MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 72-2015
2015 Annual Report

Title: Managing charcoal rot of soybean through supplementing secondary nutrients

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Summary

Charcoal rot as a disease has been documented from over 500 host plants and can cause severe yield loss in years conducive to disease development. *Macrophomina phaseolina*, the fungus that causes charcoal rot, has a vast geographical distribution, including tropical and subtropical climates, but is most often observed in hot arid locations where there is little rainfall or parts of fields that do not have adequate moisture (Abawi and Pastor-Corrales 1990; Diourte et al. 1995; Gray et al. 1990; Kaur et al. 2011).

Macrophomina phaseolina is primarily soilborne, with heterogeneous host specificity, which means the fungus has the ability to infect monocots as well as dicots (Su et al. 2001). In addition, *M. phaseolina* has a non-uniform distribution within the soil profile (Mayek-Perez et al. 2001; Su et al. 2001). Soybean is an important host of charcoal rot, also referred to as seedling blight, dry root rot, ashy stem blight, and dry weather wilt (Mengistu et al. 2011). Not only can charcoal rot cause yield loss as a result of infection, but the disease can significantly reduce soybean seed quality in years when the infection is severe (Bowers and Russin 1999). In addition to infecting soybean, in MS charcoal rot can result in yield loss in corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), grain sorghum (*Sorghum bicolor* L. Moench), and sweetpotato (*Ipomoea batatas* (L.) Lam.). However, yield loss in other hosts is rare in MS and soybean is considered to be the predominant host of the fungus. Since there are additional hosts that can become infected by the fungus, managing the disease through rotational practices is difficult.

Symptoms of charcoal rot typically appear during hot, dry weather when plants are under drought stress; however, the disease can also occur in irrigated fields as a result of delayed or improper irrigation methods. Non-irrigated fields with random areas of prematurely dead plants that appear stunted when compared to the surrounding plants are key to identifying charcoal rot in soybean fields. Aboveground symptoms of charcoal rot on soybean generally appear after flowering (R1), particularly between R5 (beginning seed) and R7 (beginning maturity) (Mengistu et al. 2008). Diseased plants may wilt and prematurely die, with leaves and petioles remaining attached to plants (Mengistu et al. 2008; Smith and Wyllie 1999). Infected plants desiccate and the roots can appear decayed with a shredded appearance (Ilyas and Sinclair 1974). Affected plants mature prematurely, normal leaf abscission is not initiated, foliage may appear chlorotic, foliage may prematurely senesce, and pods generally fail to fill completely (Wyllie 1988). Visible symptoms of the disease in the field are most apparent under conditions that reduce plant vigor, such as poor soil fertility, high seeding rates (Pearson et al. 1984; Sinclair and Backman 1989), low soil water content (Ali and Ghaffar 1991; Kendig et al. 2000; Meyer et al. 1974; Sheikh and Ghaffar 1979), high temperatures (Odvody and Dunkle 1979; Mihail 1989), and root injury (Canaday et al. 1986).

Materials and Methods

Objective 1: Evaluate the response of soybean to *M. phaseolina* with the addition of soil-applied secondary nutrients in a controlled environment.

Greenhouse inoculum preparation. For the purposes of inoculum production (greenhouse), a sand corn-meal medium will be prepared and used for inoculum as described by Kirkpatrick et al. (2006). Briefly, 400 g of non-silicone sand, 12 g of freshly ground corn meal and 80 ml of distilled water will be combined in a 1,000 ml flask. Flasks will be autoclaved at 121°C for 15 min on three consecutive days prior to infesting with *M. phaseolina*. Once cooled, 25, 5 mm disks of a 5 day old *M. phaseolina* culture as described above will be added to flasks. Flasks will be incubated in the laboratory at ambient temperature (22°C) and a 12 h light:dark cycle for 14 days. Flasks will be agitated daily by hand to evenly distribute the inoculum and provide even growth throughout the substrate.

Rate-response greenhouse trial. Greenhouse experiments will be established to determine the most effective rate of nutrition to suppress *M. phaseolina* in vivo. Soil characterized as a Bosket very fine sandy loam will be collected from a field that will be used for field trials and steam sterilized for 72 h at 80°C. Experiments will be conducted using a randomized complete block design in a 3 × 6 × 3 factorial arrangement with two non-treated controls (n=56 treatments). The three treatments consist of calcium, magnesium and calcium + magnesium. Six different rates, 28, 112, 280, 560, 840, and 1,120 kg/ha of each product (either gypsum ($CaSO_4$) or Epsom salts ($MgSO_4$)) relating to 120, 511, 1,279, 2,557, 3,836, and 5,114 kg/ha of Ca and 283, 1,132, 2,831, 5,662, 8,493, and 11,325 kg/ha of Mg. Treatments will be applied at each of three timings: at plant, R1 and at plant followed by (fb) R1. The non-treated controls will consist of one without *M. phaseolina* inoculum (completely non-treated) and one without nutrition and inoculum for the purposes of comparison.

A charcoal rot susceptible hybrid, Pioneer 46T21R, will be used for greenhouse trials. Inoculum and treatment to be applied at planting will be incorporated into the soil with a small hand rake no more than 5 cm deep and 10 seed will be sown into 5 gallon pots in a uniform pattern. Once plants have emerged, days to emergence ratings will be recorded and plants will be thinned to 5 plants per pot to decrease competition between plants, but to allow one plant per sampling period per replicate pot. Vigor ratings will be recorded at V2 to V4 timing using a 1 to 9 (1 = healthy, 9 = dead) scale and plant heights will be recorded at the V3, R1, R3, R5, and R7 growth stages.

The R1 application will be cultivated into pots so as not to disturb the roots and plants will be irrigated every 1 to 2 days until physiological maturity (R8). Individual plant sampling will initiate at R3 and continue at R5, R7 and R8. Individual plants will be gently removed from pots and the roots will be cut off approximately 2.5 cm above the soil line, washed thoroughly with a gentle stream of tap water, air dried and stored in paper bags in a cold room, Munster Corporation Dehumidification Division (Amesbury, MA) (5°C; 35% RH) at the Delta Research and Extension Center, Stoneville, MS. Entire soybean roots will be rated using a 0 to 5 scale for disease severity using a modified version adapted from Baird et al. (1996). Roots will be rated for lesions caused by the pathogen where root disease is based on a 0 to 5 scale with 0 being no root symptoms and 5 being dead plants. Plants exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5. Reductions in the overall root mass will be assessed using an Epson Perfection

V700 Scanner and WinRhizo software (Regent Instruments, Inc., Quebec, Canada). The remaining plant shoots will be stored in paper bags in cold storage. Pod counts and number of soybean seed per pod will occur at harvest along with seed weight recorded in grams. The individual root and shoot samples will be ground using a Wiley mill (No. 5, Thomas Wiley Mills, Thomas Scientific, Swedesboro, NJ), fitted with a 1-mm mesh screen and the mill will be cleaned between each sample using a 10 gallon Shop-Vac.

Colony forming units. Colony forming units will be determined for each sample by mixing ground root tissue with 1% bleach solution for 3 min in a Waring mini-sample blender (Fisher Scientific, Pittsburgh, PA), plant tissue was collected using a 45 um pore size screen and rinsed with 10 ml of distilled water and combined with 100 ml of molten, cooled agar (≈ 55°C) to be equally dispensed into five plates (Mengistu 2008). The agar was amended with 100 mg of rifampicin, 224 mg a.i. metalaxyl (as Apron XL LS, Syngenta Crop Protection, Greensboro, NC), 1 ml of tergitol, 160 mg of chloroneb (as Teremec SP, Advanced Turf Solutions, Fishers, IN), and 250 mg of streptomycin sulfate (Henn, *unpublished data*). Plates will be incubated for 6 days at 29°C in the dark. CFU counts will be taken per plate and CFU per gram calculations according to Mengistu 2008 will be recorded.

Greenhouse trials. Upon determination of the most beneficial rate of nutrition greenhouse trials will consist of 11 treatments using a randomized complete block arrangement as a 3 × 3 factorial with two non-treated controls with 5 replications. Five gallon pots will be used as outlined above. The variables to be measured for greenhouse trials will be conducted as outlined above. Data analysis will be conducted using SAS.

	Greenhouse field repeat treatments
1	Non-Inoculated- untreated
2	Inoculated- untreated
3	Calcium (pre-plant) (1000 lb/acre)
4	Calcium (at-plant) (1000 lb/acre)
5	Calcium (pre-plant + at plant) (1000 lb/acre)
6	Magnesium (pre-plant) (1000 lb/acre)
7	Magnesium (at-plant) (1000 lb/acre)
8	Magnesium (pre-plant + at-plant) (1000 lb/acre)
9	Calcium + Magnesium (pre-plant) (1000 lb/acre)
10	Calcium + Magnesium (at-plant) (1000 lb/acre)
11	Calcium + Magnesium (pre-plant + at-plant) (1000 lb/acre)

Objective 2: Determine the efficacy of secondary nutrients in the suppression of *M. phaseolina*.

Field inoculum preparation. A culture of *M. phaseolina* originating from Bolivar County will be grown in a PDA/potato dextrose broth (PDB) (8g PDA: 20g PDB) mixture for 7 days. Four hundred and fifty four grams of Pearl millet will be soaked in 5.7 liters of distilled water along with 40 g of sugar, 4 g of yeast extract and 4 g of tartaric acid in a Nalgene tub (50.8 × 43.18 × 12.7 cm) for 24 hours. After 24 hours the millet will be drained and put into an autoclave bag (30.5 × 60.9 cm; Bel-Art Products, Wayne, NJ) with 118 ml of the water mixture. The bags

containing the pearl millet will subsequently be autoclaved for 30 minutes (121°C) for 3 consecutive days. The 7 day old *M. phaseolina* broth solution will be ground using a Waring blender on high speed for 1 min and poured into the autoclaved millet bags and allowed to grow for 2 weeks at ambient temperature and approximately 12 h of light: dark. Millet will then be spread on clean paper and allowed to dry at ambient conditions. Once dried the millet will be run through a No. I-P 8/64" round screen (Seedburo Equipment Company, Chicago, IL) to prevent clumping.

Field trials. Two field trials will be established at the DREC in Stoneville, MS. Trials will be conducted as a randomized complete block design with a 3 × 3 factorial arrangement and two non-treated controls (n=11) with 5 replications and a split-plot constraint by variety. Plots will be 12.2 meters long and 4 rows wide on a 1.02 meter row spacing. One trial will be planted to a charcoal rot susceptible variety, Pioneer 46T21R, and the second trial will be planted to a charcoal rot moderately resistant variety, Pioneer 49T80. Thirty six seed per meter will be planted and 100 ml of *M. phaseolina* inoculum will be applied to each plot row at planting in-furrow through the cones on the plot planter. Once plants have emerged, days to emergence ratings will be recorded and stand counts will be taken.

Vigor ratings will be recorded at V2 using a 1 to 9 scale (1= healthy and 9 = dead plants) and plant heights will be recorded at V3, R1, R3, R5, and R7 growth stages. The nutrient application will be distributed with fertilizer spreaders and incorporated into the top layer of the soil with a four row hipper (W&A Manufacturing Co., Pine Bluff, AR). Root and shoot sampling will occur from rows 1 and 4 beginning at R3 and will continue at R5, R7 and harvest (R8). Pod counts and number of soybeans per pod will occur at harvest. Yield will be harvested from the center two rows of each plot and adjusted to 13% moisture. Data analysis will be conducted using SAS.

Roots will be rated for disease severity on a 0 to 5 scale looking at root lesions caused by the pathogen and decreased root mass. Root ratings will be conducted using a modified scale as outlined by Baird et al. (1996) by considering root lesions. Plants exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5. Reductions in the overall root mass will be assessed using an Epson Perfection V700 Scanner and WinRhizo software (Regent Instruments, Inc., Quebec, Canada). The individual root and shoot samples will be ground using a Wiley mill (No. 4, Thomas Wiley Mills, Thomas Scientific, Swedesboro, NJ), fitted with a 1-mm mesh screen and the mill will be cleaned between each sample using a 10 gallon Shop-Vac.

Colony forming units. Colony forming units will be determined for each sample by mixing ground root tissue with 1% bleach solution for 3 min in a Waring mini-sample blender (Fisher Scientific, Pittsburgh, PA), plant tissue was collected using a 45 um pore size screen and rinsed with 10 ml of distilled water and combined with 100 ml of molten, cooled agar (≈ 55°C) to be equally dispensed into five plates (Mengistu 2008). The agar was amended with 100 mg of rifampicin, 224 mg a.i. metalaxyl (as Apron XL LS, Syngenta Crop Protection, Greensboro, NC), 1 ml of tergitol, 160 mg of chloroneb (as Teremec SP, Advanced Turf Solutions, Fishers, IN), and 250 mg of streptomycin sulfate (Henn, *unpublished data*). Plates will be incubated for 6 days at 29°C in the dark. CFU counts will be taken per plate and CFU per gram calculations according to Mengistu 2008 will be recorded and data will be analyzed using SAS.

	Field Treatments
1	Non-Inoculated- untreated
2	Inoculated- untreated
3	Calcium (pre-plant) (1000 lb/acre)
4	Calcium (at-plant) (1000 lb/acre)
5	Calcium (pre-plant + at plant) (1000 lb/acre)
6	Magnesium (pre-plant) (1000 lb/acre)
7	Magnesium (at-plant) (1000 lb/acre)
8	Magnesium (pre-plant + at-plant) (1000 lb/acre)
9	Calcium + Magnesium (pre-plant) (1000 lb/acre)
10	Calcium + Magnesium (at-plant) (1000 lb/acre)
11	Calcium + Magnesium (pre-plant + at-plant) (1000 lb/acre)

2013-2015 Results

Greenhouse results

2013-2014 rate response trial. Although additional agronomic evaluations were assessed throughout the season no significant differences between treatments were observed. However, seed weights were analyzed using PROC GLM in SAS with means separated using Fisher's LSD at a 95% confidence level. Results suggest a 1000 lb. per acre rate of either calcium or magnesium alone and in combination yielded the greatest seed weight over the control treatment (Figure 1., Figure 2., and Figure 3.). Therefore this rate of nutrients was used for future greenhouse and field research.

None of the three application timings were significantly different across treatments therefore no decisions could be made as to which provided the greatest benefit from this data.

Fig.1. Seed weight per plant with the addition of calcium

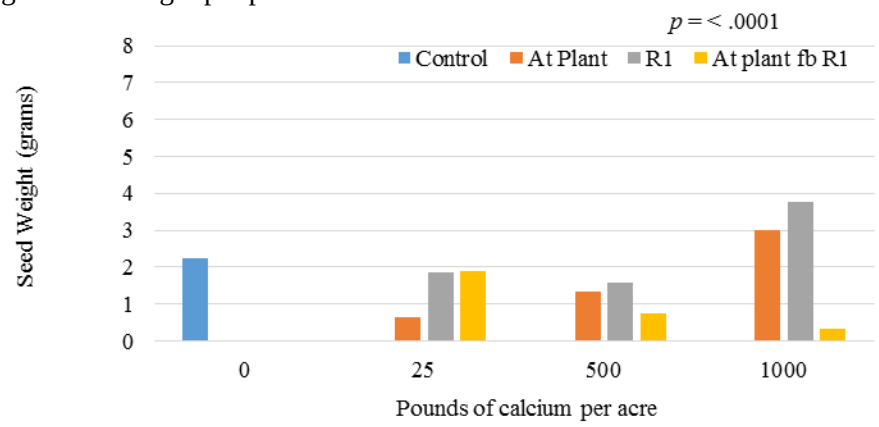


Fig.2. Seed weight per plant with the addition of magnesium

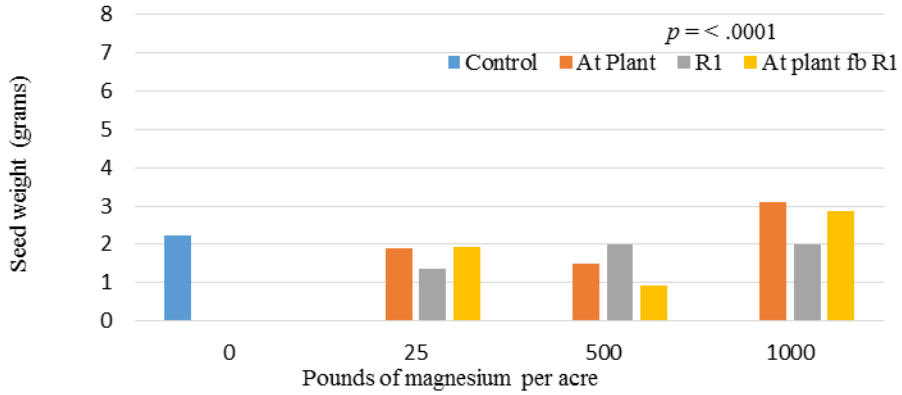
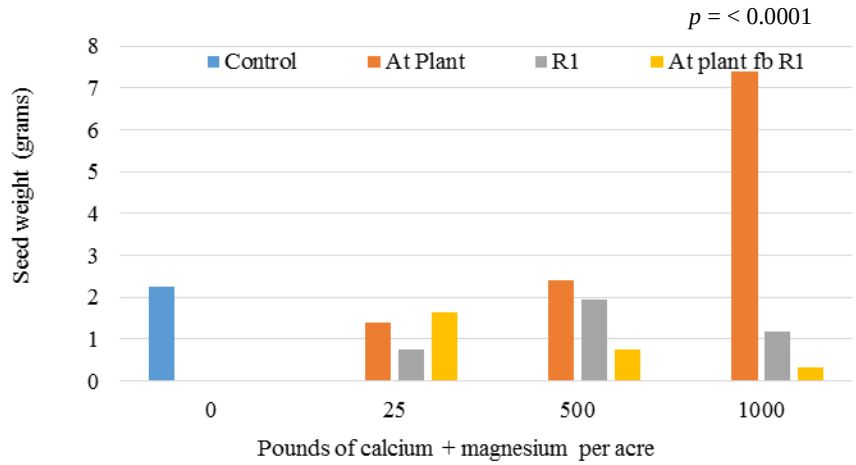


Fig.3. Seed weight per plant with the addition of calcium plus magnesium



2014-2015 Greenhouse field repeat trial. Data from one year of greenhouse research (Table 1, Table 2. Table 3) suggests that in most cases magnesium applied pre-planting followed by an at planting application significantly provided the greatest benefit over the control treatments with respect to disease severity, colony forming units and seed weight. Magnesium (1000 lb/a) applied pre-planting followed by at-planting decreased disease severity 62% compared to the inoculated untreated control at both the R5 and R7 sample timings (Table 1). Numerical reductions, between 75%-91%, in colonization at the R5 growth stage were seen with magnesium (1000 lb/a) applied alone at pre-planting, at-planting, and pre-planting followed by at-planting (Table 2). Colonization was significantly reduced between 80-82% with applications of magnesium (1000 lb/a) applied alone at pre-planting, at-planting, and pre-planting followed by at-planting when compared to the inoculated control at the R7 growth stage (Table 2). Yield was significantly increased by 51% over the inoculated non-treated control with magnesium applied (1000 lb/a) pre-planting followed by at planting (Table 3).

Table 1.Effect of fertilizer on disease severity during a greenhouse study at Stoneville, MS		
	Disease severity[a]/Growth stage[b]	
Treatment	R5*	R7**
Non-Inoc- untreated	2.2 abc	1.2
Inoc-untreated	2.6 abc	2.6
Ca (pre-plant)	3 ab	2.6
Ca (at-plant)	3.4 a	3
Ca (pre-plant + at plant)	2.2 abc	3.8
Mg (pre-plant)	1.25 c	3
Mg (at-plant)	3.5 a	2.6
Mg (pre-plant + at-plant)	1 c	1
Ca + Mg (pre-plant)	1.25 c	2.5
Ca + Mg (at-plant)	1.4 bc	3
Ca + Mg (pre-plant + at-plant)	1.5 bc	1.5
*p = 0.0199; **p =0.1786 [a]Root disease rated for soybean using a scale of plants exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5. [b]R5 (beginning seed), R7 (beginning maturity).		

Table 2. Effect of fertilizer on colony forming units in a greenhouse study at Stoneville, MS		
	Colony forming units[a]/Growth stage[b]	
Treatment	R5*	R7**
Non-Inoc- untreated	0	0 a
Inoc-untreated	1050	3980 a
Ca (pre-plant)	640	3220 ab
Ca (at-plant)	300	1560 ab
Ca (pre-plant + at plant)	2020	2600 ab
Mg (pre-plant)	267	820 bc
Mg (at-plant)	100	780 bc
Mg (pre-plant + at-plant)	100	734 bc
Ca + Mg (pre-plant)	375	950 bc
Ca + Mg (at-plant)	120	2680 ab
Ca + Mg (pre-plant + at-plant)	250	2000 ab
* p= 0.7069; **p= 0.0173 [a]Colony forming units calculated per gram of tissue [b]R5 (beginning seed), R7 (beginning maturity)		

Table 3. Effect of fertilizer on seed weight

	Seed weight
Treatment	Grams/plant*
Non-Inoc- untreated	3.027 dc
Inoc-untreated	3.07902 dc
Ca (pre-plant)	1.9964 d
Ca (at-plant)	2.80522 dc
Ca (pre-plant + at plant)	2.65706 dc
Mg (pre-plant)	4.1201 bcd
Mg (at-plant)	1.6818 d
Mg (pre-plant + at-plant)	6.1616 ab
Ca + Mg (pre-plant)	4.9368 abc
Ca + Mg (at-plant)	2.99442 dc
Ca + Mg (pre-plant + at-plant)	6.49225 a
*p= 0.0014	

2013-2015 Field Results

Statistical analysis. Data obtained from field experiments in 2014 and 2015 were analyzed across years using PROC GLIMMIX and PROC CORRELATION. Means were compared at the 0.05 significance level. Statistical analysis were performed using SAS (Statistical Analysis System) version 9.4 (SAS Institute Inc., Cary, NC., USA).

Pioneer 49T80 results (resistant variety). Data collected for 2014 and 2015 field trials using a resistant variety suggest a negative correlation between charcoal rot severity, kernel weight and yield. As disease severity increases, kernel weight and yield both decrease (Table 1a.). Applications of 1000 lb/acre of magnesium applied at planting provided a 38% decrease in colony forming units and a numerical decrease in disease severity in tissues samples taken at the R5 growth stage when compared to the inoculated untreated control (Table 1a and Table 2a). Although not significant, an approximate 35% reduction in colony forming units was seen with applications of magnesium applied at 1000 lb/ acre at the at-planting timing in tissue samples collected at R7 when compared to the inoculated untreated control (Table 4a). However, no benefit was seen with any of the treatments in regard to reduction in disease severity at this timing (Table 5a). Yield data showed no significant differences between any treatments when compared to the controls (Table 6a).

Agronomic evaluations

Table 1a. Effect of fertilizer on the correlation of height, yield, kernel weight, and disease severity in a Stoneville MS field trial

	HEIGHT	YIELD	KERNEL	CSR
HEIGHT		0.83731***	0.67883***	-0.69341***
YIELD	0.83731***		0.57795***	-0.66057***
KERNEL	0.67883***	0.57795***		-0.51415***
CSR	-0.69341***	-0.66057	-0.51415***	

*** $p < .0001$. Pearson correlation used for data analysis

Disease Evaluations

Table 2a. Fertilizer effects on colonization of *M. phaseolina* at an early reproductive stage in soybean

	Colony forming units[a] (R5 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	180			
Inoc/non-treated	80			
Pre-planting		100	200	100
At-planting		100	50	80
Pre-plant fb at-planting		342	480	100

[a]Colony forming units calculated per gram of tissue
[b]R5 (beginning seed)
*Not significantly different according to LSD test (p= 0.05)

Table 3a. Fertilizer effects on disease severity at an early soybean growth stage

	Charcoal Rot Severity Rating[a] (CRS) (R5 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	1.58 cd			
Inoc/non-treated	1.61 cd			
Pre-plant		1.64 bcd	1.42 cd	1.88 ab
At-plant		1.98 a	1.49 cd	1.80 abcd
Pre-plant fb at-plant		1.82 ab	1.82 abcd	1.38 d

p= 0.0171
[a]Root disease rated for soybean using a scale of plants: exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5.
[b]R5 (beginning seed)

Table 4a. Effects of fertilizer applications on colonization of *M. phaseolina*

	Colony forming units[a] (R7 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	258			
Inoc/non-treated	377			
Pre-plant		1350	230	188
At-plant		153	248	215
Pre-plant fb at-plant		50	130	415

[a]Colony forming units calculated per gram of tissue
[b]R7 (beginning maturity)
*Not significantly different according to LSD test (p= 0.05)

Table 5a. Reducing disease severity using secondary nutrients				
	Charcoal rot severity[a] (R7 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	1.64			
Inoc/non-treated	1.60			
Pre-plant		1.64	1.74	1.66
At-plt		1.69	1.74	1.69
Pre-plant fb at-plant		1.63	1.69	1.76

[a]Root disease rated for soybean using a scale of plants: exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5.
[b]R7 (beginning maturity)
*Not significantly different according to LSD test (p= 0.05)

Table 6a. Secondary nutrition effects on yield of a moderately resistant charcoal rot variety

	Yield[a] (2014-2015)			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	57.7			
Inoc/non-treated	55.9			
Pre-plant		54.9	57.2	56.4
At-plant		56.4	58.1	56.0
Pre-plant fb at-plant		55.4	56.4	56.8

[a]Yield calculated as bushels per acre at 13% moisture
*Not significantly different according to LSD test (p= 0.05)

Pioneer 46T21R results (susceptible variety). Results from field trials using a susceptible charcoal rot variety suggest a negative correlation between charcoal rot severity, plant height and kernel weight. As disease severity increases, plant height and kernel weight significantly decrease (Table 1b.). Samples taken at the R5 sample timing provided no benefit in reducing colony forming units over the inoculated non-treated control. Applications of 1000 lb/a of magnesium applied at planting when compared to the inoculated non-treated control provided a 23% numerical reduction in colony forming units at the R7 growth stage. The greatest reduction in colony forming units was observed with the at-plant application of 1000 lb/a of calcium with a 64% numerical decrease over the inoculated non-treated control (Table 3b). Numerical benefits were seen with disease observations over the inoculated non-treated control with some of the treatments. An approximate 9% reduction was observed with 1000 lb/a of magnesium and 1000 lb/a of calcium applied together at-planting in samples collected at the R5 growth stage (Table 3b). All applications of magnesium, calcium and magnesium plus calcium numerically reduced disease severity between 2-4% with samples taken at the R7 sample timing (Table 5b). Yield was not significantly impacted with any of the treatments (Table 6b).

Agronomic Evaluations

Table 1b. Effect of secondary nutrition on correlation of plant height, yield, 100 kernel weight and disease severity in an inoculated charcoal rot susceptible variety

	HEIGHT	YIELD	KERNEL	CSR
HEIGHT			0.85779***	-0.67116***
YIELD			0.27183**	
KERNEL	0.85779***	0.27183**		-0.70675***
CSR	-0.67116***		-0.70675***	

*** $p < .0001$
** $p = .0041$
Pearson correlation used for data analysis

Disease Evaluations

Table 2b. Nutrition effects on colonization of *M. phaseolina* in a charcoal rot susceptible variety during early reproductive stages

	Colony forming units[a] (R5 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	300			
Inoc/non-treated	220			
Pre-plant		365	255	338
At-plant		338	270	250
Pre-plant fb at-plant		280	550	650

[a]Colony forming units calculated per gram of tissue
[a]R5 (beginning seed)
*Not significantly different according to LSD test (p= 0.05)

Table 3b**.** Fertilizer effects on colonization of *M. phaseolina* in a charcoal rot susceptible variety at an early reproductive stage.

	Disease severity ratings[a] (R5 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	1.60			
Inoc/non-treated	1.40			
Pre-plant		1.42	1.40	1.41
At-plant		1.38	1.62	1.28
Pre-plant fb at-plant		1.57	1.52	1.50

[a]Root disease rated for soybean using a scale plants: exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5.
[b]R5 (beginning seed)
*Not significantly different according to LSD test (p= 0.05)

Table 4b. Secondary nutrition effects on colonization of *M. phaseolina* in a charcoal rot susceptible variety at late reproductive growth stages				
	Colony forming units[a] (R7 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	1460			
Inoc/non-treated	1980			
Pre-plant		1190	748	1160
At-plant		723	1538	810
Pre-plant fb at-plant		980	1500	1290
[a]Colony forming units calculated per gram of tissue [b]R7 (beginning maturity) *Not significantly different according to LSD test (p= 0.05)				

Table 5b**.** Fertilizer effects on colonization of *M. phaseolina* in a charcoal rot susceptible variety at a late reproductive stage.				
	Disease severity ratings[a] (R7 growth stage[b])			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	2.08			
Inoc/non-treated	1.74			
Pre-plant		1.90	1.56	1.60
At-plant		1.66	1.68	1.72
Pre-plant fb at-plant		1.74	1.84	1.88
[a]Root disease rated for soybean using a scale plants: exhibiting no symptoms = 0, < 2% discoloration and necrosis on roots =1, 2 to 10% discoloration and necrosis on roots = 2, 11 to 50% discoloration and necrosis on roots = 3, >50% discoloration and necrosis in roots = 4, dead or dying plants = 5. [b]R7 beginning maturity *Not significantly different according to LSD test (p= 0.05)				

Table 6b. Effect of secondary nutrition on yield of a charcoal rot susceptible variety				
	Yield[a] (2014-2015)			
Treatments	Control	Calcium	Magnesium	Calcium + Magnesium
Non-inoc/non-treated	58.7			
Inoc/non-treated	65.3			
Pre-plant		64.2	64.7	61.1
At-plant		65.7	65.4	60.8
Pre-plant fb at-plant		64.1	62.3	65.4

[a]Yield calculated as bushels per acre at 13% moisture
*Not significantly different according to LSD test (p= 0.05)

Discussion

Results during 2013-2015 greenhouse research trials suggested that applications of magnesium at pre-plant and at planting reduced colonization of *Macrophomina phaseolina* and disease severity when compared to the non-inoculated and inoculated control. In both years of study, seed

weights were significantly increased with some nutrient applications. In some cases, nutrient combinations may have caused some antagonism or negative impacts. A third year of greenhouse research is currently in place to confirm greenhouse results.

Field trial results indicate that there are significant treatment effects between nutrients and timing with disease severity. In every instance there was colonization of *Macrophomina phaseolina* in plant tissue and some level of disease severity regardless of variety. Although, signs of the disease were present, there were no visual symptoms present regardless of variety or research year. Currently a third year of field research is in progress to confirm field results from 2014 and 2015.

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