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Effects of Iron on Cercospora Leaf Blight of Soybean

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EFFECTS OF IRON ON CERCOSPORA LEAF BLIGHT OF SOYBEAN

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TO MY UNCLE TOM

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ABSTRACT

Cercospora leaf blight (CLB) of soybean caused by *Cercospora kikuchii* is an important disease in Lousiana. Preliminary screening of foliar applications of the micronutrients Fe, Mn, Cu, Zn, B, Mo and Al, showed that Fe decreased CLB severity consistently. The objective of this work was to test the effects of commercial formulations of Fe, Manny Plex Fe and Fe EDTA (Brandt Consolidated, Springfield, IL) on leaf colonization by *C. kikuchii*, symptom development (blight and purple leaves), and yield. Four rates of Manny Plex Fe and four rates of Fe EDTA were applied to field plots at R5 growth stage. Leaf tissue analyses for microelements and qPCR were performed. Leaf blight and purple leaf symptom severity was assessed quantitatively, and yield was measured. Results showed there was a poor relationship between leaf colonization and symptoms (neither purple nor blight). Moreover, Fe concentration in leaves did not affect biomass of *C. kikuchii*. Severity of purple leaf symptoms increased as Fe concentration in leaves increased, but severity decreased as Fe concentrations surpassed 230 mg/kg of dry matter. Blight symptoms were suppressed as the Fe concentration in soybean leaves increased. There was no correlation between purple leaf and blight symptoms. There was a positive relationship between leaf concentrations of Fe and yield. Negative relationships between yield and Cercospora biomass and severity of blight and purple leaf symptoms were observed.

1. INTRODUCTION

1.1. Soybeans Overview

Soybean (*Glycine max* (L.) Merr.) was domesticated in northern China during 1700-1100 B.C. (Sinclair *et al*. 1989). In 1765, an employee from the East India Company, Samuel Bowen, brought soybean from China via London to the United States (Sinclair *et al*. 1989).

Nowadays, soybeans are grown in most parts of the world, especially Argentina, Brazil, China, and the United States, which has been the leading producer of soybean worldwide. In 2014, the U.S. planted over 34 million hectares of soybeans, with a total yield of 0.104 billion tonnes, which contributed $45 billion to the American economy (USDA, 2014). In Louisiana, soybean plays a crucial role in the economy. The state planted almost 0.5 million hectares in 2013, with a total yield of 1,465,172.043 tonnes, contributing nearly $775 million to the state's farm gate income (LSU AgCenter, 2013).

1.2. Purple Seed Stain and Cercospora Leaf Blight

First reported in Korea in 1921 (Suzuki, 1921), purple seed stain (PSS) was found to be caused by *Cercospora kikuchii* (Matsumoto & Tomoyasu) M. W. Gardner. Symptoms consisted of light to dark purple irregular blotches ranging from a tiny spot to the entire area of seed coat (Murakishi, 1951). PSS was first observed in the United States in 1924 (Gardner 1926), but now this disease has a worldwide distribution.

Cercospora leaf blight (CLB) also is caused by *C. kikuchii*, the same pathogen that causes PSS (Walters, 1980). Symptoms of CLB begin to appear on upper leaves exposed to sunlight at the late stages of reproductive growth, usually after R5 (Walters,

1980). Leaves from the upper canopy show a leathery appearance and purple bronze color on the upper surface (Figure 1A). Chlorosis and blight of leaf tissue result in defoliation starting with the younger upper leaves (Figure 1B) (Walters, 1980). These symptoms are frequently mistaken for senescing defoliation, however, in the CLB-related defoliation, green leaves usually remain below the defoliated area (Sinclair, 1989). Previous publications (Walters, 1980) affirmed that the initial symptom of CLB is the bronzing of leaves and as the disease progresses the bronzing becomes blight. These symptoms appear during late reproductive stages, and all plants in a field may show symptoms simultaneously. However, in previous studies fungal biomass of *C. kikuchii* was detected by real time PCR in soybean leaf tissue during R2 and R3 growth stages (Chanda, 2014).

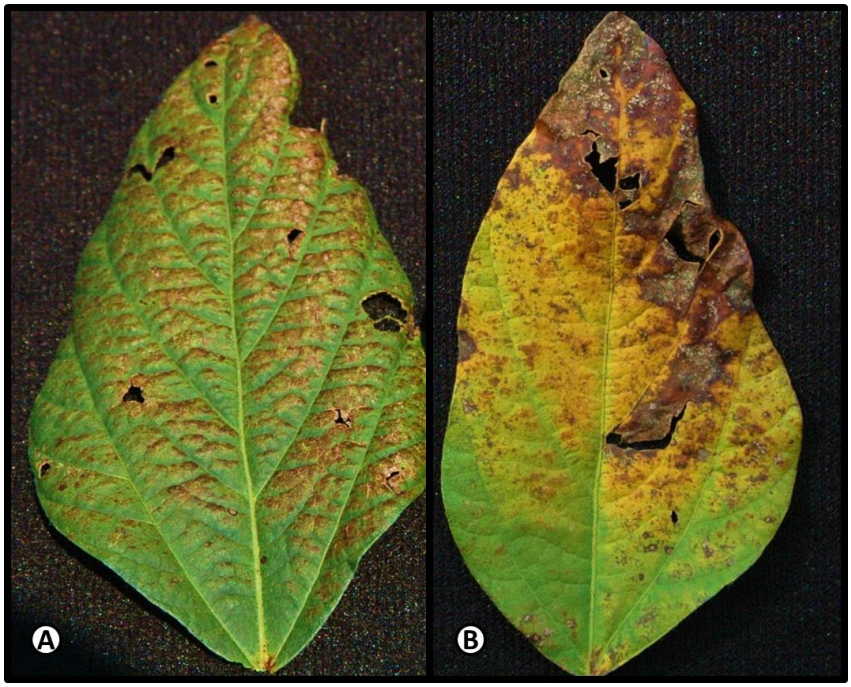

Figure 1. A) Soybean leaves showing the purple CLB symptom. B) Soybean blighted leaf symptom.

Orth first reported latent infection (1992). It begins with fungal penetration of the epidermal cells followed by colonization of surrounding cells. Further work reported that latent infecting hyphae resumed growth followed by leaf drop and senescence and death of host tissue, which may contribute to inoculum load. These findings suggested that soybean plants are infected at early growth stages, but symptom expression is triggered during late reproductive growth stages.

The optimum environmental conditions for infection of soybean by *C. kikuchii* are 20 to 30^{o}C and 8 to 24h of leaf wetness (Martin, 1982; Walters, 1980; Boyette, 1985; Schuh, 1991). Conidia of *C. kikuchii* germinate, and hyphae enter through stomata without forming appressoria; however, direct penetration of the cuticle following the formation of appressoria also has been observed (Fugita, 1990). Infection of young developing pods and seed coats were reported (Roy, 1976; Fugita, 1990; Velicheti, 1992), but infection of flowers is not known (Kilpatrick, 1956; Roy, 1976),

Once infected, germination and emergence of soybean seeds are reduced (Yeh, 1982). Germination of infected seeds can range from 0 to 49%, while seedling emergence may be reduced by 0 to 15% (Murakishi, 1951; Sherwin, 1952; Wilcox, 1973; Roy, 1976; Chen, 1979; Hepperly, 1981; Yeh, 1982). Moreover, *C. kikuchii* was isolated from 87 to 99% of PSS and 3 to 11% of symptomless seeds. (Murakishi, 1951; Wilcox, 1973; Imazaki, 2007).

1.3. Importance of Purple Seed Stain and Cercospora Leaf Blight

Cercospora kikuchii can cause significant losses in soybean fields (Wrather, 1997). In 1994, this fungus reduced soybean yield by 930,000 metric tons in Brazil and 74,800 metric tons in the U.S. In total, CLB/PPS reduced yield by 1,087,000 tonnes in the top 10 soybean producing countries during 1994 (Wrather, 1997). Among all soybean diseases studied by Wrather (1997) in the U.S., CLB/PPS was ranked as 11th most important disease in 1994.

In 1996, CLB/PPS was ranked as fifth most important disease; which caused an estimated 23% yield losses in the U.S. (Wrather, 2009). However, in 1999 the disease began to increase in severity and incidence in the Mid-South of the U.S., especially in Louisiana (Moore, 2000). In 2006, CLB/PPS were the most serious diseases in Mississippi, Texas, and Louisiana, especially (Wrather, 2009).

The proportion of soybean cultivars that are susceptible to these diseases in the field increased from 1999 to 2005 (Moore, 2000; Schneider, 2003; Levy, 2013). During the 1999 growing season, only a small proportion of the cultivars were susceptible (Moore, 2000). In contrast, 59 of 62 cultivars were susceptible in 2002 (Schneider, 2003), and in 2005, all 285 entries were identified as susceptible to PSS and CLB (unpublished). Recently, no cultivars were found to be resistant in Louisiana (Levy, 2013).

Since CLB became a serious issue in the Mid-South, investigators have been trying to determine the cause for increased disease severity. Some suggested that Louisiana had more damaging strains of the pathogen, and unlike other fungal diseases, fungicides had not been as effective in controlling CLB/PPS (Schultz, 2006). Studies

conducted by Cai and Schneider in 2008 found that the population of *C. kikuchii* in Louisiana was dominated by a new lineage; however, this new lineage was found to be less aggressive (Cai, 2008; Cai, 2009).

Since there are currently no CLB and PSS resistant cultivars, disease management is based on the use of fungicides such as benzimidazoles, strobilurins, and triazoles (Ferrin, 2013). Although these fungicides are efficient in suppressing disease development, Cercospora species and many other fungi can become resistant to these fungicides. Several examples of resistance by Cercospora species can be found in the literature (Table 1). These findings provide emphatic reasons for seeking alternative disease management strategies.

1.4. Cercosporin: A Phytotoxin Produced by *Cercospora* spp.

Cercosporin is a photoactivated toxin, which means light is required to activate its toxic moiety (Daub and Briggs, 1983). This compound absorbs light, and it is converted to an energetically activated triplet state that reacts with oxygen to produce toxic, reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and superoxide (O_2^-) (Daub and Briggs, 1983). These ROS are involved in peroxidation of membrane lipids causing membrane breakdown and death of the cells (Daub and Briggs, 1983). Daub hypothesized that membrane damage allows for leakage of nutrients into the leaf intercellular spaces allowing for fungal growth and sporulation (Daub and Chung, 2007). Through selection of genes that are highly induced by light, the first gene, *CFP* (*c*ercosporin *f*acilitator *p*rotein), was identified (Callahan, 1999).

Table 1. *Cercospora* species resistant to different fungicides.

Pathogen	Common name	Crop	Fungicide Group Name	Reference
Cercospora sojina	Frogeye leaf spot	Soybean	Strobilurins	Zhang, 2011
Cercospora beticola	Sugar beet leaf spot	Sugar beet	DMI fungicides	Karaoglanidis , 2000; Secor, 2010; Kirk, 2012
Cercospora kikuchii	Cercospora leaf blight and purple seed stain	Soybean	Thiophanatemethyl	Sakai, 1999; Imazaki, 2006
Cercospora apii	Early blight	Celery	Methyl Benzimidazole Carbamates	Berger, 1973
Cercospora arachidicola	Leaf spot	Peanut	Methyl Benzimidazole Carbamates	Clark, 1974; Littrell, 1974
Cercospora beticola	Leaf spot	Sugar Beet	Methyl Benzimidazole Carbamates	Georgopoulos, 1973
Cercospora musae/ Mycosphaerella musicola	Leaf spot	Banana	Methyl Benzimidazole Carbamates	Joya, 1982
Cercospora sojina	Frogeye leaf spot	Soybean	QoI fungicides	FRAC 2011

This bright red, purplish toxin was first isolated and purified in 1957 (Kuyama, 1957), and it plays a major role in pathogenicity, symptom expression, colonization of seed coats, and virulence (Kilpatrick and Johnson, 1956; Ilyas, 1975; Fajola 1978; Upchurch, 1991; Velichetti, 1994).

Photoactivated perylenequinones are produced by a number of important fungal plant pathogens other than Cercospora, such as species of *Alternaria, Cladosporium, Elsinoe,* and *Hypocrella*, among others. Red toxins are produced by these pathogens, and they share a similar structure (Daub, 2007).

The production and activity of cercosporin and many toxins are affected by several environmental and physiological factors, such as temperature, light and nutritional factors (You, 2008). Toxin synthesis may also be influenced by metal ions. Trace amounts of copper and manganese enhance phytotoxicity of toxins in filtrates of cultures of *Fusicoccum amygdali*, while cobalt and iron decrease toxicity. *Fusarium solani* requires traces of Zn and Mg for producing pigments in media (Wood, 1972). Several nonselective toxins, such as stemphyloxins, marasmins, naphtharazins, fusaric acid, and ascochitine, also have chelating proprieties. Iron also can affect their activity and biosynthesis (Barash, 1986; cited by Barton, 1993).

1.5. Plant Nutrition and Disease Development

In addition to affecting plant growth and development, a balanced mineral nutrition is essential for plant defense to pathogens and abiotic stresses (Datnoff *et al*, 2007). However, in many cases the level of micronutrients necessary for suppressing disease may be greater than what is normally required by the plant (Elmer and Datnoff,

2014). This suggests that an extra dose of micronutrients may be required to reach a desirable management of plant diseases. The adequate levels of micronutrients necessary for plant growth and development are presented in Table 2.

Table 2. Average concentrations of mineral elements in plant dry matter necessary for adequate growth according to Kirkby (2012).

Element	Chemical symbol	mg/kg
Molybdenum	Mo	0.1
Nickel	Ni	0.1
Copper	Cu	6
Zinc	Zn	20
Manganese	Mn	50
Iron	Fe	100
Boron	B	20
Chlorine	Cl	100

Some mineral nutrients can be absorbed not just by roots but also by leaves (Taiz, 2010). Foliar application reduces the lag time between application and the plant uptake. This is especially important during the phase of rapid plant growth and if the plant is threatened by a pathogen. In addition, mineral nutrients (mostly metals) can easily be adsorbed by soil particles, and hence they will be less available for plant uptake (Taiz, 2010).

The mechanism by which micronutrients suppress disease development is very specific to the micronutrient/host/pathogen system. In order to protect the plant against a pathogen, micronutrients need to meet at least one of the following conditions described by Poschenrieder and his colleagues (2006):

- The micronutrient needs to be more toxic to the pathogen than to the plant;
- The micronutrient needs to increase the resistance of the plant against the pathogen;
- The micronutrient needs to hamper the growth and/or the virulence of the pathogen.

One of the mechanisms by which micronutrients mediate plant resistance is by binding to the plant cell wall thus creating a barrier against the pathogen (Ghanmi, 2004). In addition, phenylpropanoids, known to have antimicrobial activity or to reduce pathogen growth and development, can also be trigged by some nutrients. Flavonoid and isoflavonoid concentrations increased in roots and root exudates in lupin (*Lupinus albus* L.) grown in low nitrogen (a macronutrient) (Graham, 1991; Wojtaszek, 1993), whereas low iron levels can cause increased release of phenolic acids, presumably to help solubilize metals and thereby facilitate their uptake (Marshner, 1991). Production of enzymes, such as superoxide dismutase (SOD) involved in ROS detoxification, also are induced by micronutrients. In addition to plant defense to oxidative stress, Cu and Zn are important for SOD as a virulence factor in necrotrophic fungal pathogens, (Rolke, 2004; Babitha, 2002). Micronutrients, such as Fe, Cu and Zn, also can be the center of competition between microorganisms in plants as well as between a pathogen and its host. Siderophores are efficient Fe chelators produced by several of microorganisms in order to supply themselves with Fe from other microorganisms or from the plant host (Dellagi, 2005).

The most traditional micronutrient with fungistatic effect is Cu (Poschenrieder, 2006). Copper sulfate inhibits growth and development of many plant pathogens, and it has been used since the 19th century in order to prevent infections in vineyards (Poschenrieder, 2006). Seebold (2001) demonstrated that silicon appears to delay incubation and latent periods of *Magnaporthe grisea*, the causal agent of blast in rice.

Signal transduction pathways of biotic stress very often overlap with micronutrient stress signaling (Glazebrook, 2005). Jasmonate and ethylene are involved in signaling induced by necrotrophic pathogens and the micronutrient Cd (Glazebrook, 2005; Maksymiec, 2005). Micronutrient overload and biotic stress seem to share the same signaling molecules. Reduced glutathione, for example, appears to be the key factor for overload micronutrient stress tolerance and pathogen resistance (Freeman, 2005). Clearly, a number of plant nutrients play an important role in suppressing plant disease.

2. OBJECTIVES

The main goal of this project was to develop a deeper understating of the effect of micronutrients on CLB, a devastating disease in soybeans, and to provide Louisiana soybean farmers an alternative methodology for CLB management.

Objective 1 - Screen selected micronutrients for managing CLB under field conditions.

Objective 2 - Select the best micronutrient that suppressed CLB for a more detailed study regarding its effect on CLB symptoms, *C. kikuchii* leaf colonization, and yield improvement.

3. MATERIALS AND METHODS

3.1. Experimental Design

Field experiments were performed at the Louisiana State University AgCenter Dean Lee Research Station during the 2013 growing season. The soybean cultivar Pioneer 95Y61 (late group V) was planted on June 11. Plots were three rows wide, 6.0 meters long, and planted on 0.96-meter centers. The experimental design was a randomized complete block with four replications. The middle row of each experimental unit was harvested to assess yield losses.

3.2. Screening of Selected Micronutrients to Manage CLB in the Field

Seven micronutrients were used in this research: Fe, Mn, Cu, Zn, B, Mo and Al. These were selected based on previous research which used those nutrients in reagent grade form (data not published). Two commercial formulations (Brandt EDTA and Brandt Manni-Plex) from Brandt Consolidated Inc., Springfield, IL for foliar application were selected for each of the micronutrients (Table 3). Micronutrient solutions were applied with a 10-boom sprayer (R & D Sprayers, Opelousas, Louisiana) at R5 growth stage.

Rates of application were calculated based upon the label recommendation. Doses above the recommended rates were used to test the effect of high concentrations of micronutrients on CLB severity. The treatments, label recommendations and rates are listed in Table 3.

Four controls were used for this experiment. The EDTA control was formulated using 30.76% w/w of disodium EDTA and 69.24% w/w of water. The solution was

vigorously stirred and corrected to pH 8.0 using 5M KOH. The Manni-Plex and adjuvant controls were formulated by Brandt Consolidated Inc. for use in this research. Distilled water was used for the control "None".

Iron, one of the best micronutrients that suppressed CLB symptoms, was chosen for a comprehensive study regarding leaf colonization by *C. kikuchii*, symptom development and yield. The rates of iron applied using Brandt EDTA Fe were 52.54, 105.08, 157.61 and 210.15 g/ha and Brandt Manni-Plex Fe formulations provided the following iron rates: 233.27, 350.25, 466.53 and 483.51 g/ha as shown on the Table 4. These values were chosen based upon the recommended label rates for each formulation.

3.3. Tissue Analysis

Ten middle leaflets from the third node from the top were collected in the middle row of each experimental unit 10 days after application. The leaflets were washed with a solution of 0.1M HCl and 0.5 ml of Liquinox™ (Alconox, Inc. White Planes, NY), according to Wallace (1993). After washing, leaflets were placed in paper bags (Duro Grocery paper bag, 6#, model # 80983) and dried at 60°C for two days. The dried leaflets were then ground to a powder using a coffee grinder (Mr. Coffee, model IDS77) and stored in a paper envelope (Quality Park Products, #50162, Minneapolis, MN) in a sealed Ziploc® quart freezer bag (S.C. Johnson & Son, Inc., Racine, Wisconsin).

Table 3. Micronutrient treatments used in the field trials to assess activity against Cercospora leaf blight on soybean.

Chemical	Label recommendation (L)	Product rate (L/ha)	Plot rate (ml/3L)	Treatment code
Brandt EDTA Fe	0.473- 0.946	1.169	18.45	EFe1
		2.338	36.9	EFe2
		3.508	55.35	EFe3
		4.677	73.8	EFe4
Brandt EDTA Mn	0.946- 1.892	4.677	73.8	EMn1
		9.354	147.6	EMn2
Brandt EDTA Cu	0.473 – 0.946	1.169	18.45	ECu1
		2.338	36.9	ECu2
Brandt EDTA Zn	0.473 – 0.946	1.169	18.45	EZn1
		2.338	36.9	EZn2
N-Boron	0.946 - 1.892	2.338	36.9	NB1
		4.677	73.8	NB2
		7.015	110.7	NB3
		9.354	147.6	NB4
Manni-Plex Fe	0.946 - 1.892	4.677	73.8	MFe1
		7.015	110.7	MFe2
		9.354	147.6	MFe3
		11.692	184.5	MFe4
Manni-Plex B Moly	0.473 – 0.946	1.169	18.45	MBMo1
		2.338	36.9	MBMo2
		3.508	55.35	MBMo3
		4.677	73.8	MBMo4
Manni-Plex Moly	0.946 - 1.892	2.338	36.9	MMo1
		4.677	73.8	MMo2
Manni-Plex Mn	0.946 - 1.892	2.338	36.9	MMn1
		7.015	110.7	MMn2
Manni-Plex Zn	0.946 - 1.892	2.338	36.9	MZn1
		4.677	73.8	MZn2
Hydriclear Al	x	x	20.4g/3L	HCAl1
	x	x	61.1g/3L	HCAl2
Control EDTA	x	x	36.9	EC
Control Manni-Plex	x	4.677	73.8	MC
Control Adjuvant	x	4.677	73.8	AC
Control None	x	x	x	C

Table 4. Commercial Fe formulations and rates of application used in field trials for activity against Cercospora leaf blight of soybeans.

Chemical Name	Treatment Code	Fe (g/ha)
Brandt EDTA Fe	EFe1	52.54
	EFe2	105.08
	EFe3	157.61
	EFe4	210.15
Manni-Plex Fe	MFe1	233.27
	MFe2	350.25
	MFe3	466.53
	MFe4	583.51
Control EDTA	EC	-
Control Manni-Plex	MC	-
Control None	C	-

Tissue digestion for multi-element analysis was done using the nitric acid - hydrogen peroxide method described by the US Environmental Protection Agency (USEPA method 3050B) (USEPA, 1996). Briefly, 0.5g of tissue was weighed and placed in a digestion tube with 5.0 ml of concentrated reagent grade HNO_3 (assay 67-70%) for 50 minutes. The samples were vortexed for five seconds and then placed in the digestion block at 152-155°C for five minutes to initiate vigorous boiling. The tubes were cooled for 10 minutes, and 3.0 ml of H_2O_2 (30% reagent grade) was added. The tubes were then covered with a small glass funnel and placed in a digestion block for two hours and 45 minutes. After digestion, the samples were transferred to 15 ml centrifuge tubes, and the solution was brought to 12.5 ml using distilled water. Samples were analyzed using

inductively coupled plasma-optical spectroscopy at the Soil Testing & Plant Analysis Laboratory in the School of Plant Environment and Soil Science, Louisiana State University Agricultural Center.

3.4. Real Time PCR

Ten days after treatments were applied; ten middle leaflets from the third node from the top of plants in the middle row were collected and kept on ice until frozen with liquid nitrogen. The leaves were stored at -80°C, ground to a powder using mortar and pestle, and then stored again at -80°C until qPCR was performed. The qPCR procedure was done as described by Chanda and his colleagues (2014) to verify leaf colonization by *C. kikuchii*.

3.5. Disease Assessment

Disease severity was accessed using quantitative disease severity assessments for purple and blighted leaves based on the template shown in Figure 2. Only the leaves of the upper canopy, exposed to the sun, were considered in the assessment.

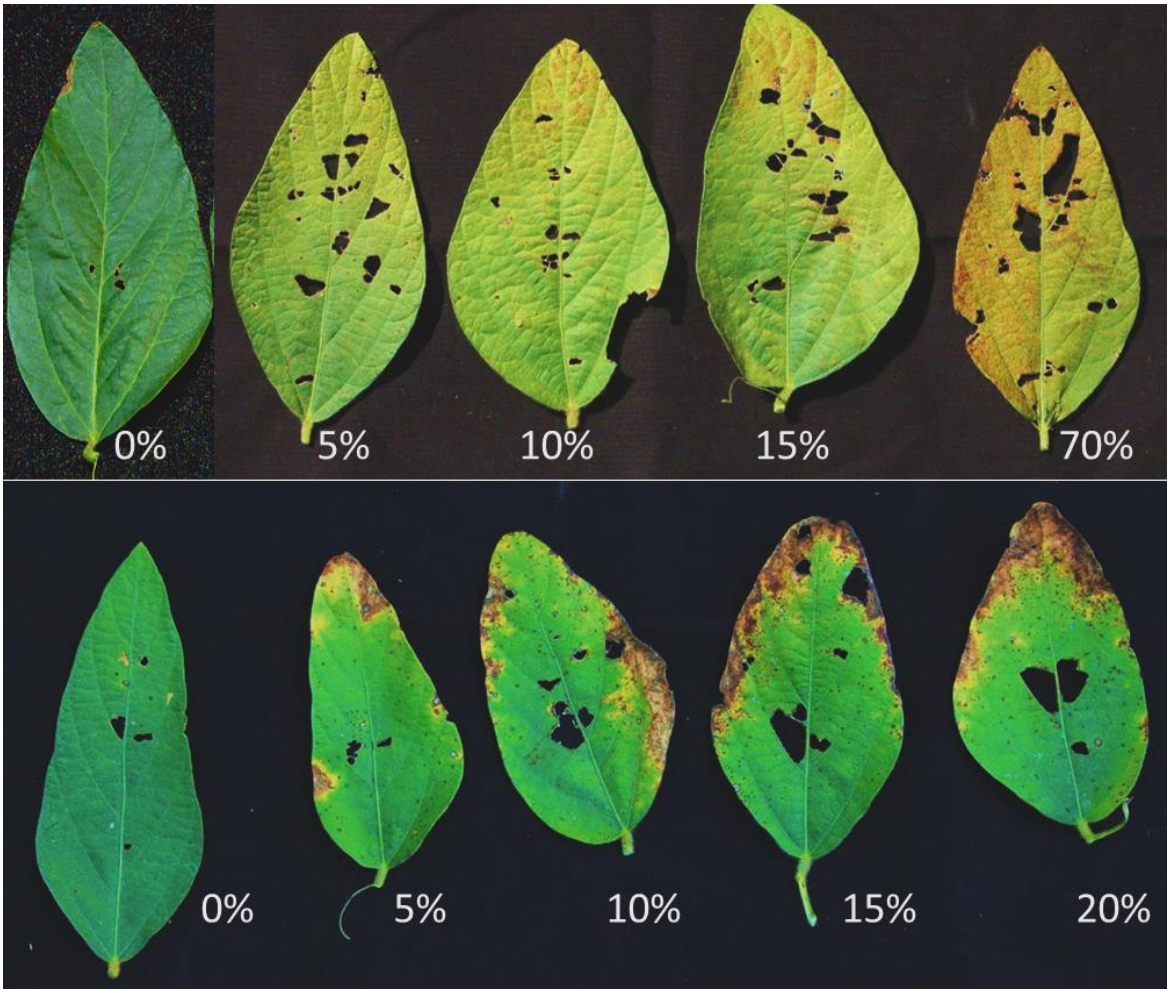

Figure 2. Cercospora leaf blight rating scale (percentage of leaf area affected) used to rate disease severity in all field experiments. The percentage of leaf area affected was calculated using ASSESS (APS Press, 2002), an image analysis for plant disease quantification.

4. RESULTS

4.1. Screening of Selected Micronutrients to Manage Cercospora Leaf Blight in Field Experiments

The first step of this research was to screen selected micronutrients that might reduce CLB symptoms under field conditions. Results showed that the highest rate of iron applied using the Manni-Plex formulation (MFe4) suppressed both purple and blight symptoms as compared to the controls (Figures 3 and 4, respectively). Because of these findings, iron was chosen for further investigations.

4.2. Tissue Analysis: Iron Accumulation in Soybean Leaves

Results from tissue analyses showed that the highest rates of Manni-Plex formulations (MFe3 and MFe4) were sufficient to significantly increase the concentration of iron in leaves compared to the control (Figure 5). The EDTA formulations (EFe1, EFe2, EFe3, EFe4) did not significantly increase iron concentrations in the leaves.

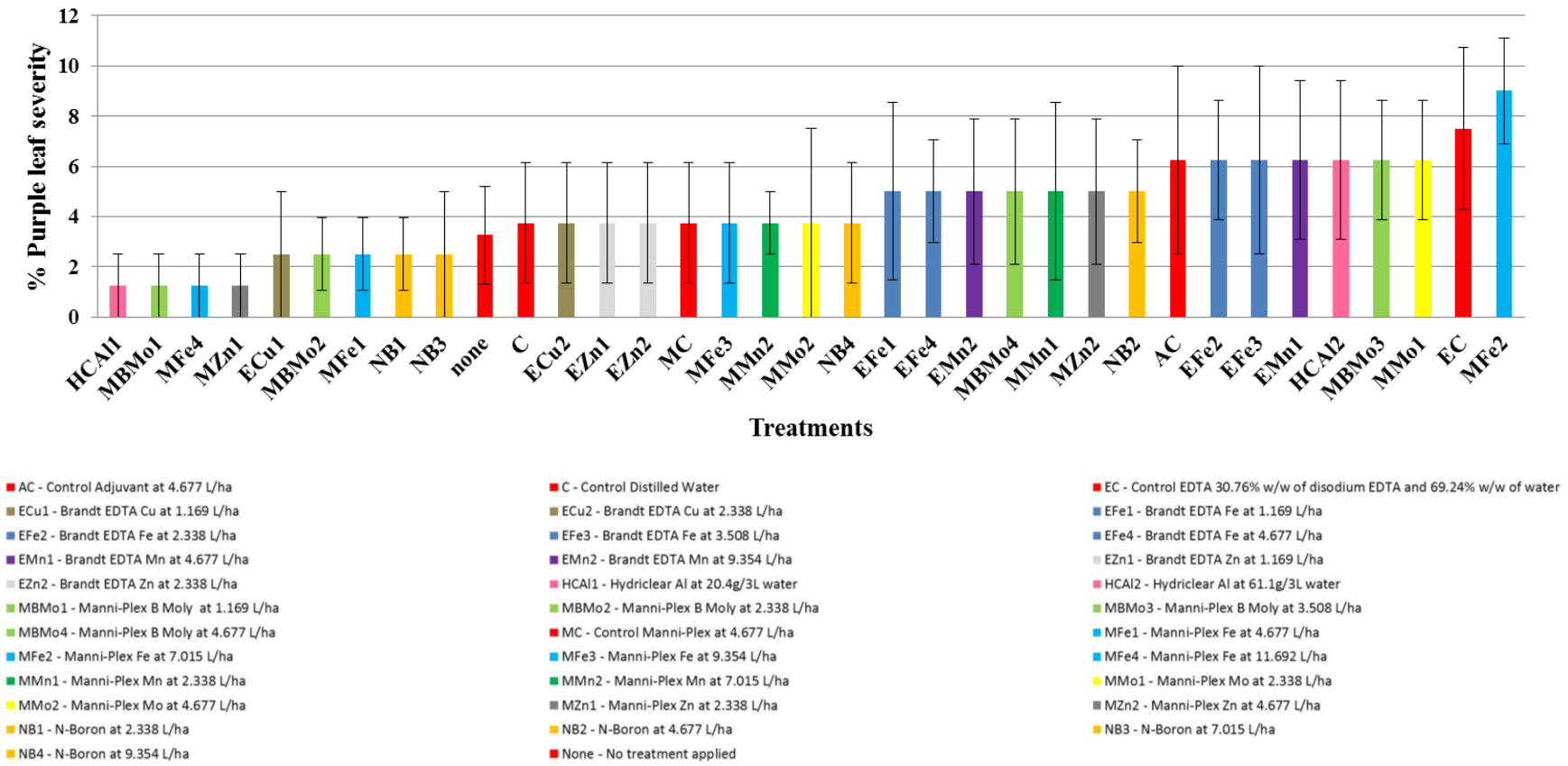

Figure 3. Effect of micronutrients on the severity of purple leaf symptoms caused by *Cercospora kikuchii* in soybean. Bars indicate standard error between four replications.

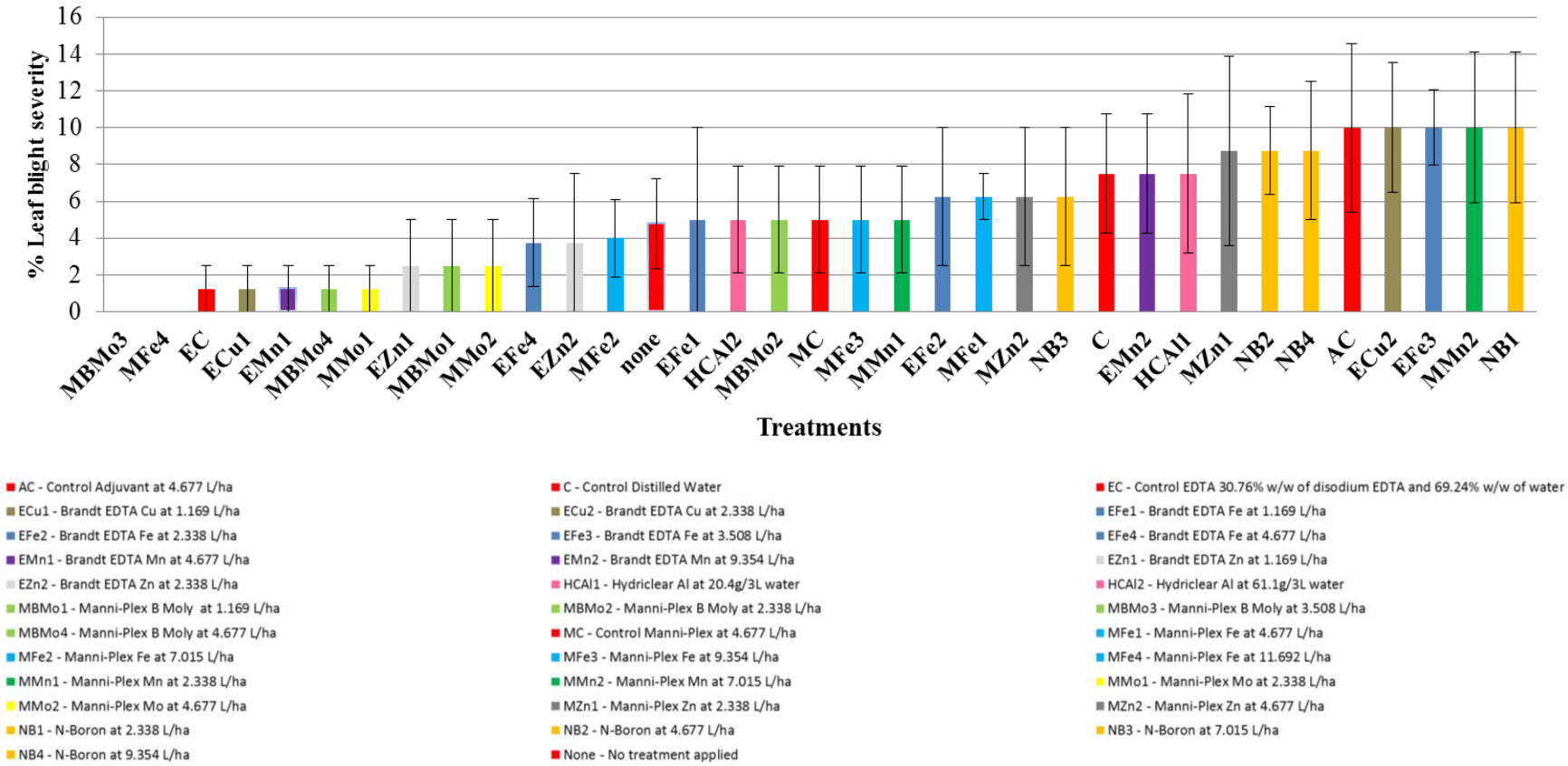

Figure 4. Effect of micronutrients on the severity of blight symptoms caused by *Cercospora kikuchii* in soybean. Bars indicate standard error between four replications.

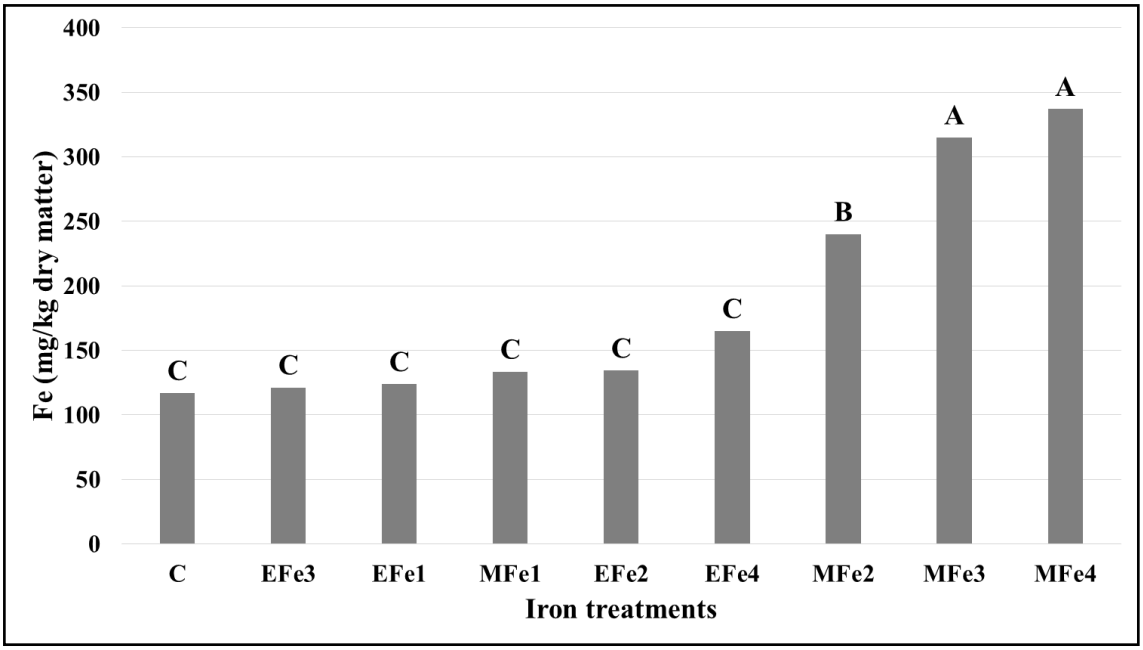

Figure 5. Iron concentrations in soybean leaves (mg/kg dry matter) as affected by foliar application of commercial formulations of two iron products. Means followed by the same letter are not significantly different (P=0.10). $LSD_{0.1}$= 58.8; $LSD_{0.05}$= 70.8

4.3. Effect of Iron Concentration in Soybean Leaves on Severity of Cercospora Leaf Blight

There were two patterns of disease suppression for each of the symptoms assessed. Severity of purple leaf discoloration initially increased as the Fe concentration in leaves increased, but severity declined as the concentration of iron exceeded values above 230 mg/kg dry matter (Figure 6). The highest disease severity assessed was 10% of leaf affected when iron concentration was about 230 mg/kg dry matter. However, iron concentrations above the threshold of 230 mg/kg dry matter suppressed blight symptoms (Figure 6).

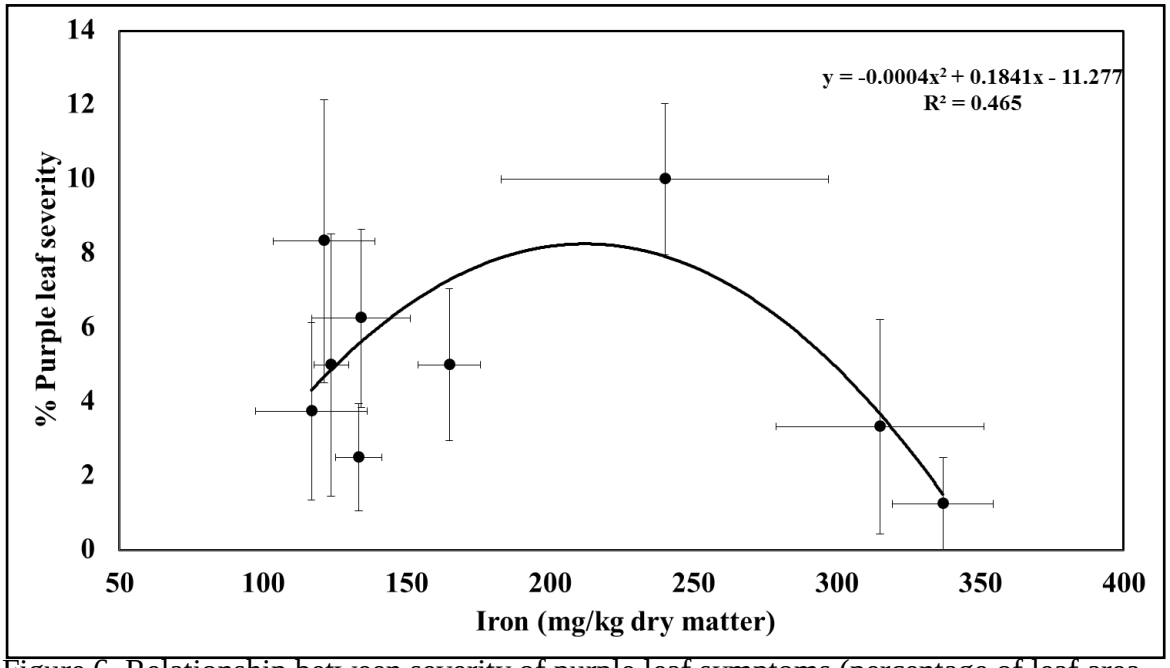

Figure 6. Relationship between severity of purple leaf symptoms (percentage of leaf area affected), and iron concentration in leaves (mg/kg dry matter). Bars indicate standard error between four replications.

Blight symptoms responded differently from purple symptoms in response to foliar iron applications. Blight symptoms were significantly suppressed as iron concentrations in leaves increased (Figure 7). The highest concentration of iron, about 350 mg/kg dry matter, completely suppressed blight symptoms. This significant negative correlation (R^2= 0.761) clearly showed that relatively high levels of iron in leaves suppressed blight symptoms under field conditions.

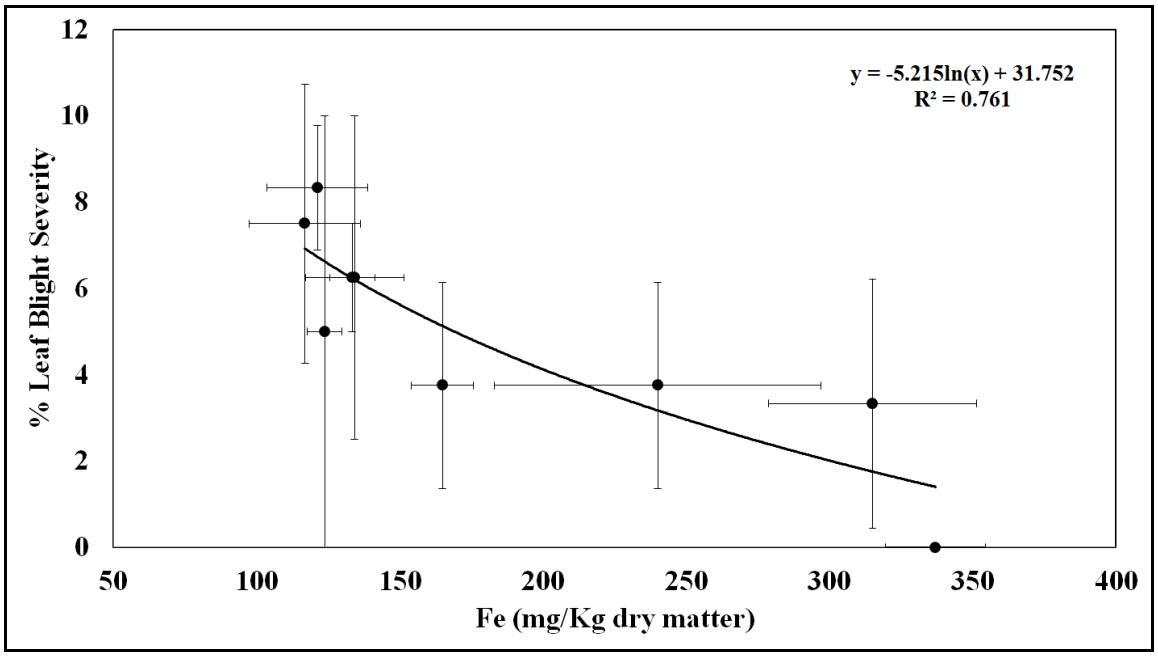

Figure 7. Relationship between severity of blight leaf symptoms (percentage of leaf area affected), and iron concentration in leaves (mg/kg dry matter). Bars indicate standard error between four replications.

Visually, soybean plants that received 483.51g Fe/ha (Figure 8B) looked considerably more robust and healthy as compared to the control (Figure 8A). The controls also had higher levels of defoliation as compared to the best treatment (MFe4) (Figure 8A and 8B).

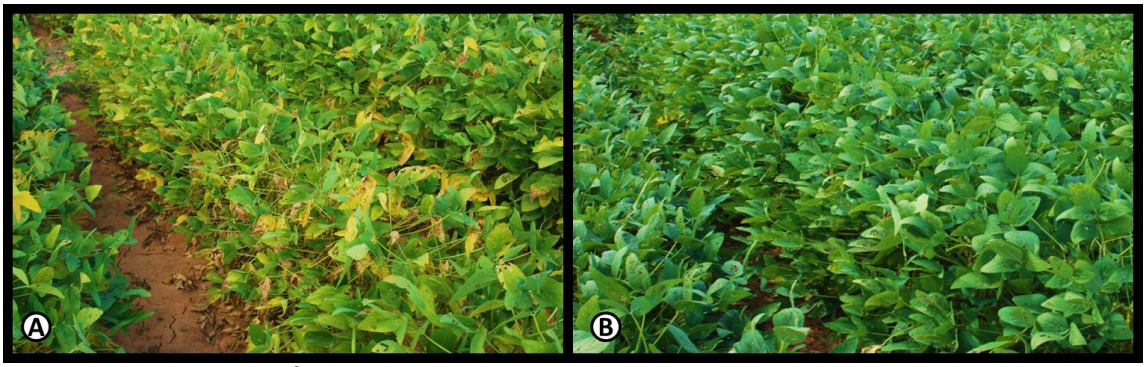

Figure 8. Photograph of soybean plants nontreated (A), and treated with MFe4. Nontreated plants showed blight and purple symptoms and were heavily defoliated.

The correlation between the two types of disease symptoms was investigated. The coefficient of determination (R^2) between purple leaf and blight symptoms was 0.063, meaning that purple and blight are not correlated (Figure 9).

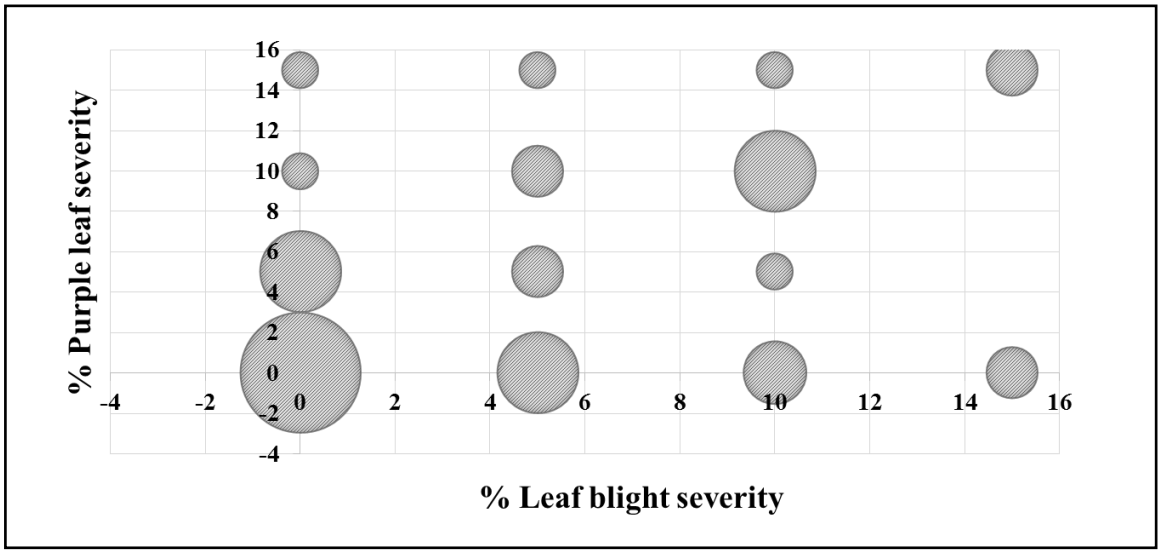

Figure 9. Bubble chart showing the relationship between purple and blight symptoms caused in soybean by *Cercospora kikuchii*. The size of each bubble reflects the number of times each assessment rating was recorded.

4.4. Biomass of *Cercospora kikuchii* in Soybean Leaves as Related to Leaf Iron Concentration and Severity of Cercospora Leaf Blight

Biomass of *C. kikuchii*, as assessed by qPCR analyses, had a poor relationship (R^2=0.167) with leaf iron content (Figure 10). This relationship suggests that Fe probably did not play a direct role on the growth of *C. kikuchii* in soybean leaf tissue.

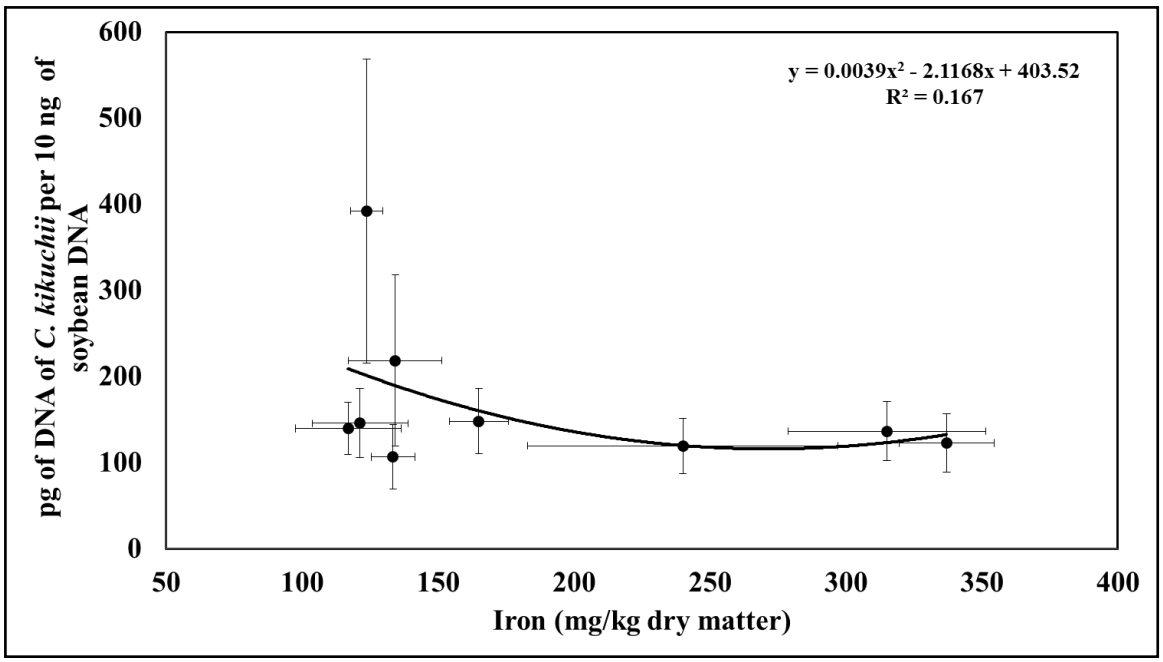

Figure 10. Relationship between biomass of *Cercospora kikuchii*, as assessed by qPCR analysis of fungal DNA, and iron concentration in soybean leaves (mg/kg dry matter). Bars indicate standard error between four replications.

4.5. Coefficient of Determination (R^2) Between Biomass of *C. kikuchii* in Soybean Leaves and Severity of Purple Leaf and Blight Symptoms.

The coefficient of determination (R^2) between biomass of *C. kikuchii* in soybean leaves and severity of purple leaf and blight symptoms were 0.109 and 0.008 respectively (Figures 11 and 12). For both symptoms, soybean plants were observed and recorded as not being infected, but DNA of *C. kikuchii* was detected by qPCR analyses in the leaves of these plants.

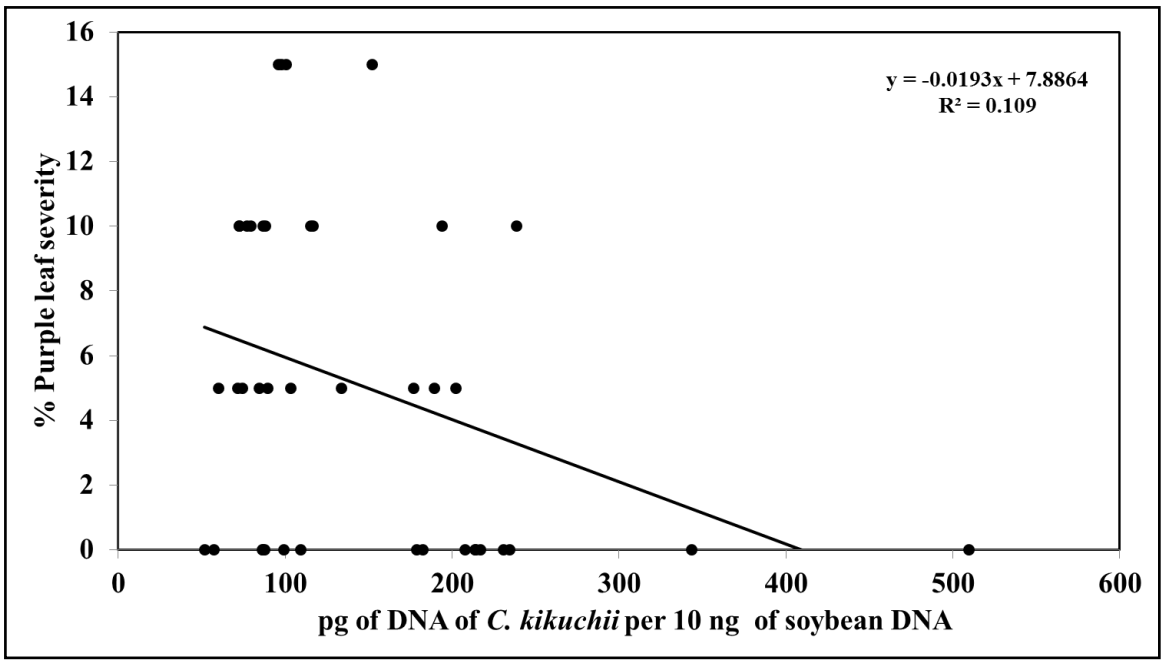

Figure 11. Relationship of purple symptom severity (% leaf area affected) in soybean caused by *Cercospora kikuchii* compared to biomass of the pathogen in leaves.

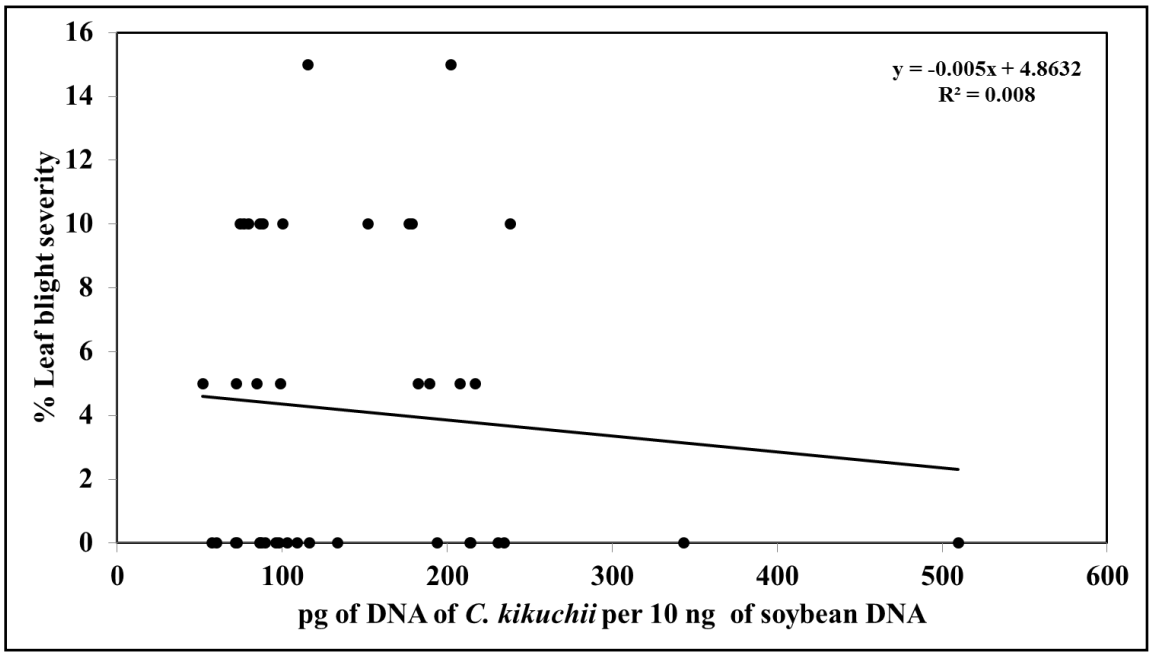

Figure 12. Relationship of blight symptom severity (% leaf area affected) in soybean caused by *Cercospora kikuchii* compared to biomass of the pathogen in leaves.

4.6. Effect of Severity of CLB and Biomass of *Cercospora kikuchii* in Leaves on Soybean Yield

Purple symptoms in soybean leaves played a significant role in affecting yield (Figure 13). The negative linear model coefficient of determination was significant (R^2=0.671) for this symptom and yield. Up to 21% of the yield was reduced (P<0.05) when the symptom of leaf purpling reached 5% (Figure 13). A similar significant relationship was observed for yield and blight symptoms (R^2=0.653) (Figure 14). Results from qPCR analysis showed that soybean yield decreased as the biomass of *C. kikuchii* increased (R^2=0.405) (Figure 15).

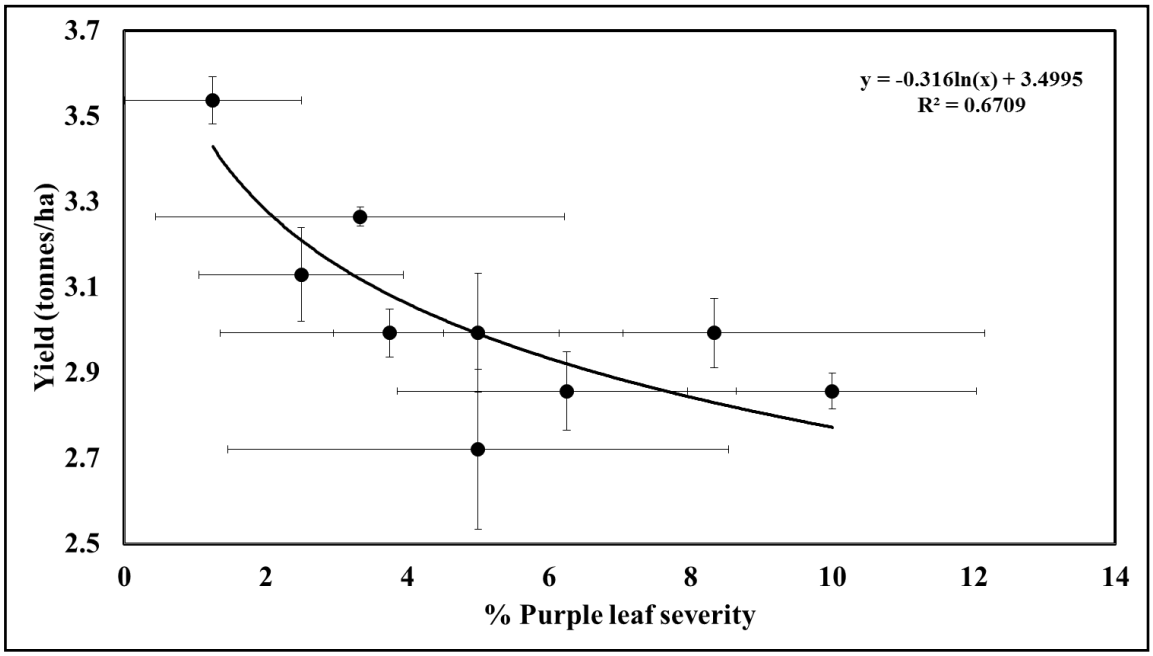

Figure 13. Relationship between yield (tonnes/ha) and % purple leaf severity caused in soybean caused by *Cercospora kikuchii.* Bars indicate standard error between four replications.

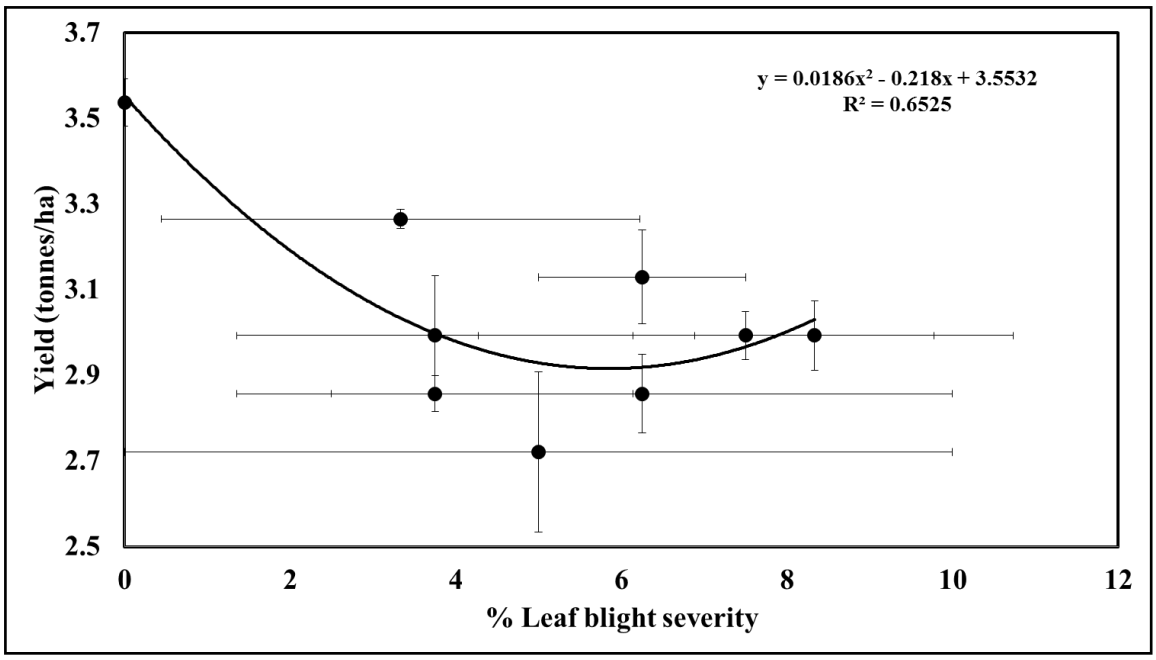

Figure 14. Relationship between yield (tonnes/ha) and % of blight leaf symptoms in soybean caused by *Cercospora kikuchii.* Bars indicates standard error between four replications.

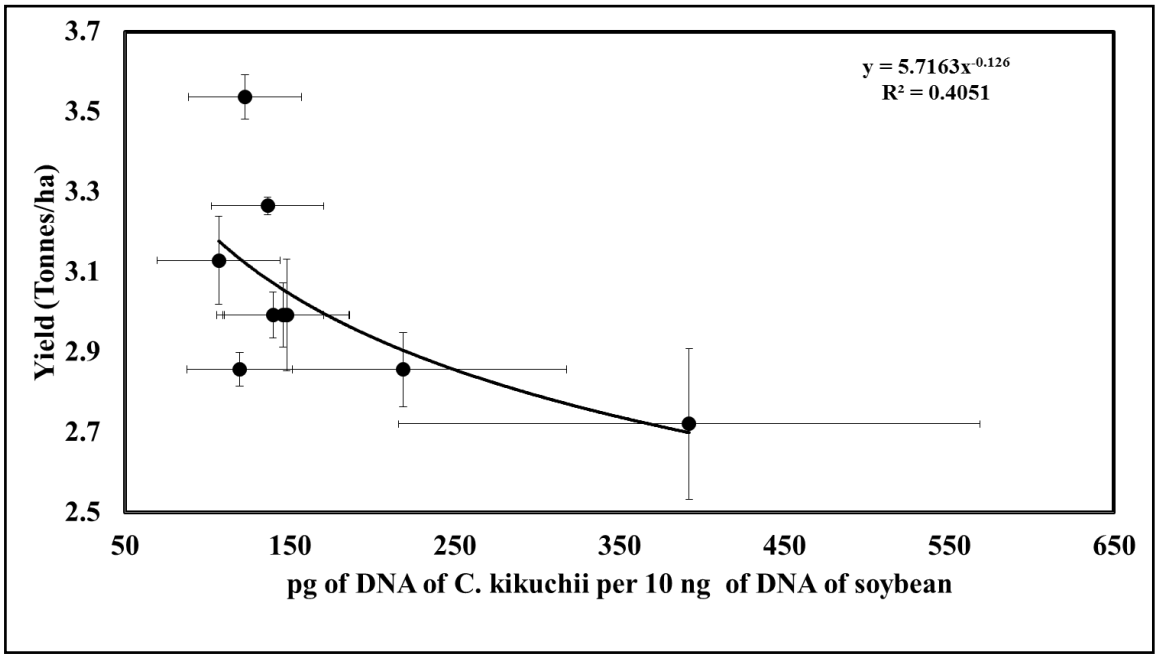

Figure 15. Relationship of yield (tonnes/ha) and biomass of *Cercospora kikuchii* in soybean in leaves. Bars indicate standard error between four replications.

4.7. Effect of Iron Concentration in Soybean Leaves on Yield

A positive relationship between iron concentration in leaves and soybean yield (Figure 16) clearly demonstrate the importance of this micronutrient not just to suppress disease, but to increase yield. The highest rate of Fe measured in leaves increased yield by 21% regardless of CLB severity and fungal biomass.

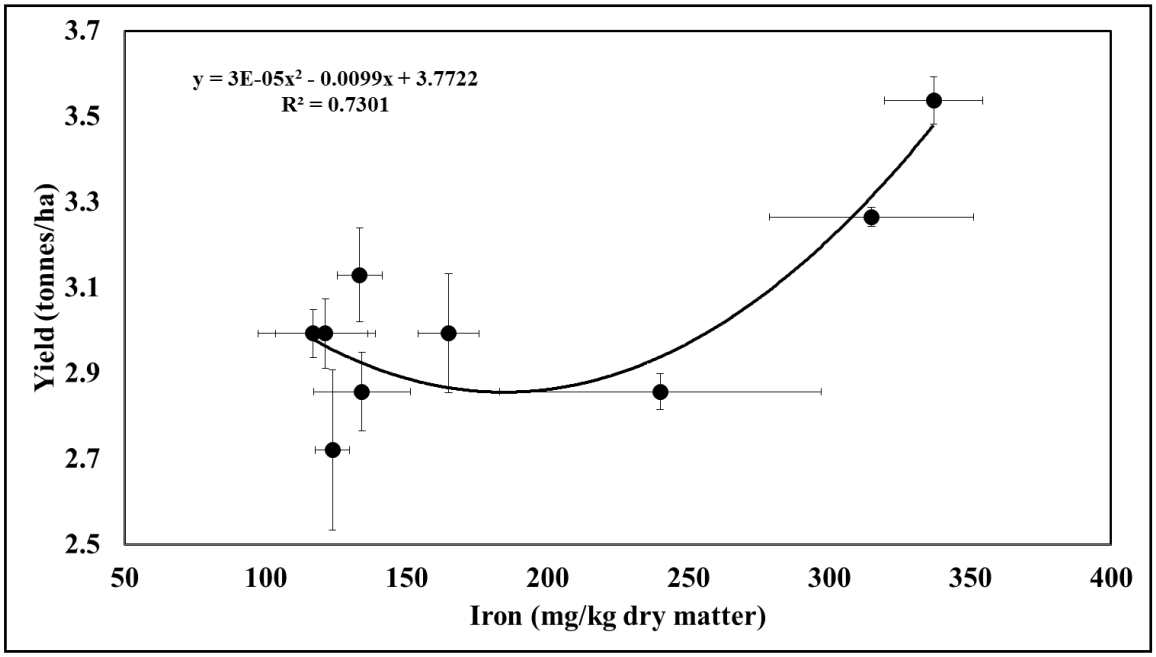

Figure 16. Relationship between soybean yield (tonnes/ha) and iron concentration in soybean leaves. Bars indicate standard error between four replications.

5. DISCUSSION

The field trial in which micronutrients were evaluated showed consistently that the highest rate of the iron-based formulation, Brandt Manni-Plex Fe (11.692 L/ha) significantly increased Fe concentration in soybean leaves as well as suppressed purple and blight symptoms of CLB. Fe-mediated CLB suppression also was documented using reagent grade iron compounds applied in field trials in 2012 and 2013 (data not published). Brandt EDTA Fe formulation did not perform was well as Manni-Plex considering the rates used in this experiment. Further experiments using higher rates of the EDTA formulation were performed during 2014 growing season. Results from this test confirmed the findings from 2013.

Tissue analyses showed that plants treated with the lowest rate of Brandt Manni-Plex Fe (MFe1, 233.27 g/ha) had the same level of Fe concentration in leaves (approximately 135 mg/kg of dry matter) as the control. On the other hand, the plants treated with MFe3 and MFe4 (466.53 g Fe/ha and 483.51 g/ha respectively) had similar foliar Fe concentrations, approximately 340 mg/kg dry matter. The concentration of Fe in plants may depend on a number of factors, including plant species, plant health, soil type and environmental conditions. Fe concentrations in plants also differ in different organs. In general, adequate tissue levels of Fe for growth and development required by plants such as soybeans is 100 mg/kg of dry matter (Taiz, 2010).

Purple and blight symptoms of CLB responded differently to the Fe treatments. Under high levels of Fe (about 350 mg/kg of dry matter), both symptoms were suppressed considerably. However, purple symptoms increased when Fe concentration

was 230 mg/kg of dry matter. It is possible to hypothesize that this Fe concentration is optimum for growth of *C. kikuchii* or for virulence. However, concentrations below 230 mg/kg of dry matter did not reduce biomass of *C. kikuchii* in leaves.

There was a significant negative relationship between blight symptoms and Fe concentration in soybean leaves, and blight was completely suppressed at an Fe concentration of 350 mg/kg of dry matter. Since qPCR results showed that biomass of *C. kikuchii* was not affected by Fe concentration, perhaps Fe interferes with pathogen virulence, and the Fe concentration must be relatively high in order to maintain the pathogen in its endophytic state. This hypothesis is supported by the fact that biomass of *C. kikuchii* was relatively high even though there were no symptoms. This confirmed the result from other studies that showed that *C. kikuchii* was detected in soybean plants during early vegetative growth stages and that the pathogen has an extended latent phase, which may be characterized as an endophytic association (Chanda, et al., 2014). This suggested that *C. kikuchii* infects soybean plants early, but the production of elicitors and virulence factors by the fungus occurs after the plant reaches reproductive growth stages.

The lack of correlation between purple and blight symptoms does not support the commonly held assumption that blight symptoms are a more severe stage of purple symptoms. These findings suggested that these symptoms may be reflective of different diseases or that the purple symptom is a plant defensive reaction and blight is a true CLB symptom. Although evidence for the former hypothesis is not presented herein, this finding has important consequences for management of CLB if confirmed. This hypothesis will be explored in further studies.

The plant defense hypothesis was formulated based on the knowledge that plants produce purple pigments in response to oxidative stress (Hahlbrock, 1981; Beggs, 1985; Li, 1993; Lois, 1994). This purpling is usually associated with anthocyanins, purple/red flavonoids, which protect plants against oxidative stresses (Li, 1993). Colorless flavonoids and other phenolics may also be involved.

Recent work done by Rodríguez-Celma and his colleagues (2013) showed that synthesis of phenylpropanoids is tightly linked to regulation of other genes encoding proteins involved in Fe uptake. Reactive Fe^{3+}, but not Fe^{2+}, is deposited at the cell wall where it accumulates and mediates the oxidative burst in wheat leaves inoculated with *Blumeria graminis* f. sp. *tritici*. The secretion of Fe^{3+} from inside to outside of the cell leads to intracellular iron depletion, which promotes the transcription of pathogenesis-related genes in concert with H_2O_2 (Liu, 2007). Bitan and his colleagues (1988), cited by Barton (1993), found that Fe deficiency, as well as infection by *Verticillium dahlia,* induced production of phytoalexins in peanuts. Medicarpin, a phytoalexin, was 18.6, 23.8 and 43.4 times higher in Fe-deficient healthy, Fe-sufficient infected, and iron-deficient infected peanuts, respectively, in comparison to the healthy control. Considering that pathogen infection by itself triggers production of ROS, and cercosporin also induces ROS production in plants (Daub, 1983), Fe may play a significant role in reducing CLB severity in soybeans. This brief review supports the hypothesis that the purple leaf symptoms may be a plant reaction to oxidative stress, and based on our results, 230 mg/kg dry matter of Fe may be an optimum concentration for plants to produce these defense compounds.

Fe is also either a component or induces the production of several antioxidant enzymes that help the plant to scavenge ROS. Fe, for example, is a component of superoxide dismutase (SOD), which constitutes the first line of defense against ROS. Since phospholipids in membranes are impermeable to O_2^- molecules, SODs are responsible for the removal of O_2^- in the compartments where O_2^- is produced, and in the process, produces H_2O_2 (Alscher, 2002). In the absence of ferritin (proteins that accommodate several thousand Fe atoms in their central cavity), Arabidopsis plants had higher levels of ROS (Ravet, 2009). Catalase and ascorbate peroxidase also are more active in tomato plants exposed to high concentration of Fe (Dasgan, 2003), and bean roots had peroxidase activity strongly depressed under Fe-deficient media (Sijmons, 1985). These two enzymes help to detoxify H_2O_2 by producing water and oxygen.

As mentioned in the introduction, Fe can bind to some fungal toxins and reduce their toxicity. *Cercospora beticola* was reported to produce a yellow pigment that is able to form a stable complex with Fe^{3+}, and this toxin also induced necrotic lesions on sugar beet leaves similar to those caused by the pathogen (reported by Eckart Schlosser and published by Wood, 1972). Biosynthesis of several toxins also is affected by Fe concentrations (Barash, 1986; cited by Barton, 1993). The cercosporin Fe-binding property and the effect of Fe on cercosporin production is not known, but if these hypothesis are confirmed in the future, it will be the first indication to suggest that cercosporin could be considered a siderophore.

Moreover, it is well known that plants under Fe deficiency have low photosynthetic activity, but leaves absorb more light in each chlorophyll molecule than is

necessary for photosynthesis (Abadia, 1999). This increased light absorption results in a high risk of photooxidative damage in leaves exposed to high solar radiation. This plant damage maybe even worse in soybean plants infected with the cercosporin-producing fungal species *C. kikuchii*. This is another reason why Fe concentrations need to be high in soybean plants to avoid oxidative damage, and prevent yield reduction.

Even though they were not correlated, purple and blight symptoms, as well as *C. kikuchii* biomass were associated with lower grain yields. This suggests that the physiological mechanism by which each disease reduces yield is different. Results also showed that, regardless of the symptoms and fungal biomass, yield was greatly increased when Fe concentrations were high.

Other Cercospora species have pathogenicity mechanisms similar to *C. kikuchii*. Results from this research may help to generate a general protocol to manage Cercospora diseases in other important crops such as banana, coffee, corn, grapes, peanut, rice, sugarcane and sweetpotato.

These Fe recommendations also may be used by farmers in developing countries where proper plant heath management is limited because of economic constraints. While complete disease control may not be achieved, fungicide applications might be minimized, from several per season to just a single application. Moreover, recommendations for soybean producers that provide inexpensive and environmentally friendly protocols for managing CLB could be better developed.

REFERENCES

Abadía, J., Morales, F. and Abadía, A. 1999. Photosystem II efficiency in low chlorophyll, iron-deficient leaves. Plant Soil 215: 183–192.

Alscher, R. G., E. Neval., and Heath L. S. 2002. Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany 53:1331-1341.

Babitha, M. P. 2002. Differential induction of superoxide dismutase in downy mildew resistant and -susceptible genotypes of pearl millet. Plant Pathol. 51: 480–486

Barash, I., and Manulis, S. 1986. Iron siderophores and Plant Diseases (T. R. Swin-burn, ed.). Plenum Press, New York. 273-281.

Barton, L. L., and Hemming, B. C. 1993. Iron chelation in plants and soil microorganisms. Academic Press, Inc. San Diego, California.

Beggs, C. J., Stoltzer-Jehle, A., and Wellman, E. 1985. Isoflavonoid formation as an indicator of UV stress in bean (P*haseolus vulgaris* L.) leaves: The significance of photo-repair in assessing potential damage by increased UV-B radiation. Plant Physiol. 79: 630-634

Berger, R. D. 1973. Disease progress of Cercospora apii resistant to benomyl. Plant Disease Reporter. 57: 837 – 840

Bitan, N. 1988.Effect of iron on *Vertililium wilt* in peanuts. M.Sc. Thesis, Tel-Aviv University, Ramat Aviv, Israel.

Boyette, C. D., and Walker, H. L. 1985. Production and storage of inoculum of *Cercospora kikuchii* for field studies. Phytopathology. 75(2): 183-184.

Cai, G., and Schneider, R. W. 2008. Population structure of *Cercospora kikuchii*, the causal agent of Cercospora leaf blight and purple seed stain in soybean. Phytopathology 98:823-829.

Cai, G., and Schneider, R. W. 2009. Assessment of Lineages of *Cercospora kikuchii* in Louisiana for Aggressiveness and Screening Soybean Cultivars for Resistance to Cercospora Leaf Blight. Plant Disease 93: 868-874.

Callahan, T., Rose, M., Meade, M., Ehrenshaft, M., and Upchurch, R. 1999. *CFP*, the putative cercosporin transporter of *Cercospora kikuchii*, is required for wild type cercosporin production, resistance, and virulence on soybean. Mol. Plant-Microbe Interact. 12:901-910.

Chanda, A. K., Ward, N. A., Robertson, C. L., Chen, Z.Y., and Schneider, R. W. 2014. Development of a quantitative polymerase chain reaction detection protocol for

Cercospora kikuchii in soybean leaves and its use for documenting latent infection as affected by fungicide applications. Phytopathology. 104(10):1118-24.

Chen, M. D., Lyda, S. D., and Halliwell, R. S. 1979. Environmental factors influencing growth and sporulation of *Cercospora kikuchii*. Mycologia. 71:1150-1157.

Clark, E. M., Backman, P. A., Rodriguez-Kabana, R. 1974. Cercospora arachidicola and Cercosporidium personatum tolerance to benomyl and related fungicides in Alabama peanut fields. Phytopathology. 64: 1476 – 1477

Dasgan, H. Y., Ozturk, L., Abak, K. and Cakmak, I. 2003. Activities of iron-containing enzymes in leaves of two tomato genotypes differing in their resistance to Fe chlorosis. J. Plant Nutr. 26:1997–2007.

Datnoff, L. E., Elmer, W. H., and Huber, D. M. 2007. Mineral Nutrition and Plant. Disease. St. Paul, MN: APS Press. 278.

Daub, M. E., and Briggs, S. P. 1983. Changes in tobacco cell membrane composition and structure caused by the fungal toxin, cercosporin. Plant Physiol. 71:763-766.

Daub, M. E., and Chung, K. R. 2007. Cercosporin: A Photoactivated Toxin in Plant Disease. Online. APSnet Feature. http://www.apsnet.org/online/feature/cercosporin/

Dellagi, A., Rigault, M., Segond, D., Roux, C., Kraepiel, Y., Cellier, F., Briat, J.F., Gaymard, F., and Expert, D. 2005. Siderophore-mediated upregulation of Arabidopsis ferritin expression in response to *Erwinia chrysanthemi* infection. Plant J. 43: 262–272

Elmer, W., and Datnoff, L. E., 2014. Mineral Nutrition and Suppression of Plant Disease. Encyclopedia pof Agriculture and Food Systems 4:231-244.

Fajola, A. O. 1978. Cercosporin, a phytotoxin from *Cercospora* spp. Physiological Plant Pathology. 13:157-164.

Ferrin, D. M., Hollier, C. A., Hoy, J. W., and Overstreet, C. 2013. Plant Disease Management Guide. Online. http://www.lsuagcenter.com/NR/rdonlyres/87A3F38A-BBB6-487B-A3B5-80E0E7D51818/90525/12013PlantDiseaseManagementGuideTEXT.pdf

Freeman, J. L., Garcia, D., Kim, D., Hopf, A., and Salt, D. 2005. Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in *Thlaspi nickel* hyperaccumulators. Plant Physiol. 137:1082–1091

Fujita, Y. 1990. Ecology and control of purple seed stain of soybean caused by *Cercospora kikuchii*. Bull. Tohoku Natl. Agric. Exp. Stn. 81:99-109.

Gardner, M. W. 1926. Indiana plant diseases, 1924. Proc. Ind. Acad. Sci. 35: 237-257.

Georgopoulos, S. G., Dovas, C. 1973. A serious outbreak of strains of cercospora beticola resistant to benzimidazole fungicides in northern Greece. Plant Disease Reporter. 62: 205 – 208

Ghanmi, D., McNally, D. J., Benhamou, N., Menzies, J. G., and Bélanger, R. R. 2004. Powdery mildew of *Arabidopsis thaliana*: a pathosystem for exploring the role of silicon in plant-microbe interactions. Physiol. Mol. Plant Pathol., 64:189-199.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43: 205–227

Graham, M. Y. and Graham, T. L. 1991. Rapid accumulation of anionic peroxidases and phenolic polymers in soybean cotyledon tissues following treatment with *Phytophthora megasperma* f. sp. *glycinea* wall glucan. Plant Physiol., 97:1445-1455.

Hahlbrock, K. 1981. Flavonoids. In Biochemistry of Plants, Vol. 7, P.K. Stumpf and E.E. Conn, eds (New York: Academic Press), 425-456.

Hepperly, P. R., and Sinclair, J. B. 1981. Relationships among *Cercospora kikuchii*, other seed mycoflora, and germination of soybeans in Puerto Rico and Illinois. Plant Disease. 65: 130-132.

Ilyas, M. B., Dhingra, O. D., Ellis, M. A., and Sinclair, J. B. 1975. Location of mycelium of *Diaporthe phaseolorum* var. sojae and Cercospora kikuchii in infected soybean seeds.

Imazaki, I., H. Iizumi, K., Ishikawa, M., Sasahara, N., Yasuda, and S. Koizumi. 2006. Effects of thiophanate-methyl and azoxystrobin on the composition of *Cercospora kikuchii* populations with thiophanate-methyl-resistant strains. Journal of General Plant Pathology. 72: 292-300.

Imazaki, I., Iizumi, H., Ishikawa, K., Sasahara, M., Yasuda, N., and Koizumi, S. 2006. Effects of thiophanate-methyl and azoxystrobin on the composition of *Cercospora kikuchii* populations with thiophanate-methyl-resistant strains. Journal of General Plant Pathology. 72: 292-300.

Joya, C. 1982. Identification of sigatoka pathogens and monitoring for benzimidazole resistance. Phytopatholog.y 72, 453

Karaoglanidis, G. S., Ioannidis, P. M., and Thanassoulopoulos, C. C. 2000. Reduced sensitivity of *Cercospora beticola* isolates to sterol-demethylation-inhibiting fungicides. Plant Pathology. 49: 567-572.

Kilpatrick, R. A. and Johnson, H. W. 1956. Sporulation of Cercospora species on carrot leaf decoction agar. Phytopathology. 46: 180-181.

Kirk, W. W., Hanson, L. E., Franc, G. D., Stump. W. L., Gachango, E., Clark, G., and Stewart, J. 2012. First report of strobilurin resistance in *Cercospora beticola* in sugar beet (Beta vulgaris) in Michigan and Nebraska, USA. New Disease Reports. 26:3. Available online: http://dx.doi.org/10.5197/j.2044-0588.2012.026.003.

Kirkby, E. 2012. Introduction, Definition and Classification of Nutrients. In Marschner's Mineral Nutrition of Higher Plants. Marschner, P. Elsevier Ltd. 3-5.

Kuyama, S. and Tamura, T. 1957. Cercosporin. A pigment of *Cercosporina kikuchii* Matsumoto et Tomoyasu. I. Cultivation of fungus, isolation and purification of pigment. Journal of the American Chemistry Society. 57: 5725-5726.

Levy, R., Padgett, B., Lofton, J., Harrell, D., Stevens, J., Stephenson, D., Viator, S., Udeigwe, T. K., Hollier, C., Haggard, B. J., Buckley, B., Davis, J., and Bollich, P. 2013. Soybean Variety Yields and Production Practices. Online. http://www.lsuagcenter.com/NR/rdonlyres/A3A3A6A7-0AFC-4106-9B27-2D2E55F93CDA/89921/pub2269SoybeansVariety2013.pdf

Li, J., Ou-Lee, T. M., Raba, R., Amundson, R.G., and Last, R. L. 1993. Arabidopsis favonoid mutants are hypersensitive to UV-B irradiation. Plant Cell 5:171-179.

Littrell, R. H. 1974. Tolerance in Cercospora arachidicola to benomyl and related fungicides. Phytopathology 64: 1377 - 1378

Liu, G., Greenshields, D. L., Sammynaiken, R., Hirji, R. N., Selvaraj G., and Wei, Y. 2007. Targeted alterations in iron homeostasis underlie plant defense responses. J. Cell Sci. 120:596-605

Lois, R. 1994. Accumulation of UV-absorbing flavonoids induced by UV-B radiation in *Arabidopsis thaliana* L. I. Mechanisms of UV-resistance in *Arabidopsis.* Planta 194: 498-503.

LSU Agcenter. Louisiana Summary Agricultural and Natural Resources 2013. LSU AgCenter's Louisiana Cooperative Extension Service. 2013. Accessed on http://www.lsuagcenter.com/agsummary

Maksymiec, W., Wianowska, D., Dawidowicz, A. L., Radkiewicz S, Mardarowicz, M., and Krupa, Z. 2005. The level of jasmonic acid in *Arabidopsis thaliana* and *Phaseolus coccineus* plants under heavy metal stress. J. Plant Physiol. 162:1338–1346

Marcshner, H. 1991. Root-induced changes in the availability of micronutrients in the rizosphere. In Plant Roots, the Hidden H., Waisel, Y., Eshel, A., and Kafkafi, U. (New York: Marcel Dekker, Inc.), 503-528.

Martin, K. F. and Walters, H. J. 1982. Infection of soybean by *Cercospora kikuchii* as affected by dew temperature and duration of dew periods. Phytopathology. 72: 974.

Moore, S. H., and Wolcott, M. C. 2000. Using yield maps to create management zones in field crops. La. Agric. 43(3):12-13.

Murakishi, H. H. 1951. Purple seed stain of soybean. Phytopathology. 41(4): 305-318.

Orth, C. E. and Schuh, W. 1992. Histological comparison of latent and active infections of soybean foliage by *Cercospora kikuchii*. Plant Disease. 78(7): 661-664.

Ravet, K., Touraine, B., Boucherez, J., Briat, J., Gaymard F., and Cellier F. 2009. Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. The Plant Journal 57: 400–412

Resende, R.S., Rodrigues, F.A., Costa, R.V., and Silva, D.D. 2013. Silicon and fungicide effects on anthracnose in moderately resistant and susceptible sorghum lines. Journal of Phytopathology 161: 11–17

Rodríguez-Celma, J., Lin, W., Fu, G., Abadía, J., López-Míllán, A. and Schmidt, W. 2013. Mutually exclusive alterations in secondary metabolism are critical for the uptake of insoluble iron compounds by arabidopsis and *Medicago truncatula* Plant Physiology Preview. Published on June 5,

Rolke, Y., Liu, S., Quidde, T., Williamson, B., Schouten, A., Weltring, K. M., Siewers, V., Tenberge, K. B., Tudzynski, B., and Tudzynski, P. 2004. Functional analysis of H_2O_2-generating systems in *Botrytis cinerea*: the major Cu-Zn-superoxide dismutase (BCSOD 1) contributes to virulence on French bean, whereas a glucose oxidase (BCGOD1) is dispensable. Mol. Plant Pathol. 5: 17–27

Roy, K. W., and Abney, T. S. 1976. Purple seed stain of soybeans. Phytopathology. 66: 1045-1049.

Sakai, Y. 1999. Optimum timing of thiophanate-methyl application in the control of purple seed stain on soybean seed with consideration of development of resistance. Ann. Phytopathol. Soc. Jpn. 65: 510-514.

Schneider, R. W., Bollich, P. K., and Harville, B. G. 2003. Evaluation of soybean cultivars for reactions to three foliar diseases. B&C Tests (online) 18:P003 (DOI:10.1094/BC18)

Schuh, W. 1991. Influence of temperature and leaf wetness period on conidial germination in vitro and infection of *Cercospora kikuchii* on soybean. Phytopathology. 81: 1315-1318.

Schultz, B. 2006. Plant Breeder Turns Focus on Cercospora. Soybean & Grain Research & Promotion Borad Report. Aug. 2006: 3

Secor, G. A., Rivera, V. V., Khan, M. F. R., and Gudmestad, N. C. 2010. Monitoring fungicide sensitivity of *Cercospora beticola* of sugar beet for disease management decisions. Plant Disease. 94(11): 1272-1282.

Seebold, K. W., Kucharek, T. A., Datnoff, L. E., Correa-Victoria, F. J., and Marchetti, M. A. 2001. The influence of silicon on components of resistance to blast in susceptible, partially resistant and resistant cultivars of rice. Phytopathology 91:63–69.

Sherwin, H. S. and Kreitlow, K. W. 1952. Discoloration of soybean seeds by the frogeye fungus, *Cercospora sojina*. Phytopathology. 42: 568-572.

Sijmons, P. C., Kolattukudy, P. E. and Bienfait, H. F. 1985. Iron-deficiency decreases suberization in bean roots through a decrease in suberin-specific peroxidase activity. Plant Physiol. 78: 115–120.

Sinclair, J. B., and Backman, P. A. 1989. Compendium of Soybean Diseases. Third edition. American Phytopathological Society, St. Paul, MN. 100p.

Suzuki, K. 1921. Studies on the cause of "Shihan" of soybean. Chosen Nakaiho 16:24-28.

Taiz, L., and Zeiger, E. 2010. Plant Physiology. Sinauer Associates, Inc.

Upchurch, R. G., Walker, D. C., Rollins, J. A., Ehrenshaft, M., and Daub, M. E. 1991. Mutants of *Cercospora kikuchii* altered in cercosporin synthesis and pathogenicity. Applied and Environmental Microbiology. 57: 2940-2945.

USDA, Oil Crops Outlook. 2014. Economic Research Service. By Mark Ash. Report OCS-14h. USDA August 14, 2014. 11

USEPA 3050B, 1996. Acid Digestion of Sludges, Solids and Soils. In SW-846 Pt 1 Office of Solid and Hazardous Wastes, USEPA, Cincinnati, OH.

Velicheti, R. K. and Sinclair, J. B. 1994. Production of cercosporin and colonization of soybean seed coats by *Cercospora kikuchii*. Plant Disease. 78(4): 342-346.

Velicheti, R. K., Kollipara, K. P., Sinclair, J. B., and Hymowitz, T. 1992. Selective degradation of proteins by *Cercospora kikuchii* and *Phomopsis longicolla* in soybean seed coats and cotyledons. Plant Disease. 76:779-782.

Wallace, A., and Jones, J. B. 1993. Sample preparation and determination of iron in biological materials. In Barton, L. L., Hemming, B. C., Iron chelation in plants and soil microorganisms. Academic Press. 447-464.

Walters, H. J. 1980. Soybean leaf blight caused by *Cercospora kikuchii*. Plant Disease. 64(10): 961-962.

Wilcox, J. R. and Abney, T. S. 1973. Effects of *Cercospora kikuchii* on soybeans. Phytopathology. 63:796-797.

Wojtaszek, P., Stobieck, M., and Gulewicz, K. 1993. Role of nitrogen and plant growth regulators in the exudation and accumulation of isoflavonoids by roots of intact white lupin (*Lupinus albus* L.) plants. J. Plant Physiology. 142: 689-694.

Wood, R. K. S., Ballio, A., and Graniti, A. 1972. Phytotoxins in Plant Diseases. London: Academic Press.

Wrather, J. A., and Koenning, S. R. 2009. Effects of diseases on soybean yields in the United States 1996 to 2007. Online. Plant Health Progress doi:10.1094/PHP-2009-0401-01-RS.

Wrather, J. A., Anderson, T. R., Arsyad, D. M., Gai, J., Ploper, L. D., Porta-Puglia, A., Ram, H. A., and Yorinori, J. T. 1997. Soybean disease loss estimates for the top 10 soybean producing countries in 1994. Plant Dis. 81:107- 110.

Yeh, C. C. and Sinclair, J. B. 1982. Effect of *Cercospora kikuchii* on soybean seed germination and its interaction with *Phomopsis sp*. Phytopathology. 105: 265-270.

You, B. J, Lee, M. H., and Chung, K. R. Production of cercosporin toxin by the phytopathogenic Cercospora fungi is affected by diverse environmental signals. Canadian Journal of Microbiology, 2008, 54(4): 259-269

Zhang, G. R. and Bradley, C. A. 2011. Development of baseline *Cercospora sojina* sensitivity levels to quinone outside inhibitor fungicides and monitoring for resistance. Proceedings of the Southern Soybean Disease Workers 2011. p. 8.

VITA

Eduardo Chagas Ferreira da Silva, son of Camilo de Lelis Ferreira da Silva and Rita de Cassia Chagas was born in 1987 in Viçosa, Minas Gerais, in Brazil. He received his B.S. degree in Agronomy Engineering at Federal University of Viçosa (UFV), Brazil in 2010. As undergraduate, he worked for 3 years at Germplasm Bank of the Center for Studies in Horticulture at UFV selecting tomato cultivars resistant to *Phytophthora infestans*, under Dr. Derly Heriques. In addition, he was Marketing Director at AgroPlan-UFV and consultant to rural entrepreneurs. In 2009, he received the Mirror Entrepreneur award for job development in the company. He also developed research procedures at the Plant-Pathogen Interaction Laboratory evaluating induced resistance in rice using Silicon, under Dr. Fabricio Ávila. In 2011 Eduardo worked at Speedling Incorporated as Grower Assistant in Georgia and Florida, through The Ohio State University. Currently he is a Graduate Research Assistant at Soybean Pathology Laboratory at Louisiana State University Agricultural Center (LSU-AgCenter), where he is researching the effect of micronutrients on Cercospora Leaf Blight in Soybean, Asian Soybean Rust and plant defense mechanisms, under Dr. Raymond Schneider.