End of Project Final Report

The long-term objective of the proposed research is to characterize the bio-control activity of a collection of fungal species isolated from soybean production fields. The ultimate goal is to use these potential bio-control agents to enhance efficiencies in managing soybean diseases caused by pathogens present in the soil, such as, *Fusarium* spp., *Phytophthora* sojae, *Pythium* spp. This could be achieved either by introducing these bio-control agents to soybean production fields, and/or by fine-tuning existing management practices so that the prevalence and activity of bio-control agents native to production fields are enhanced.

Our ultimate goal is to reduce yield losses caused by seedling diseases by characterizing native bio-control agents already present in the soil and by refining management strategies to enhance and optimize the activity of these beneficial organisms against pathogens detrimental to soybean seedlings.

PROGRESS STATED BY OBJECTIVE:

Test the effect of a set of recently identified potential bio-control agents on soil-inhabiting fungal, oomycete, and nematode pathogens of soybean – An *in-vitro* screening assay was developed to assess the activity of fifty-eight potential bio-control agents against several *Pythium sp.*, *Fusarium sp.*, *Macrophomina phaseolina*, and *Rhizoctonia solani*. In many instances, multiple isolates of the BCA species were tested against multiple isolates of the pathogen. Specific BCAs caused up to 70% decrease in radial growth of *M. phaseolina* (Figure 1) and up to 64% reduction in radial growth of *F. virguliforme* . Fourteen BCAs were tested against 12 oomycete species. Some of the BCAs demonstrated trends of plant protection against particular *Pythium* species, and specific BCAs showed activity against *Rhizoctonia solani, Fusarium virguliforme* and *M. phaseolina*.

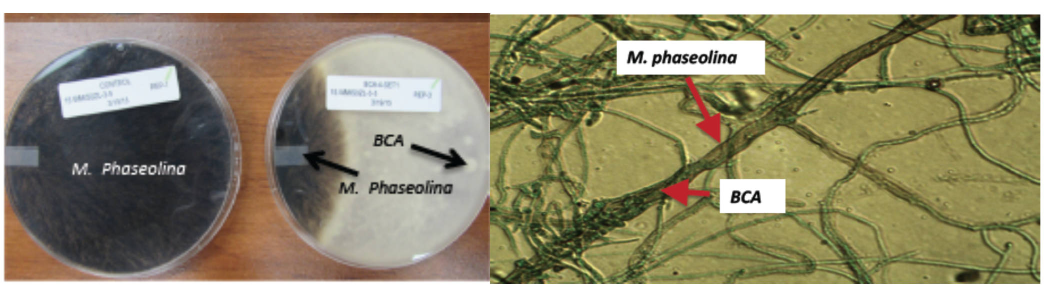

Figure 1 – *In vitro* evaluation of the antagonistic effect of BCAs on *M. phaseolina.*

Greenhouse experiments were conducted to test the effectiveness of the BCAs against *F. virguliforme*, *M. phaseolina*, and *R. solani* (Figure 2). In general, the BCAs that have shown highest antagonistic activity *in vitro* have shown similar behavior when evaluated in the greenhouse.

Figure 2 – Greenhouse screening (*F. virguliforme).*

Furthermore, growth chamber evaluation of six of the most promising BCAs was performed against four Pythium species: *P. lutarium*, *P. oopapillum*, *P. sylvaticum* and *P. torulosum*. A 'layer test' was used in which a layer of the pathogen was placed below a layer of the BCA. A preliminary study with two BCAs and *P. sylvaticum* showed extremely promising results (Figure 3).

P. sylvaticum *P. Sylvaticum* + BCA 8

Figure 3 – Greenhouse screening (*P. sylvaticum)*

Based on the *in vitro* and the greenhouse screens, the efficiency of promising BCAs was tested in field experiments in the 2016 and 2017 growing seasons. Single BCAs and combinations of different BCAs were tested against *F. virguliforme*, *M. phaseolina, R. solani,* and 6 different Pythium species. In 2016, BCAs were tested in MI and IA against Pythium spp. and in IL against *F. virguliforme* and *M. phaseolina.* In 2017, the BCAs field experiments were repeated, and the BCAs were also tested against *R. solani* and *Cercospora sojina* (the causal agent of Frogeye Leaf Spot) in KY. Collected data included stand, yield, disease incidence and severity, root colonization by BCA, and root colonization by the pathogen.

Over the two years the field experiments were conducted, the tested BCAs did not show a significant effect on Pythium, *F. virguliforme*, or *R. solani.* It is to be noted that the disease incidence was low with *F. virguliforme* in all conducted test.

In 2017, isolates of different species of *Trichoderma* were evaluated for control of frogeye leaf spot and their effect on soybean yield in field trials conducted at the University of Kentucky Research and Education Center (UKREC) in Princeton, KY. Spores produced from these potential biocontrol agents were collected from laboratory-cultured isolates and suspended in water. Spore suspensions were sprayed onto soybean plants at the R3 growth stage. A non-treated control treatment and a standard fungicide treatment (Quadris Top SBX; Syngenta Crop Protection, Greensboro, NC) applied at the R3 growth stage also were included. The trial was located in two different fields at the UKREC. The Catlett Tract field had been grown to soybean the previous year, and had high frogeye leaf spot disease pressure. The Luttrell Tract field had not been cropped for at least the previous 5 years, and had low to moderate frogeye leaf spot disease pressure. Frogeye leaf spot severity data were collected three times during the season after treatments were applied. Severity data were used to calculate area under disease progress curve (AUDPC) values. Plots were harvested with a small plot combine, and yields were calculated. The experimental design was a randomized complete block with 4 replications for each field.

For the trial at the Catlett Tract, only the Quadris Top SBX treatment significantly reduced disease compared to the non-treated control (Table 1). Yield of the *T. virens* treatment was the only treatment that had a significantly greater yield than the non-treated control. For the Luttrell Tract, all treatments had significantly lower disease than the non-treated control, and all treatments resulted in greater yields than the non-treated control (Table 2).

In a low to moderate frogeye leaf spot pressure field, the *Trichoderma* spp. evaluated significantly reduced frogeye leaf spot severity, which resulted into a significant yield increase over the non-treated control. Additional field tests across more locations and years would help confirm these results.

Table 1. Effect of Quadris Top SBX fungicide and *Trichoderma* spp. applied to soybean at the R3 growth stage on frogeye leaf spot severity area under the disease progress curve (AUDPC) values, seed moisture at harvest, and soybean yield at the Catlett Tract of the University of Kentucky Research and Education Center, Princeton, KY in 2017.

Treatment	Frogeye leaf spot severity (AUDPC)	Seed moisture (%)	Yield (kg/ha)
Non-treated control	115	16.5	28.6
Quadris Top SBX	91	16.0	29.4
T. harzianum	115	16.6	26.6
T. hamatum	107	15.9	30.7
T. virens	105	16.4	37.5
$P > F$	**0.0087**	**0.2608**	**0.0478**
LSD 0.10[z]	**11**	**NS[y]**	**6.1**

[z]Fisher's protected least significant difference test value (α = 0.10).

[y]No significant differences because of an *F*-test that was not significant ($P \geq 0.10$).

Table 2. Effect of Quadris Top SBX fungicide and *Trichoderma* spp. applied to soybean at the R3 growth stage on frogeye leaf spot severity area under the disease progress curve (AUDPC) values, seed moisture at harvest, and soybean yield at the Luttrell Tract of the University of Kentucky Research and Education Center, Princeton, KY in 2017.

Treatment	Frogeye leaf spot severity (AUDPC)	Seed moisture (%)	Yield (kg/ha)
Non-treated control	42	14.6	45.0
Quadris Top SBX	23	14.5	59.6
T. harzianum	37	14.5	56.5
T. hamatum	36	14.5	54.8
T. virens	32	14.5	54.7
P > F	**0.0011**	**0.9493**	**0.0777**
LSD 0.10[z]	**6**	**NS[y]**	**8.3**

[z]Fisher's protected least significant difference test value (α = 0.10).

[y]No significant differences because of an *F*-test that was not significant ($P \geq 0.10$).

With *M. phaseolina,* select BCAs seemed to have decreased the incidence of the disease in the field (Table 3).

Table 3 . Effect seed treatment with select BCAs on the incidence of charcoal rot. The experiment was conducted in the SIU Agronomy Research Center, Carbondale, IL.

Treatments	CFU/g	RSS
BCA 8 set 1	76.34*	3.2*
BCA 9 set 2	83.86*	4.28*
BCA 2 + BCA 3 + BCA 4 set 1	64.93*	3.24*
BCA 3 + BCA 8 + BCA 9 set 2	83.84*	3.82*
BCA 8 + BCA 9 + BCA 1 G7	77.84*	3.22*
H2O + CMC + MP (+ CONTROL)	88.86*	4.1*
H2O + CMC (- CONTROL)	28.69*	2.19*
BCA 9 set 2 + Drenching+ MP	105.07*	3.8*
H20 + CMC + Drenching+ MP (+ Control)	92.43*	3.34*
H20 + CMC + Drenching (- Control)	49.72*	2.02*

CFU: *M. phaseolina* colony forming units
RSS: Root and stem severity rating
*: Denotes significance at p < 0.0001

Assess potential roles of these agents in eliciting defense mechanisms in soybean plants - A greenhouse experiment was carried out to evaluate the induction of the expression of six genes related to Induced Systemic Resistance (ISR) using RT-PCR on the root tissue of the plants. Gene expression analysis provided evidence that the BCAs tested stimulated the plant's capacity and ability to induce defense genes in soybean seedlings upon pathogen attack. The defensive response of plants treated with the BCAs in combination with the pathogen or plants inoculated with the pathogen alone (positive controls) significantly differed from that of non-inoculated plants. Two weeks after sowing (approximately one week after germination), with all BCAs tested at the molecular level, there was an increase in the expression of genes such as CHS and PR2, which encode for chalcone synthase and beta-1,3-glucanase, respectively (Figure 4).

Chalcone synthase is a key enzyme of the flavonoid/isoflavonoid biosynthesis pathway and a member of the polyketide synthase superfamily (PKS). Its expression is commonly induced in plants under various stress conditions (i.e. fungal infection). CHS expression results in flavonoid and isoflavonoid phytoalexin accumulation. Phytoalexins are produced by plants in response to stress and contain antimicrobial properties. CHS also plays a role in the salicylic acid defense pathway. Greater expression of CHS in this study for plants treated with BCAs and inoculated with the pathogen supports evidence that the BCAs induce plant defense mechanisms in the soybean plants.

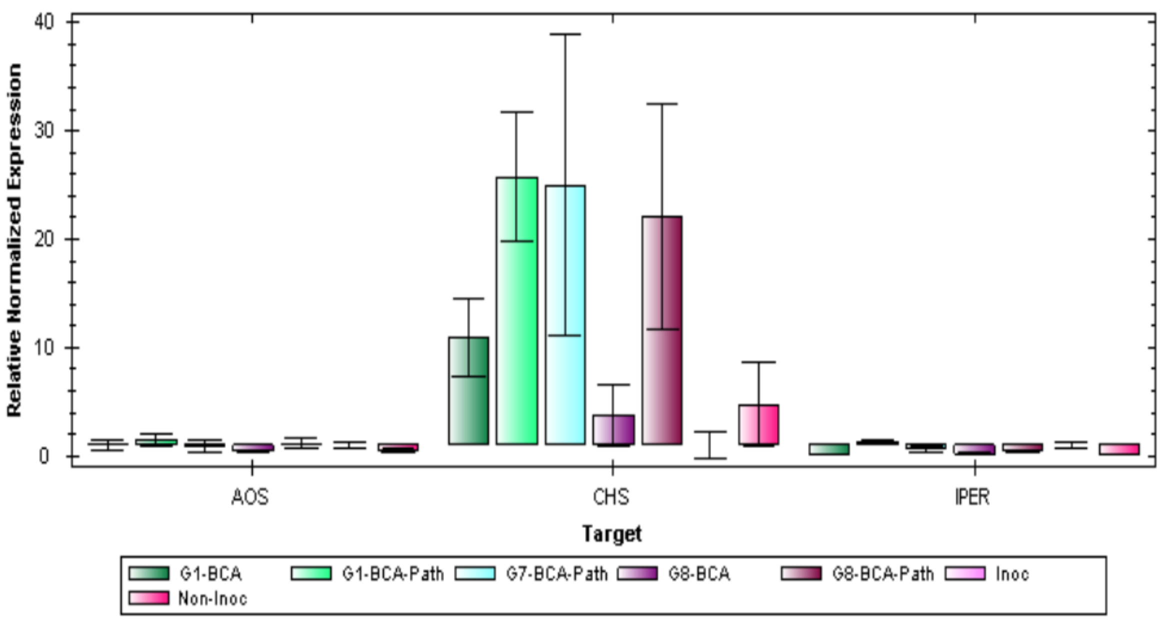

Figure 4 – BCAs elicit defense mechanisms in soybean.

***Test the effect of fungicidal seed treatments on these organisms* -** Eleven fungal isolates selected for their potential to act as bio-control agents were evaluated for their sensitivity to different fungicide active ingredients that are commonly used in seed treatment products for soybean. Six fungicide active ingredients were tested, and each represented a unique chemistry class / mode of action. The fungicides tested were pyraclostrobin (quinone outside inhibitor class), prothioconazole (demethylation inhibitor class), sedaxane (succinate dehydrogenase inhibitor class), fludioxonil (phenylpyrrole class), thiabendazole (methyl benzimidazole carbamate class), and metalaxyl (phenylamide class). Each fungicide was amended to potato dextrose agar at 1 ppm, and a 5 mm plug of an actively growing fungal culture was placed in the middle of the petri dish. A 5 mm plug was also placed on non-amended media.

Variation in the sensitivity of the fungal isolates to the different fungicides was observed. *These results indicate that some of these potential bio-control agents could be added with a fungicide seed treatment with minimum inhibition occurring*.