mn foliar fertilizer were applied at the R3 growth stage (initial pod development). Measurements of percent leaf area affected by foliar disease and insect defoliation and Mn and sulfur (S) concentration in leaves were collected at six site-years. The omission of pyraclostrobin from the enhanced production system significantly reduced yield in five of sixteen site-years by 0.21 to 0.79 Mg ha^{-1}, but its addition to a traditional system increased yield significantly at only one of sixteen site-years by 0.47 Mg ha^{-1} Soybean yield was influenced by fungicide application when fields had disease present, above average yield (>3.5 Mg ha^{-1}), and received >25 cm of precipitation in June and July. During 2013 and 2014, with established corn/soybean

rotations, no S or Mn deficiencies, and minimal insect pressure, there were limited effects of inoculant, gypsum, insecticide, and Mn foliar fertilizer on grain yield. The data indicate a very small potential for high-input production systems to enhance crop yield without the presence of diseases, insects, or nutrient deficiencies. Knowledge of potential yield limiting factors is useful in identifying inputs that will increase soybean yield on a field by field basis.

2.2 INTRODUCTION

In 2014, over 33.6 million hectares of soybean [*Glycine max* (L.) Merr.] were planted in the United States (NASS, 2014). From 200 to 2013, the average soybean commodity price United States has increased almost 300% since 2000, from an average price of \$162 Mg^{-1} in 2000 to \$522 Mg^{-1} in 2013 (NASS, 2014). However, in 2014, the average soybean commodity price went down to \$375 Mg^{-1} (NASS, 2014). With the reduction in soybean commodity price, farmers have interest in inputs that can be eliminated from their production practices

Additionally, farm management practices have been evolving and now include a greater focus on no-till management systems, high input production systems, and earlier planting dates (Conley and Christmas, 2005; Dorrance et al., 2002). High soybean prices and evolving farming practices make it necessary to evaluate input effects on soybean grain yield. Agricultural inputs of particular interest to farmers include *Rhizobia* inoculant, gypsum, fungicide, insecticide, and manganese (Mn) foliar fertilizer.

Soybean has a high nitrogen (N) demand that is partially met through a symbiotic relationship with *Bradyrhizobium japonicum* bacteria. Yield response due to seed-applied *Rhizobia* inoculant seldom occurs when fields have been planted to soybean within the past five years, because these fields typically have sufficient Rhizobia present in the soil to initiate biological N fixation (Abel and Erdman, 1964; Boonkerd et al., 1978; De Bruin et al., 2010; Ham et al., 1971; Muldoon et al., 1980; Nelson et al., 1978). A Midwest multi-state study from 2000 to 2008 evaluated 51 different inoculant products and found positive yield increases from inoculant application ranging from 5% to 23% at only six out of 73 environments with soybean cropping history (De Bruin et al., 2010). Based on a survey of farmers during 2013 and 2014, it was estimated that 60% of Ohio farmers annually apply inoculant in fields that have had soybean produced within the previous two years (L. Lindsey, unpublished results).

Gypsum (calcium sulfate) is another input that has received attention from soybean farmers. Sulfur (S) deficiency is believed to be increasing due to greater use of high concentration fertilizers with little or no S, more intensified cropping systems, elevated S removal from high yielding crops, and a reduction in S atmospheric deposition (Chen et al., 2005; McGrath and Zhao, 1995). In central Ohio, sulfate deposition from rain has been steadily decreasing from an average of 40 kg ha^{-1} deposited in 1979 to approximately 10 kg ha^{-1} in 2013 (National Atmospheric Deposition Program, 2014). Application of S as gypsum could be used as a means to increase Ohio soybean grain yield in high production systems due to the reduction of atmospheric deposition of S and to meet demands of higher yielding crops.

Yield benefits, beyond disease suppression, have been proposed from application of strobilurin fungicides which has led to increased interest in incorporating fungicides into production systems. Strobilurin fungicides may produce physiological effects such as delayed senescence, lessened inactivation of nitrate reductase, and reduced stomatal aperture (Glaab and Kaiser, 1999; Grossmann et al., 1999; Kyveryga et al., 2013). Significant yield increases from pyraclostrobin, a type of strobilurin fungicide, have been documented across the United States in the presence of low disease severity levels of brown leaf spot (caused by *Septoria glycines* Hemmi) and frogeye leaf spot (caused by

Cercospora sojina K. Hara) (Dorrance et al., 2010; Hershman et al., 2004; Kyveryga et al., 2013). In Iowa, a soybean yield response of 0.11 to 0.25 Mg ha^{-1} occurred with pyraclostrobin fungicide application across 282 fields (Kyveryga et al., 2013). Increases in yield due to fungicide application were tied to high rainfall amounts totaling over 30 cm from March to May, which would favor fungal foliar disease development (Kyveryga et al., 2013). In Kentucky, no significant yield increases occurred when pyraclostrobin was applied alone; however, a tank-mix application of pyraclostrobin and the insecticide lambda-cyhalothrin increased yield by 0.54 Mg ha^{-1} when applied at R4 (late pod) growth stage (Hershman et al., 2004).

Insecticide is commonly applied in a tank-mix with foliar fungicides. Applications of pyrethroid insecticides, particularly lambda-cyhalothrin, had varying yield effects depending on insect pressure (Dorrance et al., 2010; Hershman et al., 2004; Walker et al., 1984). In Kentucky, there were no significant yield increases from lambda-cyhalothrin applied alone (Hershman et al., 2004). In Ohio, yield increases varying between 0.202 and 1.021 Mg ha^{-1} occurred at ten out of fourteen locations where lambda-cyhalothrin was applied alone; a reduction in soybean aphid (*Aphis glycines* Matsumura) abundance occurred at 80% of the locations where a significant yield increase was observed (Dorrance et al., 2010). Current insecticide application effects must be evaluated, particularly when tank-mixed with fungicide because yield responses have been observed when the products are tank-mixed, but not when the products are applied separately (Dorrance et al., 2010; Hershman et al., 2004).

Although Mn deficiency is rare, it is the most common micronutrient deficiency in Ohio (Alley et al., 1978; Randall et al., 1975). Manganese deficiency results in earlier flower and pod formation, earlier senescence, reduced pods per plant, lower seed weight, and decreased yields (Heenan and Campbell, 1980). Manganese application increased soybean yield by 0.38 to 1.14 Mg ha^{-1} when Mn deficiency was present (Alley et al., 1978; Mascagni and Cox, 1985; Ohki et al., 1987; Randall et al., 1975). In Ohio, Mn tissue concentration is considered below sufficiency at $\leq$21 g kg^{-1} when sampled from the uppermost fully developed trifoliate at the R1 (initial flowering) growth stage (Vitosh et al., 1995). Foliar fertilizer application of Mn can be used to correct this deficiency and increase soybean grain yield (Alley et al., 1978; Mascagni and Cox, 1985).

Omission trials are a relatively new type of experimental approach which allows for the evaluation of multiple factors while minimizing the number of individual treatments. A full factorial arrangement would result in numerous treatments, consequently requiring a large field area that may not be uniform. In an omission trial, two treatment controls are used, with one control having every input factor applied (enhanced production system) and the other control having none of the input factors applied (traditional production system). To evaluate treatment effects, a factor removed from the enhanced production system is compared with the enhanced production system control containing all factors (Henninger, 2012; Florence, 2012). Conversely, a factor added into the traditional production system is compared with the traditional production system containing none of the treatment factors (Henninger, 2012; Florence, 2012). Omission trials have been used in Illinois to evaluate factors affecting corn grain yield

(Henninger, 2012) and in Ohio to study management practices that affect corn grain yield (Florence, 2012). However, a similar study has not been conducted for Ohio soybean production.

The objectives of this study were to assess if *Rhizobia* inoculant, gypsum, fungicide, insecticide, and Mn foliar fertilizer applications increase soybean grain yield. An omission trial was used to determine if the exclusion of one input factor reduced yield in an enhanced (high-input) system, or if an addition of an input factor increased yield in a traditional (low-input) system. These factors have all been evaluated individually in separate studies; however, farmers use these products together making it important to study the effects of the combined factors on yield.

2.3 MATERIALS AND METHODS

2.3.1 Site Description and Experimental Design

The study was conducted at nine Ohio locations in 2013 and seven locations in 2014 (Figure 5). Although seven of the locations were the same in 2013 and 2014, the study was not conducted in the same field. All field sites were on-farm except for the Clark, Wood, and Wayne County locations, which were conducted at Ohio Agricultural Research and Development Center (OARDC) research stations. The previous crop was corn, and all sites were no-till except for the 2013 Clinton County site which was minimally tilled to a depth of approximately 2.5 cm. At least eight soil cores were collected at planting to a 20-cm depth and homogenized for analysis of soil chemical and physical properties (Table 3). Average monthly temperature and cumulative monthly precipitation from May through October were collected from the National Oceanic and Atmospheric Administration (NOAA, 2014) with weather stations located within the county of interest, except for the 2013 Delaware site-year whose weather station was located one county south in Franklin County, approximately 25 km from the site.

Five agronomic inputs were evaluated: *Rhizobia* inoculant, pelletized gypsum, pyraclostrobin fungicide ([2-[[[1-(4-chlorophenyl-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]methoxy- methyl ester), lambda-cyhalothrin insecticide ([1a(S*), 3a(Z)-cyano(3-phenoxyphenly) methyl-3-(2-chloro-3, 3, 3-trifluoro-1-propenyl-2, 2-dimethylcyclopropanecarboxylate), and Mn foliar fertilizer. The studies were arranged as an omission trial, randomized complete block experimental design with four replications of treatments (Table 4). Plots were 8.5 m long at on-farm locations and 9.1 m long at OARDC field sites and planted using a six-row Almaco plot planter (Almaco, Nevada, IA). Planting dates ranged from 15 May to 3 June (Table 5). Plots were six rows wide with 38 cm row spacing with the center four rows harvested for yield. A Massey Ferguson Kincaid 8XP plot combine (Kincaid Equipment Manufacturing, Haven, KS) was used to harvest soybeans and grain yield was adjusted to 130 mg kg^{-1} moisture content.

2.3.2 Cultural Practices and Treatments

Pre-emergence herbicides and post-emergence herbicides were applied to appropriately manage weeds. Soybean seed of ‘Asgrow 3231’ (maturity group 3.2) was planted in May or June at 358,000 seeds ha^{-1} with an Almaco plot planter. The seed was treated with Acceleron® containing pyraclostrobin ([2-[[[1-(4-chlorophenyl-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester), metalaxyl (N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alanine methyl ester), fluxapyroxad (1*H*-Pyrazole-4-carboxamide, 3-(difluoromethyl)-1-methyl-N-(3', 4', 5'-trifluoro[1,1'-biphenyl]-2-yl), and imidicloprid (1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) (Monsanto Company, St. Louis, MO). For the Acceleron® seed treatment, in 2013 and 2014 pyraclostrobin was applied at an active ingredient rate of 0.05 ml kg^{-1} and 0.07 ml kg^{-1} seed, respectively, and metalaxyl was applied at an active ingredient rate of 0.15 ml kg^{-1} seed and 0.07 ml kg^{-1} seed, respectively. In both years, the fluxapyroxad and imidacloprid components of Acceleron® were applied at active ingredient rates of 0.05 and 0.63 ml kg^{-1} seed, respectively. Seed receiving the inoculant input was treated using TagTeam® LCO Liquid MultiAction® Legume Fertility (Novozymes BioAg Inc., Brookfield, WI), a *Bradyrhizobium japonicum* inoculant, at 0.06 g kg^{-1} of *Bradyrhizobium japonicum* seed^{-1} on 7 May 2013 and 1 May 2014.

NutraSoft Pelletized Gypsum (The Andersons, Maumee, OH) was applied by hand at the VC growth stage at 4.47 Mg ha^{-1} of product which was at a rate of 0.94 Mg ha^{-1} of Ca and 0.72 Mg ha^{-1} of S (Table 5). The fungicide, insecticide, and Mn foliar fertilizer were applied at the R3 growth stage using a carbon dioxide pressurized backpack sprayer calibrated at 186.7 L ha^{-1} with TeeJet tt1102 nozzles (TeeJet Technologies, Wheaton, IL) with 0.5 m spacing on a 1.5 m spray boom (Table 5). The fungicide applied was pyraclostrobin (Headline®, BASF Corporation, Florham Park, NJ), at the label recommended active ingredient rate of 103.49 ml ha^{-1}. The insecticide applied was the synthetic pyrethroid, lambda-cyhalothrin (Warrior II with Zeon Technology®, Syngenta, Wilmington, DE) at the label recommended active ingredient rate of 26.65 ml ha^{-1}. The Mn foliar fertilizer applied was the EDTA Max-In Ultra Manganese (Winfield Solutions LLC, St. Paul, MN) at the label recommended active ingredient rate of 0.23 L ha^{-1}. When two or three of these chemicals were applied in combination the products were tank-mixed.

2.3.3 In-Season Measurements at Selected Sites

Detailed leaf nutrient analysis and pest ratings were collected at three locations per year and are referred to as 'intensive measurement site-years' (Figure 5). In 2013, the intensive measurement sites included Clinton, Delaware, and Henry County, and in 2014 the sites included Clinton, Preble, and Sandusky County. Leaf sampling for Mn and S concentration was conducted at the R1 growth stage. The uppermost, fully developed trifoliate was collected from ten plants from one of the middle four rows per plot, dried at 65°C in a forced-air dryer, ground, and analyzed for Mn and S concentration (Latimer, 2012).

Percent leaf area affected by foliar disease was rated visually four weeks after fungicide application in the bottom, middle, and top third of the plant canopy by evaluating a minimum of five trifoliate leaves per canopy section to estimate an average percent leaf area affected. The two most predominant diseases in both years of the study were brown leaf spot and frogeye leaf spot. Percent leaf area affected (LAA) by brown leaf spot was assessed visually in the bottom third and middle third of the plant canopy using an assessment scale to evaluate severity as described by Cruz et al. (2010). Frogeye leaf spot was assessed visually in the middle third and top third of the plant canopy using an assessment scale to evaluate severity as described by Dorrance and Mills (2010).

Percent insect defoliation was assessed visually at the same time in the bottom, middle, and top third of the plant canopy by evaluating a minimum of five trifoliate leaves from the middle and top third canopy sections to calculate an average percent leaf area affected. Severity ratings were performed using an assessment scale as described by Ackley et al. (2010).

2.3.4 Statistical Analysis

Data were analyzed using the ANOVA and MIXED procedures of SAS at $\alpha = 0.05$ with treatment as the fixed effect and replication as the random effect (SAS Institute, Cary, NC; version 9.3). Mean separations were determined using single degree of freedom contrasts. To evaluate treatment effects, a factor removed from an enhanced production system was compared to the enhanced production system containing all factors, and conversely, a factor added onto the traditional production system was then compared to the traditional production system containing none of the treatment factors (Florence, 2012; Henninger, 2012). Each site-year was analyzed separately due to treatment by year interactions.

2.4 RESULTS AND DISCUSSION

2.4.1 Growing conditions

Average monthly temperatures for both years of this experiment generally were within two degrees of the thirty year average (Table 6). Changes in mean monthly precipitation from the thirty year average varied depending on site-year. In general, precipitation was above the thirty year average by up to 7.5 cm in June 2013, 16.9 cm in July 2013, and 7.7 cm June 2014. The 2013 Delaware and Erie sites experienced heavy rainfall on 9 July resulting in standing water for 24 hours.

2.4.2 *Rhizobia* inoculant

Omission of inoculant from the enhanced production and addition of inoculant to the traditional system did not result in any significant yield changes at all site-years (Tables 7 and 8). Lack of yield response from inoculant is likely attributed to all site-years being in a corn-soybean rotation for several years. These findings corresponded to other studies, where yield response due to inoculant was rare when soybean had been grown in a field within the previous five years (Abel and Erdman, 1964; Boonkerd et al., 1978; De Bruin et al., 2010; Ham et al., 1971; Muldoon et al., 1980; and Nelson et al., 1978).

2.4.3 Gypsum

Omission of gypsum from the enhanced production system did not result in any significant yield decrease and addition of gypsum to the traditional production system did not result in any significant yield increase (Tables 7 and 8). Since there was a single application of gypsum, yield gains would have likely been due to a S response. Ohio fertilizer guidelines recommend 2.1 to 4.0 g kg^{-1} of S in the uppermost fully developed trifoliate at the R1 growth stage (Vitosh et al., 1995). All of the trifoliates collected from the intensive measurement site-years were within this range (data not shown). For the remaining ten site-years, no visual symptoms of S deficiency were observed. Sulfur deficiency is most likely to occur on soils with a pH ≤5.5 and/or <10 mg kg^{-1} of organic matter (Barker et al., 2005). All locations were above those criteria, with the exception of the 2014 Clark site-year which had a pH of 5.5 (Table 3).

2.4.4 Fungicide

In 2013, omission of fungicide from the enhanced production system resulted in significant yield decreases of 0.21 to 0.79 Mg ha^{-1} at three of nine site-years (Table 7). Similarly, in 2014, omission of fungicide from the enhanced production system reduced yield by 0.36 and 0.71 Mg ha^{-1} at two of seven site-years (Table 8). Conversely, in 2014, the addition of pyraclostrobin fungicide to the traditional production system resulted in a yield increase of 0.47 Mg ha^{-1} at one of 16 site-years.

The enhanced production system may have resulted in more yield responses than the traditional production system due to tank-mixing the fungicide with the insecticide in the enhanced production system. Similar results have been found in other studies where application of pyrclostrobin fungicide alone did not result in any yield gains, but when pyraclostrobin was applied in a tank-mix with the insecticide lambda-cyhalothrin yield increases of 0.54 Mg ha^{-1} were observed (Hershman et al., 2004). A similar response was also observed in an Ohio study where in locations without aphid pressure, application of azoxystrobin fungicide alone increased yield at one of five locations by 0.28 Mg ha^{-1} and a tank-mix application of azoxystrobin fungicide and lambda-cyhalothrin insecticide increased yield at three of five locations from 0.34 to 0.40 Mg ha^{-1} (Dorrance et al., 2010).

Site-years with statistically significant yield changes in response to the fungicide input may have had greater disease pressure due to above normal rainfall in the growing season, specifically June and July (Table 6). Site-years where there was a yield response to fungicide application had rainfall that exceeded the 30-year average in June by 3.23 to 8.64 cm and in July by 3.30 to 14.02 cm. Brown leaf spot and frogeye leaf spot are favored by wet conditions and heavy rainfall, as rain splashes the fungus upward in the plant canopy, spreading the disease (Culman et al., 2014). Greater disease pressure created by wet growing conditions may have resulted in the yield responses from the fungicide input.

At the six site-years where disease levels were measured, brown leaf spot LAA ranged from 1.1% to 15.9% in the bottom third of the plant canopy, and 0% to 2.5% LAA in the middle third of the plant canopy (Table 9). Frogeye leaf spot LAA ranged from 0% to 0.9% in the middle third of the plant canopy, and 0% to 6.4% LAA in the top third of the plant canopy (Table 10). Percent LAA by brown leaf spot and frogeye leaf spot were similar between both years of the study, with the exception of 2013 Clinton observing the highest levels of brown leaf spot and 2013 Henry with the highest levels of frogeye leaf spot. Fungicide applications reduced disease pressure for both brown leaf spot and frogeye leaf spot. Fungicide significantly reduced brown leaf spot LAA for 29% of treatment comparisons and reduced frogeye leaf spot LAA for 58% of treatment comparisons, and these reductions were most commonly found where disease pressure was the highest. Brown leaf spot LAA reductions following fungicide application for statistically significant comparisons ranged from 0.3% to 1.6% in the middle third of the plant canopy and 2.9% to 11.2% in the bottom third of the plant canopy. Frogeye leaf spot LAA reductions following fungicide application for statistically significant comparisons ranged from 0.3% to 0.8% in the middle third of the plant canopy and 0.1% to 5.2% for the top third of the plant canopy. Although not measured intensively, all remaining site-years had some degree of brown leaf spot and frogeye leaf spot present.

Fungicide results in this study are consistent with other studies, where it has been documented brown leaf spot can cause yield losses of 0.20 to 0.67 Mg ha^{-1} in Ohio (Cruz, 2008; Cruz et al., 2010; Dorrance et al., 2010). Yield losses attributed to frogeye leaf spot of up to 35% on susceptible cultivars occurred in Ohio during 2005 (Cruz and Dorrance, 2009). Although the action threshold for frogeye leaf spot has not yet been established in Ohio (Dorrance et al., 2010), studies have found reduced yield from final disease severity levels. In a 2006 Ohio study, out of two locations with frogeye leaf spot disease present, one of the locations had a significant decrease in frogeye leaf spot disease pressure following application of pyraclostrobin from 2.4% to 0.5% and a significant increase in yield by 0.25 Mg ha^{-1} (Dorrance et al., 2010). In this study, reductions of frogeye leaf spot and/or brown leaf spot from pyraclostrobin application likely resulted in yield increases.

2.4.5 Insecticide

There was a significant reduction in yield of 0.32 Mg ha^{-1} due to omission of insecticide from the enhanced production system at one of 16 site-years. This was most likely due to a reduction in insect defoliation damage when the insecticide was applied. Limited yield response due to the insecticide input may be attributed to low insect pressure and low defoliation severity during the two years of the study. Soybean aphid was not present at any of the site-years, and there was minimal feeding from bean leaf beetle (*Cerotoma trifurcata* Forster). At the intensive measurement sites, trifoliate defoliation in the mid to upper canopy was less than 15% (data not shown). Soybean can tolerate defoliation rates between bloom and pod fill of up to 15%, and between pod fill and maturity defoliation levels of up to 25% without significant losses in yield; generally, an insecticide treatment is warranted when defoliation exceeds 25% LAA from beginning pod formation to pod fill (Ackley et al., 2010; Culman et al., 2014; Hadi et al., 2012). All site-years except for one were well below the defoliation thresholds as described by Ackley et al. (2010) and Culman et al., (2014).

2.4.6 Manganese foliar fertilizer

Omission of manganese foliar fertilizer from the enhanced production system was found to significantly reduce yield by 0.54 Mg ha^{-1} in 2014 at Sandusky County.

Although the leaf samples at the Sandusky County location were within the recommended sufficiency range of 21 to 100 mg kg^{-1} of Mn and did not exhibit visual deficiency symptoms (data not shown), the soil at this site-year had a large concentration of sand (630 mg kg^{-1}). Due to this high sand content, the soil was very likely dry and may have lost moisture easily. In dry soil, Mn is readily oxidized and so it is present in the soil in the plant unavailable form Mn^{4+} rather than the plant available form of Mn^{2+} (Hong et al., 2010). Additionally, that site-year had the lowest organic matter content, which may have affected Mn uptake (Barker et al., 2005).

2.4.7 Enhanced vs. traditional production system

Limited yield response occurred when the enhanced production system with all of the inputs was compared with the traditional production system having none of the inputs. In only two out of 16 site-years was there a significant yield increase in the enhanced system compared to the traditional system (Tables 7 and 8). At the 2013 Wood County location and at the 2014 Mercer County location, the enhanced production system yielded 5.5% and 10.8% higher, respectively, than the traditional production system. Limited yield effects were likely due to a general lack of input response. Generally, the field sites in both years were highly productive. All fields were in a corn-soybean rotation, most had low levels of insect and disease severity, and were within the range of Ohio soil fertility recommendations. All of those factors combined contributed to the lack of observed yield responses, since yield-limiting factors were minimal.

The enhanced production system had an estimated product and application cost of \$150 ha^{-1} (Table 2). At the 2013 United States average soybean grain price of \$517 Mg^{-1}, a yield benefit of at least 0.29 Mg ha^{-1} would be required to break-even with the product and application cost (NASS, 2014). While a yield benefit of at least 0.40 Mg ha^{-1} would be required to break-even with the product and application cost of the enhanced treatment at the 2014 United States average soybean grain price of \$375 Mg^{-1} (NASS, 2014). The 2013 and 2014 break-even economic thresholds were met at three out of 16 site-years (Delaware 2013, Mercer 2014, and Sandusky 2014) with an average yield increase of 0.45 Mg ha^{-1} compared to the traditional production system (Tables 4 and 5). At the other 13 site-years, the enhanced production system resulted in an average yield increase of 0.06 Mg ha^{-1} compared to the traditional system.

2.5 CONCLUSIONS

During 2013 and 2014, there were limited effects of inoculant, gypsum, insecticide, and Mn foliar fertilizer on grain yield of soybean in fields with established corn-soybean rotations, no Mn or S deficiencies, and reduced insect pressure. Pyraclostrobin fungicide was effective at reducing frogeye leaf spot and brown leaf spot disease severity. Omission of pyraclostrobin from an enhanced production system resulted in a decrease in yield at five of sixteen site-years, and addition of pyraclostrobin to a traditional production system resulted in a significant increase in yield at one of sixteen site-years. Excessive rainfall in June and July created optimum environments for development of brown leaf spot and frogeye leaf spot, and disease severity appeared to be the driving force in determination of yield response to fungicide. Therefore, findings suggest pyraclostrobin fungicide yield responses are likely attributed to reduction in disease severity and are most likely to be observed in high yielding systems. Crop scouting is a useful tool to identify fields where disease pressure levels are higher in order to determine when fungicide applications may be justified, as well as to scout for nutrient deficiencies and insect pressure to warrant the application of inoculant, gypsum, insecticide, and Mn foliar fertilizer.

2.6 ACKNOWLEDEMENTS

We would like to thank the Ohio Soybean Council for funding this study, as well as Monsanto® for donating the soybean seed. Salary and research support provided in part by state and federal funds appropriated to the Ohio Agricultural Research and Development Center (OARDC) and The Ohio State University, manuscript number HCS05-02. Thanks are extended for field assistance from JD Bethel, Aaron Brooker, Matthew Hankinson, Chris Kroon Van Diest, Alex Lindsey, John McCormick, Sin Joe Ng, and Stephanie Verhoff, along with OARDC staff. Also, thank you to the farmer-cooperators who participated in this study.

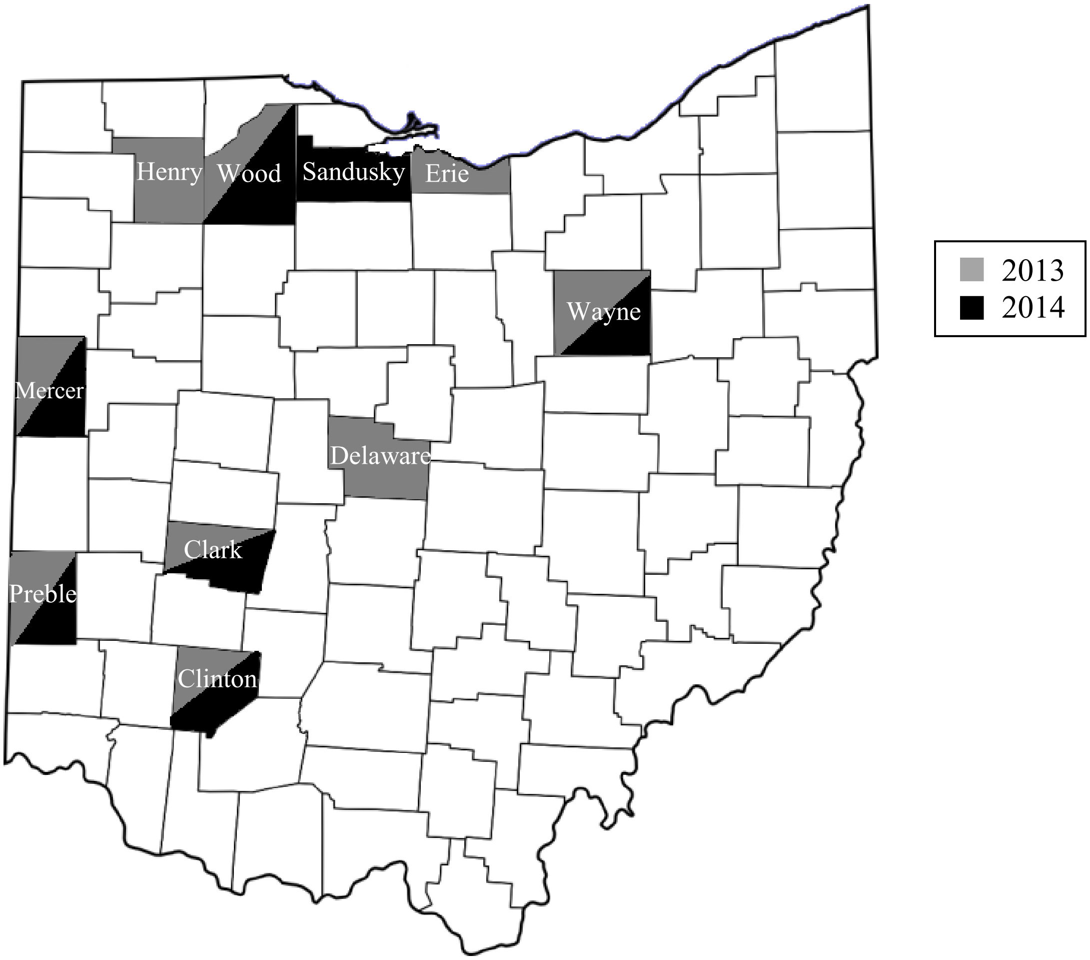

Figure 5. Ohio counties sites were located in 2013 and 2014. Intensive measurement sites for detailed leaf nutrient analysis of manganese (Mn) and sulfur (S) and pest ratings including percent leaf area affected by foliar disease and insect defoliation were in 2013 Clinton, Delaware, and Henry and in 2014 were Clinton, Preble, and Sandusky.

Table 3. Trial locations by site (Ohio county name) with soil chemical and physical properties including organic matter (OM), available phosphorus (P), exchangeable potassium (K), magnesium content (Mg), calcium content (Ca), soil pH, cation exchange capacity (CEC), and sand, silt, and clay content.

Year	Site	OM	P†	K	Mg	Ca	pH	CEC	Sand	Silt	Clay
		g kg^{-1}	----------------mg kg^{-1}----------------					cmol$_c$ kg^{-1}	-----------------g kg^{-1}-----------------		
2013	Clark	40	50	135	575	2050	6.2	19.0	220	420	360
2013	Clinton	29	41	148	350	1400	6.5	11.5	240	440	320
2013	Delaware	27	32	159	265	1200	6.3	9.8	260	400	340
2013	Erie	51	30	157	260	1900	6.3	14.5	420	320	260
2013	Henry	45	25	133	390	2500	6.7	17.3	260	280	460
2013	Mercer	33	40	147	370	1450	6.5	11.9	240	400	360
2013	Preble	34	17	115	320	1250	6.2	11.6	260	440	300
2013	Wayne	20	20	68	220	900	6.2	7.7	160	600	240
2013	Wood	40	27	175	375	2100	6.6	15.3	260	280	460
2014	Clark	27	19	110	295	1650	5.5	15.9	170	510	320
2014	Clinton	21	25	122	235	1100	6.3	9.0	210	530	260
2014	Mercer	32	53	163	415	2100	6.8	14.9	170	510	320
2014	Preble	37	91	199	415	1950	5.8	18.6	290	330	380
2014	Sandusky	20	28	91	150	850	6.1	7.0	630	190	180
2014	Wayne	22	28	121	191	942	6.5	6.6	330	520	150
2014	Wood	35	30	174	470	2400	6.7	17.7	260	440	300

† Mehlich-3 extractant was used and reported results were converted to Bray-1 extractant to correspond with state soil fertility recommendations. Potassium, Mg, and Ca were extracted using Mehlich-3 and reported results were converted to ammonium acetate extraction to correspond with state soil fertility recommendations.

Table 4. Omission trial design, treatment names, and list of inputs applied in 2013 and 2014.

Trt #	Treatment name	Inputs				
		Inoculant†	**Gypsum‡**	**Fungicide∫**	**Insecticide§**	**Mn^{2+}ℓ**
1	Enhanced (E)	Yes	Yes	Yes	Yes	Yes
2	E – inoculant	No	Yes	Yes	Yes	Yes
3	E – gypsum	Yes	No	Yes	Yes	Yes
4	E – fungicide	Yes	Yes	No	Yes	Yes
5	E – insecticide	Yes	Yes	Yes	No	Yes
6	E – Mn	Yes	Yes	Yes	Yes	No
7	Traditional (T)	No	No	No	No	No
8	T + inoculant	Yes	No	No	No	No
9	T + gypsum	No	Yes	No	No	No
10	T + fungicide	No	No	Yes	No	No
11	T + insecticide	No	No	No	Yes	No
12	T + Mn	No	No	No	No	Yes

†*Bradyrhizobia japonicum* inoculant applied at *Bradyrhizobia japonicum* active ingredient rate of 0.06 g kg^{-1} of seed within sixty days before planting.

‡Pelletized gypsum applied at a rate of 4.47 Mg ha^{-1} at VC growth stage.

∫ Pyraclostrobin fungicide applied at active ingredient rate of 103.49 ml ha^{-1} at R3 growth stage.

§ Lambda-cyhalothrin insecticide applied at active ingredient rate of 26.65 ml ha^{-1} at R3 growth stage.

ℓ Mn^{2+} foliar fertilizer applied at active ingredient rate of 0.23 L ha^{-1} at R3 growth stage.

Table 5. Dates field activities were performed including planting date, gypsum application date, chemical application date at R3 of the fungicide, insecticide, and manganese (Mn) foliar fertilizer, and harvest date for all site-years.

Year	Site	Planting	Gypsum	Chemical	Harvest
		-------------------------------date	--------------------------------		
2013	Clark	21 May	29 May	30 July	11 Oct.
2013	Clinton	22 May	3 June	29 July	27 Oct.
2013	Delaware	20 May	5 June	5 Aug.	28 Oct
2013	Erie	29 May	11 June	5 Aug.	30 Oct.
2013	Henry	16 May	31 May	30 July	14 Oct.
2013	Mercer	17 May	29 May	31 July	15 Oct.
2013	Preble	15 May	24 May	30 July	21 Oct.
2013	Wayne	21 May	11 June	2 Aug.	5 Nov.
2013	Wood	16 May	4 June	1 Aug.	2 Oct.
2014	Clark	31 May	19 June	5 Aug.	4 Nov.
2014	Clinton	28 May	10 June	5 Aug.	30 Oct.
2014	Mercer	25 May	9 June	4 Aug.	25 Oct.
2014	Preble	22 May	29 May	4 Aug.	3 Nov.
2014	Sandusky	30 May	18 June	6 Aug.	27 Oct.
2014	Wayne	3 June	29 June	7 Aug.	11 Nov.
2014	Wood	29 May	18 June	6 Aug.	10 Nov.

Table 6. Average monthly temperature and cumulative monthly precipitation for 2013 and 2014. Average temperature and precipitation shown for thirty year average (30 yr avg.) from 1981 to 2010. Deviation in the 30 yr avg. for temperature and precipitation from respective site and month shown for 2013 and 2014.

		Average temperature						Cumulative precipitation					
Site	**Year**	**May**	**June**	**July**	**Aug.**	**Sept.**	**Oct.**	**May**	**June**	**July**	**Aug.**	**Sept.**	**Oct.**
		°Celsius						cm					
Clark	30 yr avg.	15.78	21.00	22.83	22.00	18.00	11.44	11.89	10.64	11.63	8.46	7.98	7.04
	2013	+1.44	-0.22	-0.72	-1.22	+0.11	+0.28	-7.72	+1.63	+1.45	-3.45	+0.03	+4.72
	2014	+0.50	+0.28	-3.11	-0.72	-1.28	-0.17	+2.44	+7.21	-2.18	-4.09	-6.07	-2.67
Clinton	30 yr avg.	16.22	21.22	22.94	22.00	18.22	12.11	13.44	9.78	10.72	7.70	7.24	7.70
	2013	+1.67	+0.06	-0.33	-0.72	+0.39	+0.17	-4.85	+7.54	+8.48	-2.51	+1.14	+1.27
	2014	+0.78	+0.56	-2.33	-0.11	-0.44	+0.28	-2.13	+3.23	+1.60	-2.16	-1.45	+2.18
Delaware	30 yr avg.	16.22	21.22	23.39	22.50	18.44	12.00	11.20	11.40	11.10	8.61	7.42	6.35
	2013	+2.67	+1.28	+0.50	+0.72	+1.33	+1.61	-7.09	-0.08	+6.35	-1.37	-1.02	+8.66
Erie	30 yr avg.	15.06	20.39	22.56	21.67	17.83	11.44	9.65	10.69	9.91	9.25	8.53	7.01
	2013	+2.56	+0.61	+0.22	-0.56	+0.39	+0.44	-5.16	+3.73	+16.94	-2.74	-2.84	+1.70
Henry	30 yr avg.	15.44	21.06	23.17	22.06	18.11	11.39	10.06	9.25	10.39	7.67	8.26	7.49
	2013	+2.44	+0.22	-0.56	-0.67	+0.61	+0.61	-3.56	+5.99	+3.30	-4.04	-4.52	-1.63
Mercer	30 yr avg.	16.50	21.44	23.11	22.17	18.61	12.28	10.11	10.34	12.22	9.02	6.60	6.73
	2013	+2.00	-0.44	-0.83	-0.44	+0.67	+0.22	-5.11	+2.31	-4.19	-5.69	-0.18	-0.61
	2014	+0.72	+0.83	-2.39	-0.28	-1.00	-0.28	+2.51	+7.70	-3.71	-1.27	+0.30	-1.12
Preble	30 yr avg.	16.28	21.39	23.28	22.56	18.72	11.83	12.95	10.54	11.00	7.44	7.11	7.65
	2013	+1.22	-0.17	-1.06	-1.06	+0.50	-0.33	-2.90	+2.74	+4.55	-3.68	+0.33	+5.89
	2014	+0.44	+0.72	-2.78	-0.56	-1.00	-0.06	-3.56	+4.90	-1.35	+3.76	-2.74	-0.56

Continued

Table 6, continued

Sandusky	30 yr avg.	15.50	21.06	23.17	22.11	18.06	11.39	10.06	10.34	8.99	8.00	7.90	7.32
	2014	+0.72	+0.83	-2.06	-0.33	-0.17	+0.00	-4.39	+1.50	-2.67	-4.78	+4.93	-2.41
Wayne	30 yr avg.	15.28	20.17	22.11	20.78	17.00	10.94	9.32	9.58	10.87	8.59	8.53	7.06
	2013	+1.11	-0.39	-0.39	-1.28	-0.78	+0.67	-4.75	+6.05	+3.07	-1.85	-1.37	+0.79
	2014	+0.44	+0.72	-1.89	-0.28	-0.39	+0.83	+1.30	+6.10	-6.73	+2.18	-5.03	-1.19
Wood	30 yr avg.	16.28	21.56	23.56	22.56	18.56	12.00	10.52	10.62	9.93	9.25	6.88	6.76
	2013	+1.61	-0.28	-0.83	-1.28	-0.17	+0.28	-6.55	+1.12	+14.02	-1.02	-0.13	+4.17
	2014	+0.33	+0.44	-2.83	-0.44	-0.78	-1.72	-5.77	+1.04	-4.78	-2.74	+4.65	-5.44

Table 7. Soybean grain yield in 2013. Average yield shown for enhanced and traditional treatments. Changes in yield from respective enhanced or traditional system shown for all other treatments.

Treatment	**Site**								
	Clark	**Clinton**	**Delaware**	**Erie**	**Henry**	**Mercer**	**Preble**	**Wayne**	**Wood**
	------------------------	------------------------	------------------------	------------------------	Mg ha^{-1}	------------------------	------------------------	------------------------	------------------------
Enhanced (E)	4.72	5.00	3.20	2.48	4.07	3.75	4.33	4.03	4.21
E – inoculant†	-0.13	-0.24	-0.52	-0.36	-0.04	+0.29	+0.14	+0.08	-0.07
E – gypsum	+0.53*	-0.11	+0.46	+0.39	+0.06	+0.06	+0.16	-0.04	-0.13
E – fungicide	-0.12	-0.79*	+0.13	+0.32	-0.35*	+0.05	+0.39	-0.11	-0.21*
E – insecticide	+0.05	-0.27	-0.55	-0.20	-0.01	+0.50*	+0.24	-0.01	-0.14
E –Mn	+0.03	+0.13	-0.28	+0.01	-0.05	+0.17	+0.25	+0.06	-0.16
Traditional (T)	4.78	4.76	2.72	2.57	3.88	3.92	4.58	3.87	3.98
T + inoculant‡	-0.47	+0.06	-0.35	-0.32	+0.08	-0.14	-0.02	+0.11	-0.01
T + gypsum	-0.08	-0.57*	+0.21	-0.41	-0.23	-0.26	+0.12	+0.10	+0.00
T + fungicide	+0.10	-0.14	+1.01	+0.43	+0.19	+0.07	-0.27	+0.16	+0.07
T + insecticide	-0.50	+0.00	-0.01	+0.37	-0.06	-0.14	+0.01	-0.09	+0.08
T + Mn	+0.19	+0.08	-0.21	+0.20	-0.09	-0.37	+0.06	-0.19	-0.01
E vs T	ns	ns	ns	ns	ns	ns	ns	ns	*

† Yield values in 'E minus input' rows signify a change in yield (Mg ha^{-1}) from the respective 'Enhanced (E)' treatment.

‡ Yield values in 'T plus input' rows signify a change in yield (Mg ha^{-1}) from the respective 'Traditional (T)' treatment.

* Significantly different at α = 0.05 using single degree of freedom contrasts.

Table 8. Soybean grain yield in 2014. Average yield shown for enhanced and traditional treatments. Changes in yield from respective enhanced or traditional system shown for all other treatments.

	Site						
	Clark	**Clinton**	**Mercer**	**Preble**	**Sandusky**	**Wayne**	**Wood**
Treatment				----Mg ha^{-1}----			
Enhanced (E)	3.51	4.57	4.06	5.50	4.16	4.13	3.42
E – inoculant†	-0.04	-0.04	-0.03	-0.11	-0.09	-0.13	+0.26
E – gypsum	-0.02	+0.21	+0.05	-0.16	-0.21	+0.10	+0.03
E – fungicide	+0.03	-0.71*	-0.21	-0.09	-0.36	-0.36*	+0.09
E – insecticide	+0.12	-0.33	-0.22	-0.04	-0.46	-0.32*	-0.01
E –Mn	-0.03	+0.16	-0.06	+0.04	-0.54*	-0.06	-0.01
Traditional (T)	3.49	4.32	3.62	5.55	3.73	4.09	3.21
T + inoculant‡	+0.01	+0.06	+0.30	-0.19	+0.30	-0.27	-0.20
T + gypsum	+0.08	-0.26	+0.03	-0.50*	+0.02	-0.31*	-0.06
T + fungicide	+0.08	+0.22	+0.47*	+0.13	+0.25	-0.05	+0.22
T + insecticide	+0.07	+0.06	+0.24	+0.05	-0.07	-0.30	+0.25
T + Mn	+0.05	+0.14	+0.05	-0.19	+0.01	-0.24	+0.13
E vs T	ns	ns	*	ns	ns	ns	ns

† Yield values in ‘E minus input’ rows signify a change in yield (Mg ha^{-1}) from the respective ‘Enhanced (E)’ treatment.

‡ Yield values in ‘T plus input’ rows signify a change in yield (Mg ha^{-1}) from the respective ‘Traditional (T)’ treatment.

* Significantly different at α = 0.05 using single degree of freedom contrasts.

Table 9. Percent leaf area affected by *Septoria glycines* (brown leaf spot) in the middle and bottom third of the plant canopy four weeks after fungicide application at R6 in 2013 and 2014. Average percent leaf area affected shown for enhanced and traditional treatments. Changes in percent leaf area affected from respective enhanced or traditional system shown for all other treatments.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – fungicide**	**Δ**	**Traditional (T)**	**T + fungicide**	**Δ**	**E vs. T†**
		------------------------------------% leaf area affected in middle third canopy------------------------------------						
2013	Clinton	0.9	2.5	+1.6*	1.4	0.3	-1.1	ns
	Delaware	0.2	0.7	+0.5	1.5	0.0	-1.5*	*
	Henry	0.3	0.5	+0.2	0.4	0.3	-0.1	ns
2014	Clinton	0.2	0.1	-0.1	0.4	0.1	-0.3*	*
	Preble	0.2	0.2	0.0	0.3	0.2	-0.1	ns
	Sandusky	0.1	0.1	0.0	0.1	0.03	-0.07	ns
		------------------------------------% leaf area affected in bottom third canopy------------------------------------						
2013	Clinton	4.7	15.9	+11.2*	12.4	4.5	-7.9*	*
	Delaware	2.5	4.2	+1.7	1.9	2.2	+0.3	ns
	Henry	3.9	3.9	0.0	4.5	3.8	-0.7	ns
2014	Clinton	3.3	2.5	-0.8	3.4	3.3	-0.1	ns
	Preble	1.4	1.5	+0.1	1.9	1.1	-0.8	ns
	Sandusky	3.1	6.0	+2.9*	6.5	3.5	-3.0*	*

Δ Change in percent leaf area affected between 'enhanced – fungicide' and 'enhanced' treatments or the change between 'traditional + fungicide' and 'traditional' treatments.

† Single degree of freedom contrast used to compare 'enhanced' treatment to 'traditional' treatment.

*Significantly different at $P \leq 0.05$ using single degree of freedom contrasts comparing 'enhanced – fungicide' to the 'enhanced' treatment or 'traditional + fungicide' to the 'traditional' treatment or 'enhanced' to the 'traditional' treatment.

Table 10. Percent leaf area affected by *Cercospora sojina* (frogeye leaf spot) in the top and middle third of the plant canopy four weeks after fungicide application at R6 in 2013 and 2014. Average percent leaf area affected shown for enhanced and traditional treatments. Changes in percent leaf area affected from respective enhanced or traditional system shown for all other treatments.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – fungicide**	**Δ**	**Traditional (T)**	**T + fungicide**	**Δ**	**E vs. T†**
		----------------------------------% leaf area affected in top third canopy----------------------------------						
2013	Clinton	0.2	1.9	+1.7*	1.6	0.1	-1.5	ns
	Delaware	0.3	0.9	+0.6*	0.7	0.2	-0.5*	ns
	Henry	0.3	3.4	+3.1*	6.4	1.2	-5.2*	*
2014	Clinton	0.1	0.5	+0.4	0.4	0.1	-0.3	ns
	Preble	0.0	0.1	+0.1*	0.2	0.0	-0.2*	*
	Sandusky	0.1	0.5	+0.4*	0.5	0.1	-0.4*	*
		----------------------------------% leaf area affected in middle third canopy----------------------------------						
2013	Clinton	0.0	0.1	+0.1	0.4	0	-0.4*	*
	Delaware	0.1	0.9	+0.8*	0.4	0.1	-0.3	ns
	Henry	0.1	0.4	+0.3	0.9	0.1	-0.8*	*
2014	Clinton	0.1	0.2	+0.1	0.2	0.2	0.0	ns
	Preble	0.5	0.7	+0.2	0.7	0.6	-0.1	ns
	Sandusky	0.01	0.3	+0.29*	0.5	0.1	-0.4*	*

Δ Change in percent leaf area affected between 'enhanced – fungicide' and 'enhanced' treatments or the change between 'traditional + fungicide' and 'traditional' treatments.

† Single degree of freedom contrast used to compare 'enhanced' treatment to 'traditional' treatment.

*Significantly different at $P \leq 0.05$ using single degree of freedom contrasts comparing 'enhanced – fungicide' to the 'enhanced' treatment or 'traditional + fungicide' to the 'traditional' treatment or 'enhanced' to the 'traditional' treatment.

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Appendix A: Indexed chlorophyll content for inoculant treatments

Appendix A: Indexed chlorophyll content for inoculant treatments

Indexed chlorophyll content from uppermost, fully unrolled trifoliate in 2013 and 2014 at R1, R4, and R6 growth stages.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – inoculant**	**Δ**	**Traditional (T)**	**T + inoculant**	**Δ**	**E vs.T†**
		R1						
2013	Clinton	-‡	-	-	32.1	33.5	+1.4	-
	Delaware	27.6	28.3	+0.7	28.6	29.3	+0.7	ns
	Henry	39.1	40.4	+1.3	41.9	39.0	-2.9*	*
2014	Clinton	33.9	32.8	-1.1	35.1	34.6	-0.5	ns
	Preble	39.8	39.6	-0.2	39.8	39.5	-0.3	ns
		R4						
2013	Clinton	48.9	48.1	-0.8	48.8	49.0	+0.2	ns
	Delaware	42.1	41.8	-0.3	40.9	41.1	+0.2	ns
	Henry	43.7	45.1	+1.4	43.7	43.6	-0.1	ns
2014	Clinton	50.4	49.9	-0.5	49.4	49.3	-0.1	ns
	Preble	48.5	48.7	+0.2	47.9	48.0	+0.1	ns
	Sandusky	44.1	43.4	-0.7	43.6	43.7	+0.1	ns
		R6						
2013	Clinton	47.9	47.4	-0.5	47.1	47.6	+0.5	ns
	Delaware	48.3	48.8	+0.5	48.9	48.7	-0.2	ns
	Henry	46.4	47.1	+0.7	46.3	46.2	-0.1	ns
2014	Clinton	42.4	41.7	-0.7	43.2	44.5	+1.3	ns
	Preble	48.3	47.9	-0.4	44.9	44.6	-0.3	ns
	Sandusky	47.1	45.8	-1.3	46.9	46.4	-0.5	ns

Continued

Appendix A, continued

Δ Change in indexed chlorophyll content between ‘Enhanced (E)’ and ‘E – inoculant’ treatments or the change between ‘Traditional (T)’ and ‘(T + inoculant)’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment.

‡ Data unavailable.

[*] Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – inoculant’ treatments or ‘Traditional (T)’ and ‘(T + inoculant)’ treatments, or Enhanced and Traditional treatments.

Appendix B. Indexed chlorophyll content for gypsum treatments

Appendix B. Indexed chlorophyll content for gypsum treatments

Indexed chlorophyll content from uppermost, fully unrolled trifoliate in 2013 and 2014 at R1, R4, and R6 growth stages.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – gypsum**	**Δ**	**Traditional (T)**	**T + gypsum**	**Δ**	**E vs. T†**
		----------R1----------						
2013	Clinton	-‡	-	-	32.1	-	-	-
	Delaware	27.6	28.8	+1.2	28.6	27.2	-1.4	ns
	Henry	39.1	-	-	41.9	-	-	*
2014	Clinton	33.9	34.3	+0.4	35.1	33.1	-2.0	ns
	Preble	39.8	38.8	-1.0	39.8	39.9	+0.1	ns
		----------R4----------						
2013	Clinton	48.9	48.7	-0.2	48.8	47.9	-0.9	ns
	Delaware	42.1	41.4	-0.7	40.9	42.5	+1.6	ns
	Henry	43.7	44.4	+0.7	43.7	44.5	+0.8	ns
2014	Clinton	50.4	49.6	-0.8	49.4	50.3	+0.9	ns
	Preble	48.5	46.3	-2.2*	47.9	47.3	-0.6	ns
	Sandusky	44.1	43.4	-0.7	43.6	43.9	+0.3	ns
		----------R6----------						
2013	Clinton	47.9	47.4	-0.5	47.1	46.4	-0.7	ns
	Delaware	48.3	48.7	+0.4	48.9	48.7	-0.2	ns
	Henry	46.4	46.3	-0.1	46.3	47.3	+1.0*	ns
2014	Clinton	42.4	43.4	+1.0	43.2	42.3	-0.9	ns
	Preble	48.3	46.1	-2.2	44.9	46.1	+1.2	ns
	Sandusky	47.1	47.1	0.0	46.9	47.5	+0.6	ns

Continued

Appendix B, continued

Δ Change in indexed chlorophyll content between ‘Enhanced (E)’ and ‘E – gypsum’ treatments or the change between ‘Traditional (T)’ and ‘T + gypsum’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment.

‡ Data unavailable.

[*] Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – gypsum’ treatments or ‘Traditional (T)’ and ‘T + gypsum’ treatments, or Enhanced and Traditional treatments.

Appendix C: Protein content 2013

Appendix C: Protein content 2013

Protein content from all sites in 2013. Average protein content shown for enhanced and traditional treatments. Changes in protein content from respective enhanced or traditional system shown for all other treatments.

Treatment	**Site**								
	Clark	**Clinton**	**Delaware**	**Erie**	**Henry**	**Mercer**	**Preble**	**Wayne**	**Wood**
	g kg^{-1}								
Enhanced (E)	349	346	349	353	356	346	343	354	345
E – inoculant†	+2	-1	+1	-2	-3	0	-1	+2	0
E – gypsum	+4	-1	0	+1	0	+2	0	+1	-1
E – fungicide	+3	+3	-5*	+4*	-2	-3	+1	+5*	+3
E – insecticide	+2	-3	0	-1	-1	-2	-1	-1	+2
E –Mn	0	-4	+3	-1	0	-4	0	0	+2
Traditional (T)	350	347	356	352	358	347	344	355	347
T + inoculant‡	+1	+2	0	0	+1	+1	-2	0	-1
T + gypsum	+1	0	0	+2	-1	-3	0	+4	0
T + fungicide	+1	-1	-8*	+3	-7*	-4	-3	-4	-4
T + insecticide	+1	0	-1	+1	-2	-6*	-1	+1	-1
T + Mn	-4	-2	-2	+2	-2	+2	-1	-1	-2
E vs T	ns	ns	*	ns	ns	ns	ns	ns	ns

† Protein values in ‘E minus input’ rows signify a change in protein from the respective ‘Enhanced (E)’ treatment.

‡ Protein values in ‘T plus input’ rows signify a change in protein from the respective ‘Traditional (T)’ treatment.

* Significantly different at α = 0.05 using single degree of freedom contrasts.

Appendix D: Protein content 2014

Appendix D: Protein content 2014

Protein content from all sites in 2014. Average protein content shown for enhanced and traditional treatments. Changes in protein content from respective enhanced or traditional system shown for all other treatments.

	Site						
	Clark	**Clinton**	**Mercer**	**Preble**	**Sandusky**	**Wayne**	**Wood**
Treatment	--g kg^{-1}						--
Enhanced (E)	351	356	348	359	354	360	342
E – inoculant†	+2	-1	-1	-2	-2	0	-2
E – gypsum	0	-2	0	-3	-1	-1	+2
E – fungicide	-2	-3	+1	-1	0	-2	-2
E – insecticide	-2	-2	-4	-1	+1	-2	-1
E –Mn	-1	-6	0	-1	-3	-2	-1
Traditional (T)	349	355	350	360	355	359	341
T + inoculant‡	+2	-1	-1	-2	+2	0	+3
T + gypsum	0	+2	-2	0	-2	-2	0
T + fungicide	0	-2	-3	-2	+2	-1	+1
T + insecticide	0	+2	+1	0	0	-2	+1
T + Mn	+1	0	-2	+1	+1	-2	+1
E vs T	ns	ns	ns	ns	ns	ns	ns

† Protein values in ‘E minus input’ rows signify a change in protein from the respective ‘Enhanced (E)’ treatment.

‡ Protein values in ‘T plus input’ rows signify a change in protein from the respective ‘Traditional (T)’ treatment.

* Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts.

Appendix E: Oil content 2013

Appendix E: Oil content 2013

Oil content from all sites in 2013. Average oil content shown for enhanced and traditional treatments. Changes in oil content from respective enhanced or traditional system shown for all other treatments.

Treatment	**Site**								
	Clark	**Clinton**	**Delaware**	**Erie**	**Henry**	**Mercer**	**Preble**	**Wayne**	**Wood**
					----g kg^{-1}----				
Enhanced (E)	183	185	182	175	177	183	186	179	181
E – inoculant†	-3*	0	0	+1	+1	+1	0	-1	0
E – gypsum	-2	0	0	0	0	0	+1	-1	+1
E – fungicide	-4*	-2	-2	-1	+2*	+1	0	-1	-1
E – insecticide	0	+1	0	+1	+1	+2	0	0	-1
E –Mn	-1	+1	-1	+1	+1	+1	+1	+1	0
Traditional (T)	182	184	180	177	178	183	187	179	180
T + inoculant‡	0	0	+1	0	-2	0	+1	0	+1
T + gypsum	-1	0	-1	-2	-1	+1	0	0	+1
T + fungicide	-1	0	+2*	-2	+1	+1	+1	+1*	+2
T + insecticide	0	0	0	-1	0	+1	-1	0	0
T + Mn	+1	+1	0	-1	-1	0	+1	0	+2
E vs T	ns	ns	ns	ns	ns	ns	ns	ns	ns

† Oil values in ‘E minus input’ rows signify a change in oil from the respective ‘Enhanced (E)’ treatment.

‡ Oil values in ‘T plus input’ rows signify a change in oil from the respective ‘Traditional (T)’ treatment.

* Significantly different at α = 0.05 using single degree of freedom contrasts.

Appendix F: Oil content 2014

Appendix F: Oil content 2014

Oil content from all sites in 2014. Average oil content shown for enhanced and traditional treatments. Changes in oil content from respective enhanced or traditional system shown for all other treatments.

	Site						
	Clark	**Clinton**	**Mercer**	**Preble**	**Sandusky**	**Wayne**	**Wood**
Treatment	g kg^{-1}						
Enhanced (E)	174	179	177	176	176	167	180
E – inoculant†	-1	0	+2	+2	0	0	+1
E – gypsum	-1	-1	0	+1	0	+1	-1
E – fungicide	+2	0	0	0	0	0	+2
E – insecticide	0	0	+3	0	-1	0	+1
E –Mn	0	+2	+2	0	+2	0	+1
Traditional (T)	174	180	177	175	177	168	180
T + inoculant‡	-1	+2	+1	+2	-2	0	+1
T + gypsum	+2	-1	+1	+3	+1	0	+1
T + fungicide	0	-2	+2	+1	-2	-1	0
T + insecticide	+1	-3	0	+1	-1	0	0
T + Mn	0	-2	+1	0	-1	+1	0
E vs T	ns	ns	ns	ns	ns	ns	ns

† Oil values in ‘E minus input’ rows signify a change in oil from the respective ‘Enhanced (E)’ treatment.

‡ Oil values in ‘T plus input’ rows signify a change in oil from the respective ‘Traditional (T)’ treatment.

* Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts.

Appendix G: Yield components: pods per plant

Appendix G: Yield components: pods per plant

Pods per plant from all yield component collection sites in 2013 and 2014. Average number of pods per plant shown for enhanced and traditional treatments. Changes in pods per plant from respective enhanced or traditional system shown for all other treatments.

	2013			**2014**		
Treatment	**Clinton**	**Delaware**	**Henry**	**Clinton**	**Preble**	**Sandusky**
	--pods plant^{-1}--					
Enhanced (E)	32.4	32.5	28.3	27.3	30.3	28.6
E – inoculant†	+6.6	-4.5	+3.7	+2.2	+1.9	-0.9
E – gypsum	+7.7	+2.4	+0.9	+3.7	-0.6	0.0
E – fungicide	0.0	+0.2	+8.7*	-0.2	+0.4	+4.3
E – insecticide	+5.9	-1.4	+6.4	+0.2	-1.1	+0.2
E –Mn	0.0	-0.9	+11.2*	-1.0	-1.6	+0.4
Traditional (T)	40.8	28.1	35.2	29.5	31.1	27.7
T + inoculant‡	-5.0	-4.1	-6.5	-2.4	-3.7	+3.1
T + gypsum	-1.4	+8.8*	-3.8	+0.3	-2.7	-0.5
T + fungicide	-1.1	+8.7*	-3.6	-2.8	+1.7	-1.0
T + insecticide	-0.9	+2.5	-3.2	-1.2	-4.3	0.0
T + Mn	-2.4	+1.6	-3.6	+0.6	+0.8	0.0
E vs T	ns	ns	ns	ns	ns	ns

† Pods plant^{-1} in 'E minus input' rows signify a change in pods plant^{-1} from the respective 'Enhanced (E)' treatment.

‡ Pods plant^{-1} in 'T plus input' rows signify a change in pods plant^{-1} from the respective 'Traditional (T)' treatment.

* Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts.

Appendix H: Yield components: seeds per pod

Appendix H: Yield components: seeds per pod

Seeds per pod from all yield component collection sites in 2013 and 2014. Average number of seeds per pod shown for enhanced and traditional treatments. Changes in seeds per pod from respective enhanced or traditional system shown for all other treatments.

	2013			2014		
Treatment	**Clinton**	**Delaware**	**Henry**	**Clinton**	**Preble**	**Sandusky**
	----------Seeds pod^{-1}----------					
Enhanced (E)	2.59	2.39	2.63	2.7	2.80	2.7
E – inoculant†	-0.13	-0.02	-0.06	+0.1	0.0	0.0
E – gypsum	+0.02	+0.06	-0.11	+0.1	-0.1	+0.1
E – fungicide	-0.07	+0.01	-0.14	+0.2	+0.1	0.0
E – insecticide	-0.03	-0.09	-0.07	0.0	+0.1	+0.1
E –Mn	-0.04	+0.05	-0.13	+0.1	+0.1	+0.1
Traditional (T)	2.57	2.25	2.45	2.8	2.8	2.7
T + inoculant‡	-0.07	+0.07	+0.04	0.0	0.0	0.0
T + gypsum	-0.09	+0.19*	+0.03	+0.1	0.0	0.0
T + fungicide	-0.15	+0.08	+0.08	0.0	0.0	-0.1
T + insecticide	-0.07	+0.18*	+0.02	0.0	+0.1	0.0
T + Mn	-0.06	+0.13	+0.04	-0.1	0.0	0.0
E vs T	ns	ns	ns	ns	ns	ns

† Seeds pod^{-1} in ‘E minus input’ rows signify a change in seeds pod^{-1} from the respective ‘Enhanced (E)’ treatment.

‡ Seeds pod^{-1} in ‘T plus input’ rows signify a change in seeds pod^{-1} from the respective ‘Traditional (T)’ treatment.

* Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts.

Appendix I: Yield components: fifty seed weight

Appendix I: Yield components: fifty seed weight

Fifty seed weight from all yield component collection sites in 2013 and 2014. Average fifty seed weight shown for enhanced and traditional treatments. Changes in fifty seed weight from respective enhanced or traditional system shown for all other treatments.

	2013			2014		
Treatment	Clinton	Delaware	Henry	Clinton	Preble	Sandusky
	---fifty seed weight (g)			---		
Enhanced (E)	7.9	8.1	8.4	11.8	10.7	10.4
E – inoculant	+0.2	+0.6*	+0.1	-0.6	-0.1	-0.1
E – fungicide	-0.4	-0.8*	-0.2	-1.3*	-0.2	-0.4
E – insecticide	-0.1	+0.3	+0.1	0.0	+0.3	-0.1
E –Mn	+0.2	+0.3	-0.3	-0.6	0.0	0.0
E – gypsum	+0.4	+0.2	+0.2	0.0	-0.1	0.0
Traditional (T)	7.6	7.6	8.3	11.4	10.5	10.0
T + inoculant	+0.4	-0.2	-0.3	+0.5	0.0	0.0
T + fungicide	+0.7*	+0.5	+0.4	+0.4	+0.3	+0.1
T + insecticide	0	-0.2	-0.3	-0.3	-0.5	0.0
T + Mn	-0.3	-0.2	-0.1	+0.2	-0.4	-0.2
T + gypsum	-0.5	+0.3	-0.2	-0.6	+0.4	-0.2
E vs T	ns	ns	ns	ns	ns	ns

† Fifty seed weight in ‘E minus input’ rows signify a change in fifty seed weight from the respective ‘Enhanced (E)’ treatment.

‡ Fifty seed weight in ‘T plus input’ rows signify a change in fifty seed weight from the respective ‘Traditional (T)’ treatment.

* Significantly different at α = 0.05 using single degree of freedom contrasts.

Appendix J: Brown leaf spot ratings at R4

Appendix J: Brown leaf spot ratings at R4

Percent leaf area affected by brown leaf spot (*Septoria glycines*) in the middle and bottom third of the plant canopy two weeks after fungicide application at R4 in 2013 and 2014.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – fungicide**	**Δ**	**Traditional (T)**	**T + fungicide**	**Δ**	**E vs. T†**
		-----------------------------------% leaf area affected in middle third canopy-----------------------------------						
2013	Clinton	3.5	2.3	-1.2	1.5	1.9	+0.4	ns
	Delaware	0.1	0.6	+0.5*	0.2	0.1	-0.1	ns
	Henry	0.9	0.7	-0.2	1.2	0.5	-0.7	ns
2014	Clinton	1.1	0.5	-0.6	0.6	0.6	0.0	ns
	Preble	0.5	0.6	+0.1	0.5	0.6	+0.1	ns
	Sandusky	1.1	0.5	-0.6*	0.5	0.1	-0.4*	*
		-----------------------------------% leaf area affected in bottom third canopy-----------------------------------						
2013	Clinton	8.3	14.8	+6.5*	13.2	12.7	-0.5	ns
	Delaware	9.5	10.6	+1.1	16.9	10.1	-6.8*	*
	Henry	4.3	6.4	+2.1	4.1	2.9	-1.2	ns
2014	Clinton	13.0	13.2	+0.2	12.6	13.1	+0.5	ns
	Preble	1.1	0.8	-0.3	1.2	0.8	-0.4	ns
	Sandusky	1.5	1.8	+0.3	1.9	0.6	-1.3	ns

Δ Change in percent leaf area affected between ‘Enhanced (E)’ and ‘E – fungicide’ treatments or the change between ‘Traditional (T) and ‘T + fungicide’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – fungicide’ treatments or ‘Traditional (T)’ to ‘T + fungicide’ treatments or ‘Enhanced’ and ‘Traditional’ treatments.

Appendix K: Fluorescence quenching for fungicide treatments

Appendix K: Fluorescence quenching for fungicide treatments

Fluorescence quenching (Fv’ Fm’$^{-1}$) from uppermost fully unrolled trifoliate two weeks before fungicide application (2WBA) at R1, two weeks after fungicide application (2WAA) at R4, and four weeks after fungicide application (4WAA) at R6 in 2013 and 2014

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – fungicide**	**Δ**	**Traditional (T)**	**T + fungicide**	**Δ**	**E vs. T†**
		----------------------	----------------------	----------------------Fv’ Fm’$^{-1}$ 2WBA	----------------------	----------------------	----------------------	----------------------
2013	Clinton	0.7258	0.7243	-0.0015	0.7320	0.7275	-0.0045	ns
	Delaware	0.7122	0.7112	-0.0010	0.7253	0.7077	-0.0176	ns
	Henry	0.7035	0.7048	+0.0013	0.6970	0.7005	+0.0035	ns
2014	Clinton	0.6967	0.7067	+0.0100	0.6958	0.6666	-0.0292	ns
	Preble	0.7121	0.6981	-0.0140	0.7209	0.7225	+0.0016	ns
		----------------------	----------------------	---------------------- Fv’ Fm’$^{-1}$ 2WAA	----------------------	----------------------	----------------------	----------------------
2013	Clinton	0.7212	0.7255	+0.0043	0.7172	0.7242	+0.0070	ns
	Delaware	0.7405	0.7490	+0.0085	0.7485	0.7533	+0.0048	ns
	Henry	0.7060	0.6790	-0.0270*	0.6773	0.6902	+0.0129	*
2014	Clinton	0.7397	0.7423	+0.0026	0.7335	0.7429	+0.0094	ns
	Preble	0.7460	0.7469	+0.0009	0.7455	0.7486	+0.0031	ns
	Sandusky	0.7114	0.7155	+0.0041	0.7186	0.7162	-0.0024	ns
		----------------------	----------------------	---------------------- Fv’ Fm’$^{-1}$ 4WAA	----------------------	----------------------	----------------------	----------------------
2013	Clinton	0.7167	0.7175	+0.0008	0.7122	0.7127	+0.0005	ns
	Delaware	0.7175	0.7117	-0.0058	0.7217	0.7278	+0.0061	ns
	Henry	0.7083	0.7210	+0.0127	0.7218	0.7145	-0.0073	ns
2014	Clinton	0.7309	0.7233	-0.0076	0.7446	0.7319	-0.0127	ns
	Preble	0.7197	0.7170	-0.0027	0.7143	0.7231	+0.0088	ns

Continued

Appendix K, continued

2014	Sandusky	0.7121	0.7262	+0.0141	0.7220	0.7202	-0.0018	ns

Δ Change in fluorescence ratio (Fv’ Fm’$^{-1}$) between ‘Enhanced (E)’ and ‘Enhanced – fungicide’ treatments or the change between ‘Traditional (T)’ and ‘Traditional + fungicide’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘Enhanced – fungicide’ treatments or ‘Traditional (T)’ and ‘Traditional + fungicide’ treatments or ‘Enhanced’ and ‘Traditional’ treatments.

Appendix L: Manganese concentration

Appendix L: Manganese concentration

Manganese concentration (mg kg^{-1}) of uppermost, fully developed unrolled trifoliate two weeks before manganese application (2WBA) at R1, two weeks after manganese application (2WAA) at R4, and four weeks after manganese application (4WAA) at R6 in 2013 and 2014.

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – manganese**	**Δ**	**Traditional (T)**	**T + manganese**	**Δ**	**E vs. T†**
		--mg kg^{-1} 2WBA--						
2013	Clinton	91	90	-1	105	103	-2	ns
	Delaware	49	44	-5	43	48	+5	ns
	Henry	69	63	-6	59	65	+6	ns
2014	Clinton	51	55	+4	51	50	-1	ns
	Preble	64	63	-1	67	56	-11	ns
		--mg kg^{-1} 2WAA--						
2013	Clinton	87	87	0	98	98	0	ns
	Delaware	62	50	-12	59	64	+5	ns
	Henry	69	56	-13*	59	61	+2	ns
2014	Clinton	83	77	-5	66	72	+6	ns
	Preble	157	106	-51*	81	131	+50*	*
	Sandusky	115	56	-59*	54	115	+61*	*
		--mg kg^{-1} 4WAA--						
2013	Clinton	87	97	+10	95	92	-3	ns
	Delaware	41	36	-5	44	63	+19	ns
	Henry	55	46	-9*	49	46	-3	ns
2014	Clinton	101	121	+20	96	76	-20	ns
	Preble	182	172	-10	125	115	-10	*

Continued

Appendix L, continued

2014	Sandusky	68	61	-7	57	71	+14	ns

Δ Change in manganese tissue concentration between ‘Enhanced (E) and ‘E – manganese’ treatments or the change between ‘Traditional (T) and ‘T + manganese’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment

*Significantly different at $\alpha = 0.05$ using single degree of freedom contrasts comparing ‘Enhanced (E) and ‘E – manganese’ treatments or ‘Traditional (T) and ‘T + manganese’ or ‘Enhanced’ to ‘Traditional’ treatments.

Appendix M: Fluorescence quenching for manganese treatments

Appendix M: Fluorescence quenching for manganese treatments

Fluorescence quenching ($Fv' Fm'^{-1}$) from uppermost fully unrolled trifoliate two weeks before manganese application (2WBA) at R1, two weeks after manganese application (2WAA) at R4, and four weeks after manganese application (4WAA) at R6 in 2013 and 2014

		Treatment						
Year	**Site**	**Enhanced (E)**	**E – manganese**	**Δ**	**Traditional (T)**	**T + manganese**	**Δ**	**E vs. T†**
		---------- $Fv' Fm'^{-1}$ 2WBA ----------						
2013	Clinton	0.7258	0.7298	+0.0040	0.7320	0.7305	-0.0015	ns
	Delaware	0.7122	0.6997	-0.0125	0.7253	0.7207	-0.0046	ns
	Henry	0.7035	0.7055	+0.0020	0.6970	0.6925	-0.0045	ns
2014	Clinton	0.6967	0.6972	+0.0005	0.6958	0.6954	-0.0004	ns
	Preble	0.7121	0.7215	+0.0094	0.7209	0.7170	-0.0039	ns
		---------- $Fv' Fm'^{-1}$ 2WAA ----------						
2013	Clinton	0.7212	0.7227	+0.0015	0.7172	0.7237	+0.0065	ns
	Delaware	0.7405	0.7408	+0.0003	0.7485	0.7485	0.0000	ns
	Henry	0.7060	0.6692	-0.0368*	0.6773	0.6880	+0.0107	*
2014	Clinton	0.7397	0.7372	-0.0025	0.7335	0.7325	-0.0010	ns
	Preble	0.7460	0.7405	-0.0055	0.7455	0.7477	+0.0022	ns
	Sandusky	0.7114	0.7209	+0.0095	0.7186	0.7019	-0.0167	ns
		---------- $Fv' Fm'^{-1}$ 4WAA ----------						
2013	Clinton	0.7167	0.7225	+0.0058	0.7122	0.7207	+0.0085	ns
	Delaware	0.7175	0.7130	-0.0045	0.7217	0.7052	-0.0165	ns
	Henry	0.7083	0.7217	+0.0134	0.7218	0.7313	+0.0095	ns
2014	Clinton	0.7309	0.7387	+0.0078	0.7446	0.7277	-0.0169	ns

Continued

Appendix M, continued

2014	Preble	0.7197	0.7157	-0.0040	0.7143	0.7164	+0.0021	ns
	Sandusky	0.7121	0.7201	+0.0080	0.7220	0.7173	-0.0047	ns

Δ Change in fluorescence quenching (Fv’ Fm’$^{-1}$) between ‘Enhanced (E) and ‘E – manganese’ treatments or the change between ‘Traditional (T) and ‘T + manganese’ treatments.

† Single degree of freedom contrast used to compare ‘Enhanced’ treatment to ‘Traditional’ treatment.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E) and ‘E – manganese’ treatments or ‘Traditional (T) and ‘T + manganese’ or ‘Enhanced’ to ‘Traditional’ treatments.

Appendix N: Insect Defoliation

Percent leaf area affected by insect defoliation at R4 in the top and middle third of the plant canopy two weeks after (2WAA) and four weeks after (4WAA) insecticide application in 2013 and 2014.

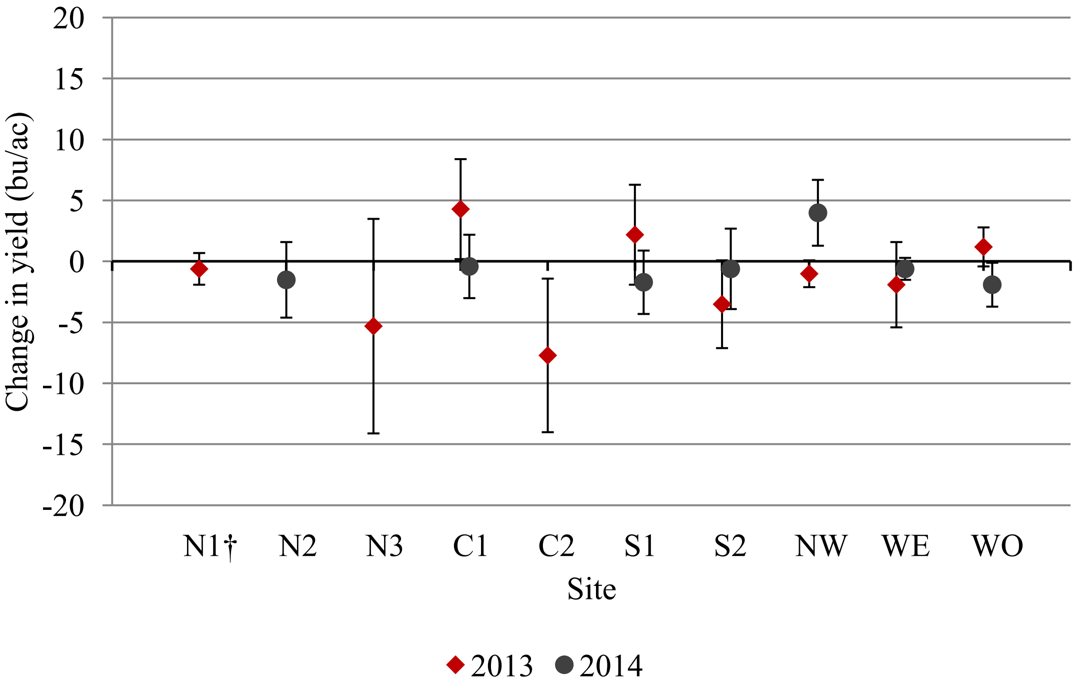
		Treatment			
Year	**Site**	**Enhanced (E)**	**E – Insecticide**	**Traditional (T)**	**T + Insecticide**
		% leaf area affected in top third canopy 2WAA			
2013	Clinton	<5%	6%	6%	6%
	Delaware	<5%	<5%	6%	<5%
	Henry	<5%	5%	5%	<5%
2014	Clinton	6%	8%	6%	8%
	Preble	<5%	<5%	<5%	<5%
	Sandusky	<5%	<5%	<5%	<5%
		% leaf area affected in top third canopy 4WAA			
2013	Clinton	<5%	<5%	<5%	<5%
	Delaware	<5%	<5%	<5%	<5%
	Henry	<5%	5%	5%	<5%
2014	Clinton	<5%	5%	<5%	<5%
	Preble	<5%	<5%	<5%	<5%
	Sandusky	<5%	5%	<5%	<5%
		% leaf area affected in middle third canopy 2WAA			
2014	Clinton	6%	9%	6%	10%
	Preble	<5%	<5%	<5%	<5%
	Sandusky	<5%	8%	<5%	7%
		% leaf area affected in middle third canopy 4WAA			
2014	Clinton	<5%	9%	<5%	5%
	Preble	<5%	<5%	<5%	<5%
	Sandusky	<5%	7%	5%	6%

Appendix O: Sulfur concentration

Sulfur concentration (S) of uppermost, fully developed unrolled trifoliate at R1, R4, and R6 in 2013 and 2014.

		Treatment	
Year	**Site**	**Enhanced (E)**	**Traditional (T)**
		-----------% S concentration at R1-----------	
2013	Clinton	0.34%	0.29%
	Delaware	0.37%	0.35%
	Henry	0.37%	0.36%
2014	Clinton	0.36%	0.33%
	Preble	0.33%	0.31%
		-----------% S concentration at R4-----------	
2013	Clinton	0.46%	0.43%
	Delaware	0.39%	0.36%
	Henry	0.44%	0.39%
2014	Clinton	0.42%	0.37%
	Preble	0.37%	0.31%
	Sandusky	0.38%	0.33%
		-----------% S concentration at R6-----------	
2013	Clinton	0.31%	0.31%
	Delaware	0.32%	0.31%
	Henry	0.37%	0.35%
2014	Clinton	0.31%	0.28%
	Preble	0.31%	0.30%
	Sandusky	0.38%	0.30%

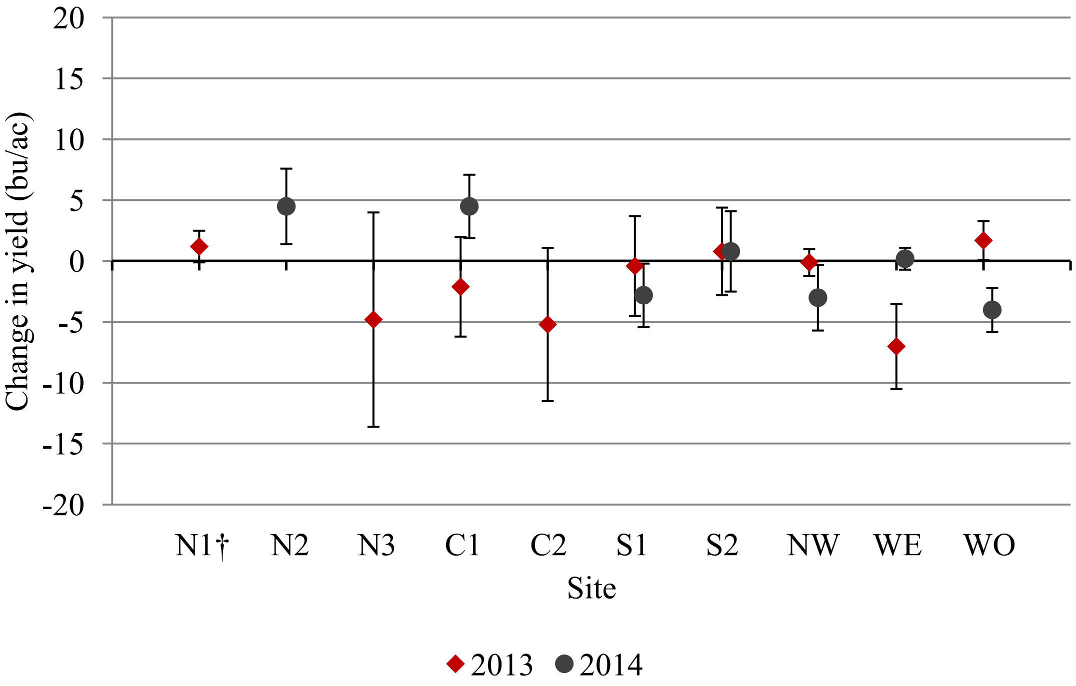
Appendix P: Inoculant yield response: enhanced system



Change in yield (bu/ac) of the 'Enhanced – inoculant' treatment from the 'Enhanced' treatment by site-year.

No significant differences at α = 0.05 using single degree of freedom contrasts comparing 'Enhanced (E)' and 'E – inoculant' treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

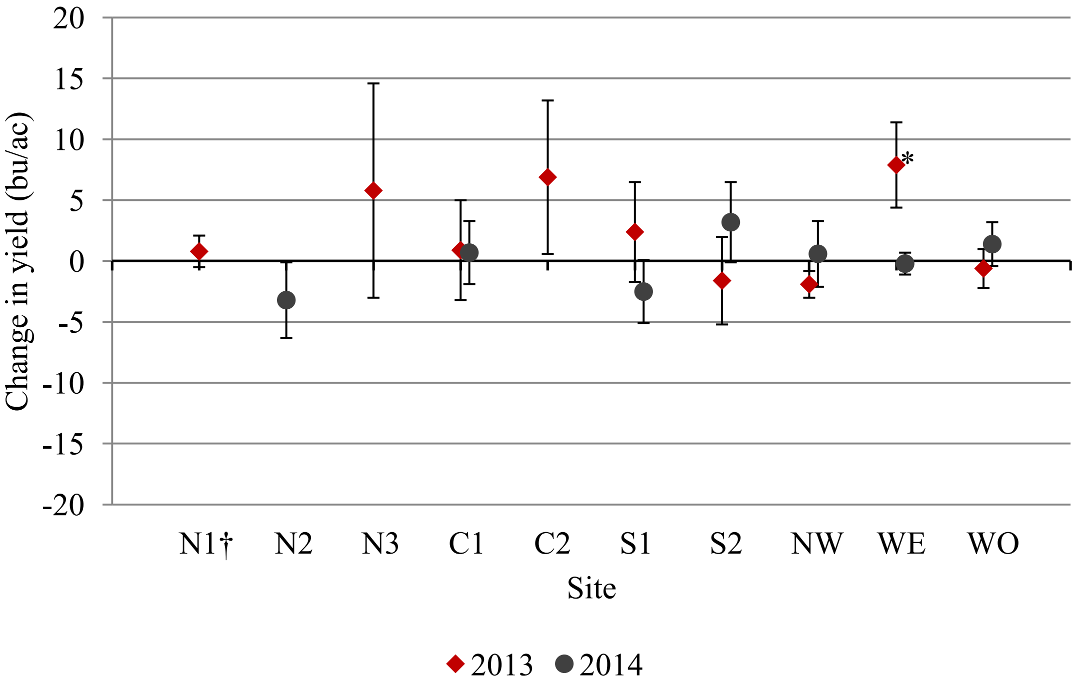
Appendix Q: Inoculant yield response: traditional system



Change in yield (bu/ac) of the 'Traditional – inoculant' treatment from the 'Traditional' treatment by site-year.

No significant differences at α = 0.05 using single degree of freedom contrasts comparing 'Traditional (T)' and 'T – inoculant' treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

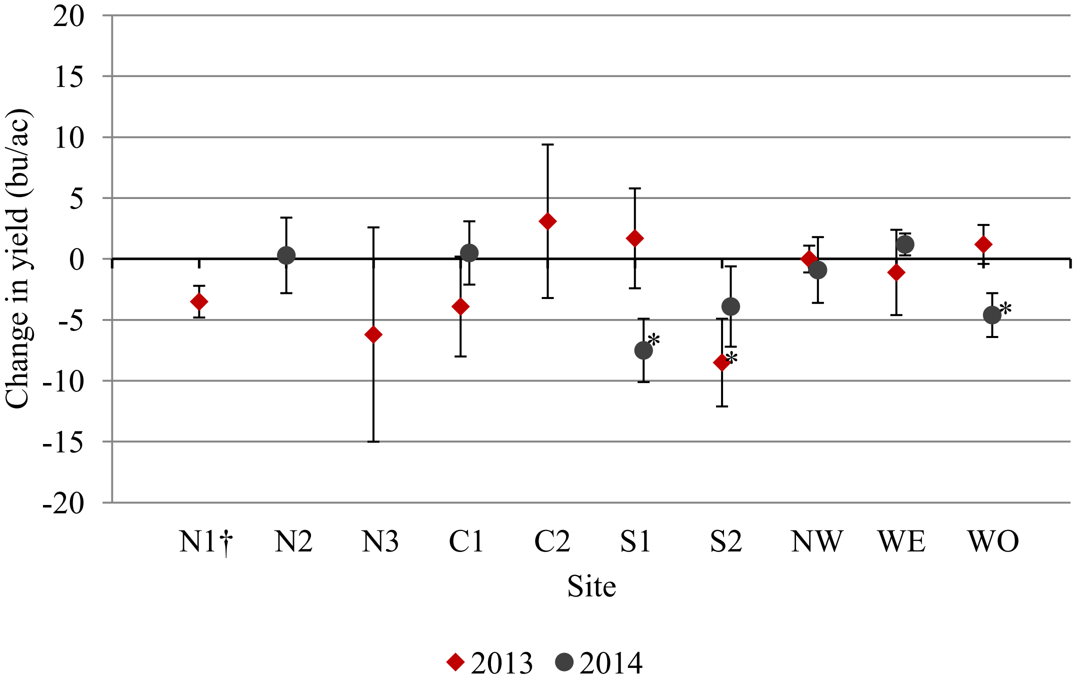
Appendix R: Gypsum yield response: enhanced system



Change in yield (bu/ac) of the ‘Enhanced – gypsum’ treatment from the ‘Enhanced’ treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – gypsum’ treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

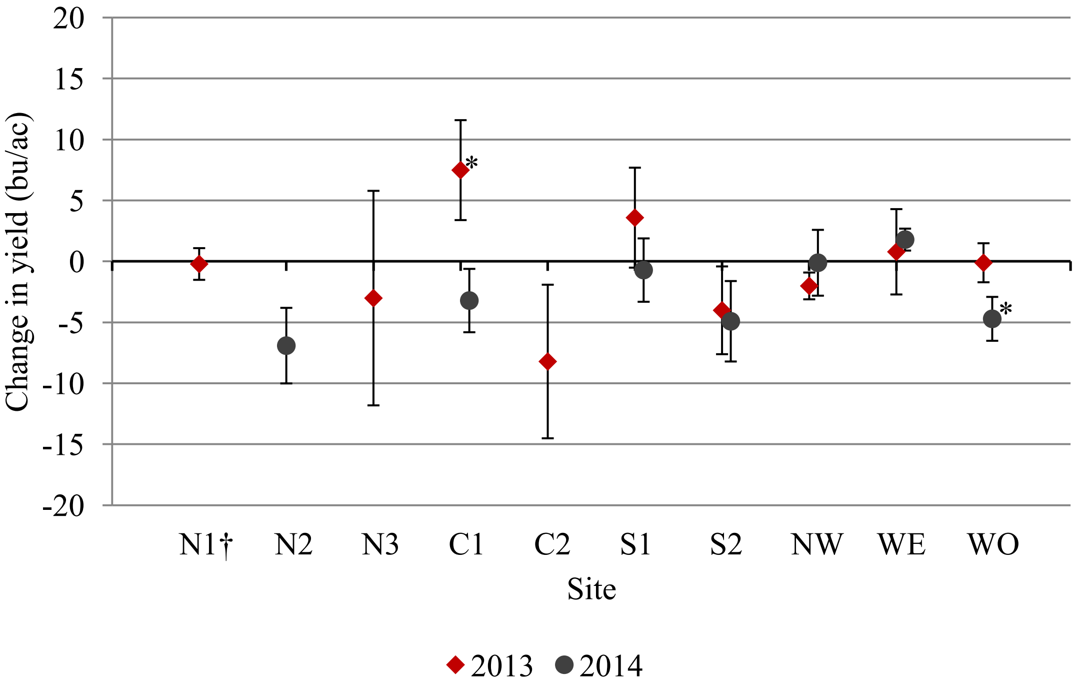
Appendix S: Gypsum yield response: traditional system



Change in yield (bu/ac) of the ‘Traditional – gypsum’ treatment from the ‘Traditional’ treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Traditional (T)’ and ‘T – gypsum’ treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

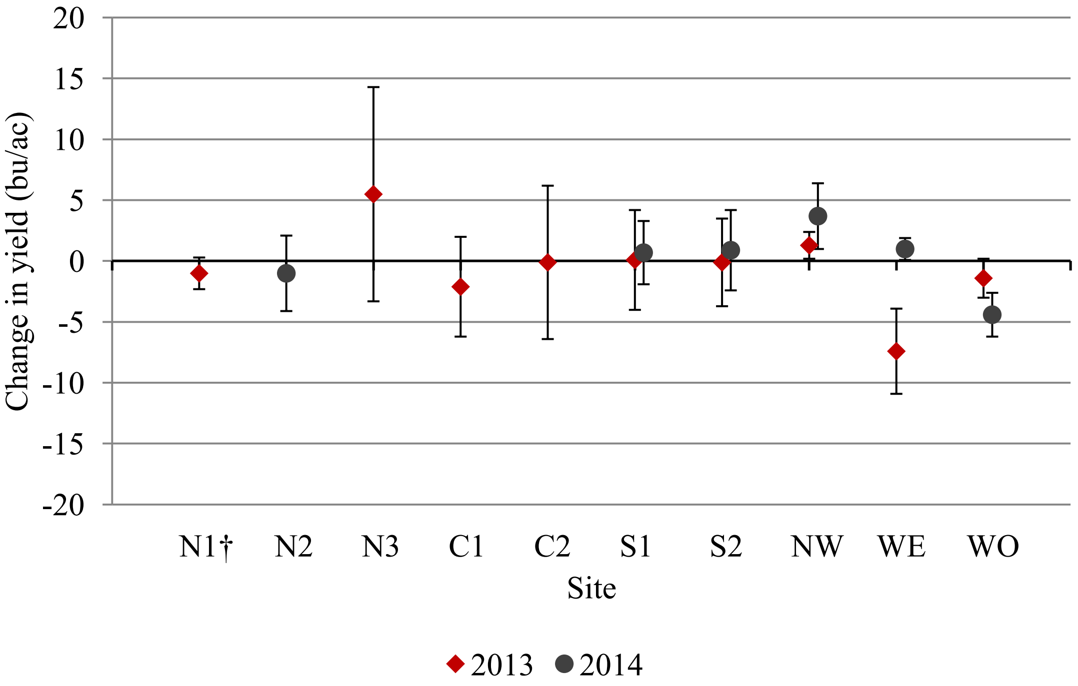
Appendix T: Insecticide yield response: enhanced system



Change in yield (bu/ac) of the ‘Enhanced – insecticide’ treatment from the ‘Enhanced’ treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – insecticide’ treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

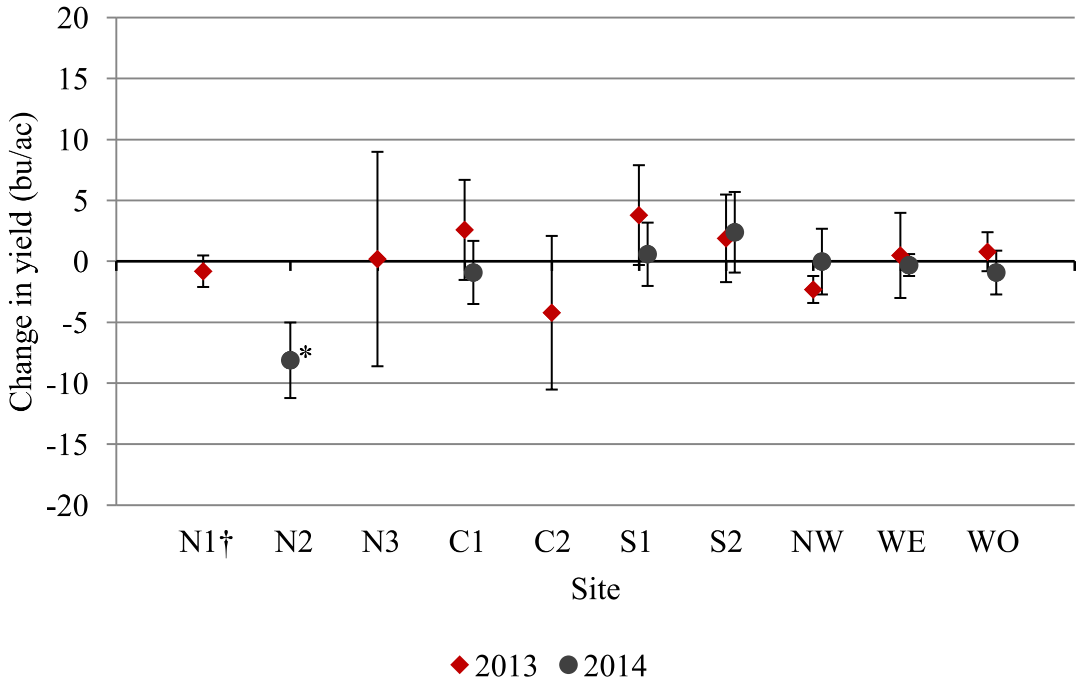
Appendix U: Insecticide yield response: traditional system



Change in yield (bu/ac) of the 'Traditional – insecticide' treatment from the 'Traditional' treatment by site-year.

No significant differences at α = 0.05 using single degree of freedom contrasts comparing 'Traditional (T)' and 'T – insecticide' treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

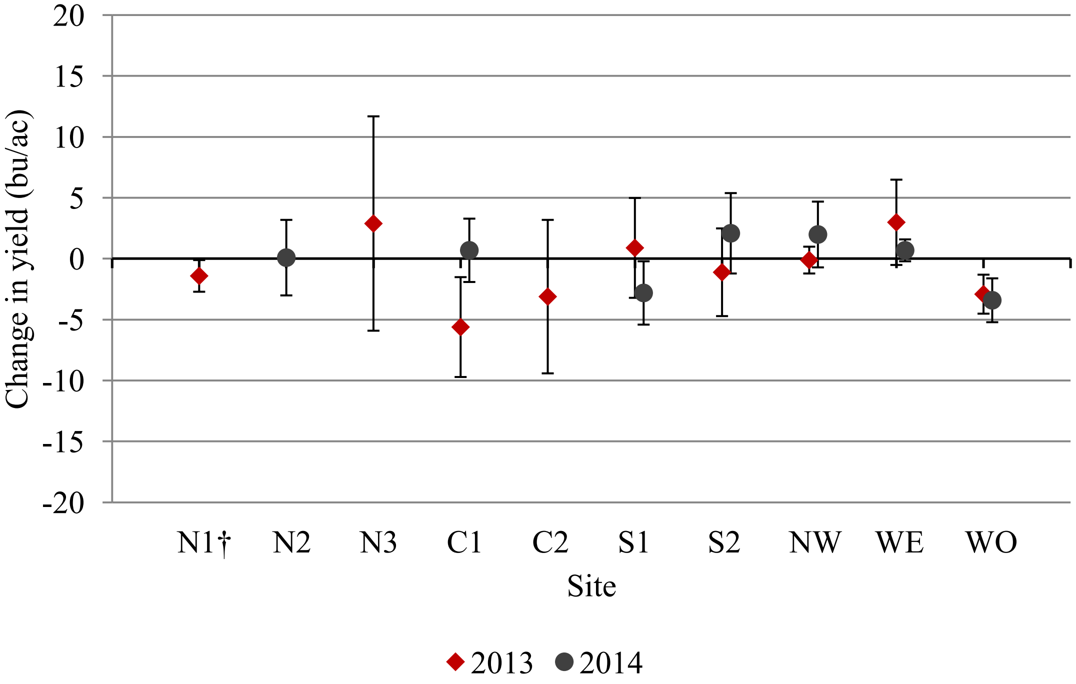
Appendix V: Manganese yield response: enhanced system



Change in yield (bu/ac) of the ‘Enhanced – manganese’ treatment from the ‘Enhanced’ treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – manganese’ treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

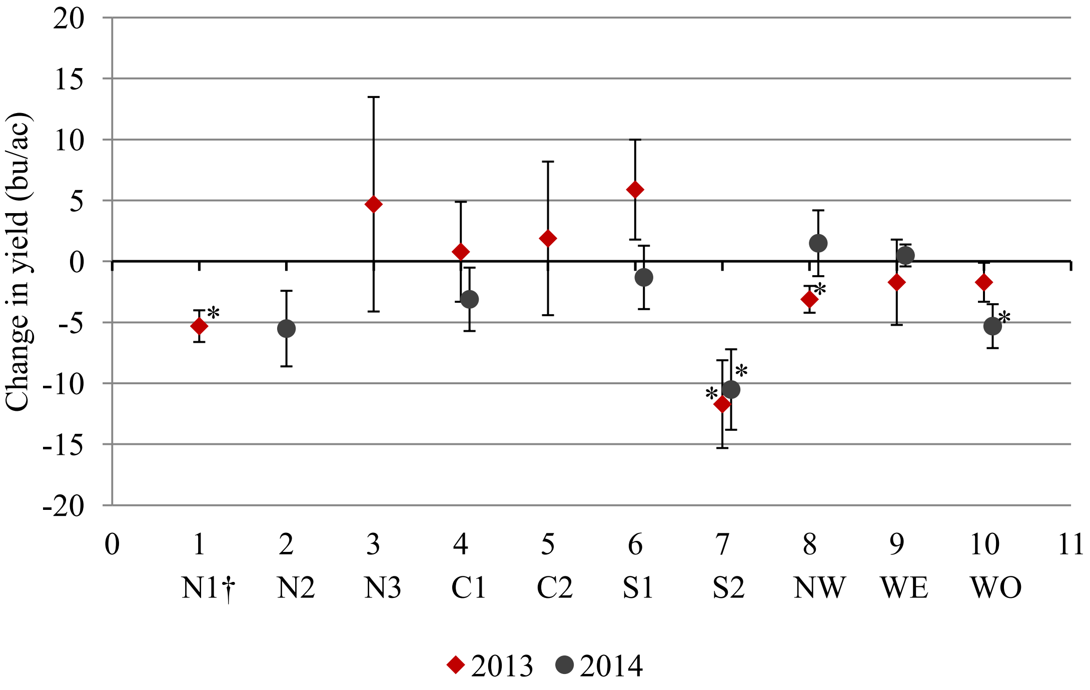
Appendix W: Manganese yield response: traditional system



Change in yield (bu/ac) of the 'Traditional – manganese' treatment from the 'Traditional' treatment by site-year.

No significant differences at α = 0.05 using single degree of freedom contrasts comparing 'Traditional (T)' and 'T – manganese' treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

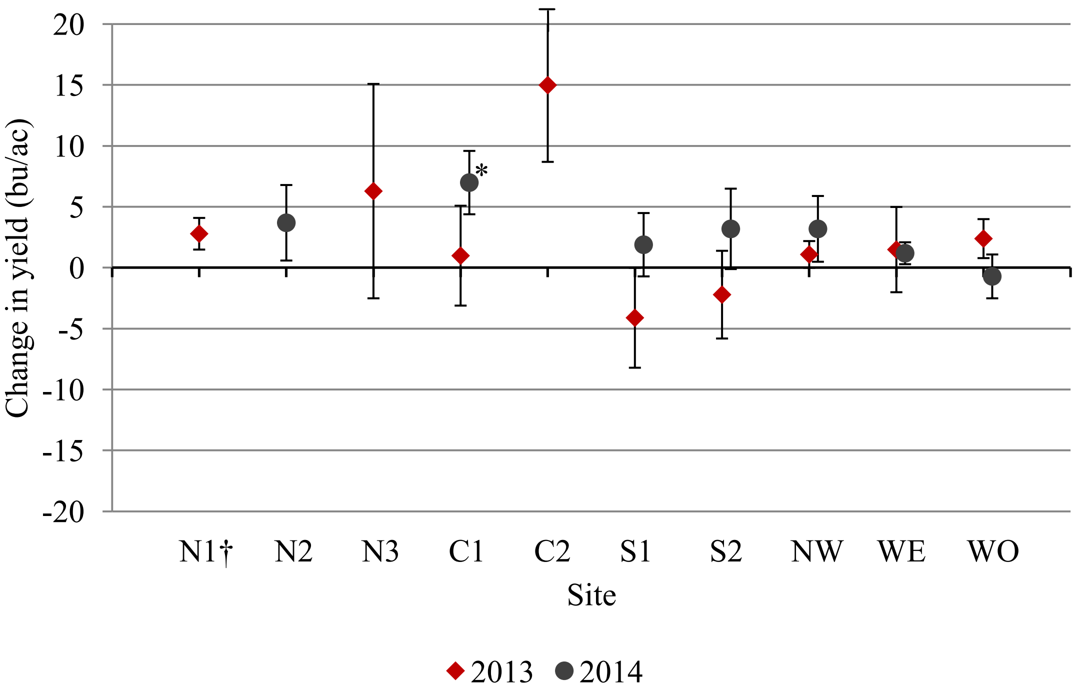
Appendix X: Fungicide yield response: enhanced system



Change in yield (bu/ac) of the ‘Enhanced – fungicide’ treatment from the ‘Enhanced’ treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing ‘Enhanced (E)’ and ‘E – fungicide’ treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

Appendix Y: Fungicide yield response: traditional system



Change in yield (bu/ac) of the 'Traditional – fungicide' treatment from the 'Traditional' treatment by site-year.

*Significantly different at α = 0.05 using single degree of freedom contrasts comparing 'Traditional (T)' and 'T – fungicide' treatments
†Sites are coded as follows: N1 (Henry), N2 (Erie), N3 (Sandusky), C1 (Mercer), C2 (Delaware), S1 (Preble), S2 (Clinton), NW (Wood/Northwest OARDC), WE (Clark/Western OARDC), and WO (Wayne/Wooster OARDC)

Appendix Z: Predicted treatment costs

Table 2. Omission trial design, treatment names, and list of inputs applied in 2013 and 2014.

Treatment name	Inputs					
	Inoculant†	Gypsum‡	Mn§	Insecticide¶	Fungicide#	Cost ha^{-1}††
Enhanced (E)	Yes	Yes	Yes	Yes	Yes	$150
E – inoculant	No	Yes	Yes	Yes	Yes	$139
E – gypsum	Yes	No	Yes	Yes	Yes	$101
E – Mn	Yes	Yes	No	Yes	Yes	$127
E – insecticide	Yes	Yes	Yes	No	Yes	$142
E – fungicide	Yes	Yes	Yes	Yes	No	$108
Traditional (T)	No	No	No	No	No	$0
T + inoculant	Yes	No	No	No	No	$11
T + gypsum	No	Yes	No	No	No	$49
T + Mn	No	No	Yes	No	No	$40
T + insecticide	No	No	No	Yes	No	$25
T + fungicide	No	No	No	No	Yes	$59

†*Bradyrhizobia japonicum* inoculant (TagTeam® LCO Liquid MultiAction® Legume Fertility) applied at 0.18 mL g^{-1} seed within sixty days before planting.

‡Pelletized gypsum applied at a rate of 4.47 Mg ha^{-1} at VC growth stage.

§Mn foliar fertilizer applied at active ingredient rate of 0.23 L ha^{-1} at R3 growth stage.

¶ Lambda-cyhalothrin insecticide applied at active ingredient rate of 26.65 ml ha^{-1} at R3 growth stage.

#Pyraclostrobin fungicide applied at active ingredient rate of 103.49 ml ha^{-1} at R3 growth stage.

††Product cost estimates of $11, $49, $23, $8, and $42 ha^{-1} for inoculant, gypsum, manganese foliar fertilizer, foliar insecticide, and foliar fungicide, respectively. An additional $17 ha^{-1} was added as application cost of the foliar manganese, insecticide, and fungicide. Product and application cost estimates were from local chemical dealers.