NCSRP Project Final Report

Project Title: Evaluation and Development of a Biological Control Product to Control Soybean Sudden Death Syndrome and White Mold

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Summary

During this three-year project period, we tested biological control agents against soybean white mold and soybean sudden death syndrome. Our data from university research farms and production farms in three north central states (IA, IL, MN) show that BCA increased soybean yields in fields severely infested with SDS or white mold. BCA can be an effective tool to manage both SDS and white mold in farms of north central regions.

In university research plots where SSD risk was high from artificial inoculation, BCA has consistently show good control of SDS and increase soybean yields over the project periods. It was effective both used for seed treatment or soil sprays.

Large field trials conducted in growers' fields have shown excellent results in control SDS or white mold. Producer reported good control to white mold with BCA-1 and an increase of 9.9 bu/acre compared with no check area. Second type of biological control agent BCA-2 also had 7 bu/acre yield increase. The control effects of BCA in growers fields were visible from satellite as images in the treated crop showed healthy soybean growth with higher NDVI values in areas treated with BCA.

Because BCA can suppress various soilborne fungi which are pathogenic to both soybean and corn, soil applications of BCA can be an economic mean of keeping soil healthy. In fields rotated soybean with corn, an application in soybean or corn should help reduce stalk rots besides control soybean diseases. This provides a potential tool for soil health. A producer told us that *"Im looking forward to using bca on all corn acres when it becomes available"*.

Brief Statement of Objectives (max 250 words):

Sudden death syndrome (SDS) and white mold are two of the most important fungal diseases in soybean production in the US, affecting 40 - 50 million acres in the North Central Region. These two diseases threaten the sustainable production of soybean in the North Central Region.

Biological control products are the future for disease management in row crops and private industry has made significant progress in development of biological fungicides for row crops. The goal of this proposal is to evaluate their effectiveness of potential biological control agent in other states of the North Central Region and develop the agent into a product for commercial use, which will provide reliable and cost-effective control of SDS and white mold of soybean.

Project Objectives:

- To conduct multi-state field evaluation for fungal biological control agents that are found effective in reducing soybean sudden death syndrome and white mold.
- To investigate a wide-host-range bacterial bio-control agent that is effective in killing SDS and white mold pathogens for management of SDS and white mold in Illinois.
- To collect data of prototype product of the biological control agent for commercialization
- To determine if application of the biocontrol agent to crop residues of corn, alfalfa, or soybean can reduce inoculums production, infection, and disease caused by *F. virguliforme* and *Sclerotinia.*
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Brief Statement of Deliverables (max 250 words):

At the end of project, we will have three sets of outcome, two product sets and one knowledge set. The first is new use of existing commercial products including these in pipeline in control SDS and white mold; the second set are biological control agents (bacterial or fungus) developed from this study for multiple disease control; the third set is knowledge on how to effectively apply the biocontrol agents to crop residues of corn, alfalfa, or soybean to control diseases caused by *F. virguliforme* and Sclerotinia.

The first set outcome will be used immediately for farmers to combat SDS and white mold as Contans does today. The most likely products will be from the new use of biological control products (Biowork or Headsup) on the market for controlling SDS and white mold.

For the second set of outcome, prototype products for control of SDS and white mold will be developed based on the discovery of the biofungicide organisms. This prototype kit will be evaluated and validated further during the process of commercialization. The products will be manufactured and validated in regional farm trials for disease control efficacy and soybean yield increase.

Brief Statement of Progress, Milestones & Deliverables (max 500 words):

Phase I

Efforts were made to evaluate biological control products against SDS and white mold by establishing multiple locations in Iowa, Illinois and Minnesota. Treatments were made on production farms with stripe plots or in university farms. Production level test was also preformed with assistance of producers. This season had a weather favorable for disease occurrence and promising results were generated from some locations.

Yield data from ISU university farms have been collected and analyzed. Yield data from commercial farms are awaited. Results on yield data from university farms show that seed treatment with biological control agents had less SDS and higher yield. Differences in treatments were also observed with some products superior to others. For post emergence application test conducted in commercial fields, observations suggest the possibility of dispersal effects from sprayed area in unsprayed area. Laboratory and green house experiments were carried in University of Illinois to determine the control mechanism biological control agent on Septoria. Experiments were also conducted to determine BCA for controlling white mold and root diseases. Initial results indicated positive control of stem, root, and foliar disease with BCA. In Minnesota, tests were made in different location and biocontrol treatments appeared effective in location with high disease pressure as white mold mushroom numbers appeared to have been affected by the treatments. An average of 6.25 apothecia (15 foot of row) was observed in untreated plots, 1.75 apothecia in treated plots and 3.5 apothecia in HeadsUp treated plots. High variability affected statistical differences, however.

Progress made in Illinois University:

Objective 1: To conduct multi-state field evaluation for fungal biological control agents those are found effective in reducing soybean sudden death syndrome and white mold.

Field trials for control of sudden death syndrome and soybean white mold (*Sclerotinia* stem rot) using a fungal bio-control agent for its efficacy were conducted in Urbana, and DeKalb, IL, respectively; and disease ratings were collected and data are currently being analyzed and summarized.

Objective 2: To investigate a wide-host-range bacterial bio-control agent that is effective in killing SDS and white mold pathogens for management of SDS and white mold in Illinois.

Efficacy of the bacterial bio-control agent (BCA) seed treatment for controlling root and stem blight, SDS, Pythium and Phytophthora root rot were tested in lab conditions. Initial results showed that BCA effectively protects seeds from those pathogens, with reduced disease severity and root lesions. The experiments will be repeated. Similarly, efficacy of the BCA for controlling white mold was performed in greenhouse using cut-petiole and spray method and initial results indicated positive control of the disease.

Efficacy of the BCA foliar spray for controlling frogeye leaf spot and *septoria* brown spot was conducted in greenhouse. Data is being currently analyzed. However, Microscopy data suggest that *C. sojina* conidia germinating and gaining entrance into stomata on untreated leaves, but fail to detect penetrating conidial hyphae on BCA-treated leaves, with substantially fewer conidia.

Efficacy of the BCA seed treatment, soil drench and foliar spray: Field trials for control of *Sclerotinia* stem rot (Dekalb, IL), sudden death syndrome (Urbana, IL), and frogeye leaf spot (Dixon Springs, IL) using BCA to evaluate its efficacy were conducted using soil drench, seed-treatment and foliar spray, respectively. Disease ratings were collected and data are currently being analyzed and summarized. However, initial results suggest that BCA could effectively reduce root, stem, and foliar soybean fungal diseases in lab and greenhouse conditions.

During this reporting period field trials for SDS and white mold were established at two ISU Research Farms and three commercial fields in Iowa. Results of SDS trial conducted at Ames location under artificial epiphytotic conditions are given in Table 1. Though no significant ($P<0.05$) differences were among the treatments, but there was two bushel yield advantage with fungus based BCA seed treatment over the untreated SDS inoculated treatment (Table 1). Results for white mold and SDS from ISU farm Nashua will be compiled after yield data are in place and will be reported in the next reporting period. Similarly, after getting the harvest data from commercial fields at Maynard (Fig 1), Humboldt (Fig 2) and Nashua (Fig 3) will be reported in the next report.

Table 1. Efficacy tests of seed treatment of biological control agents and plant products on soybean sudden death syndrome (SDS), Ames, Iowa,

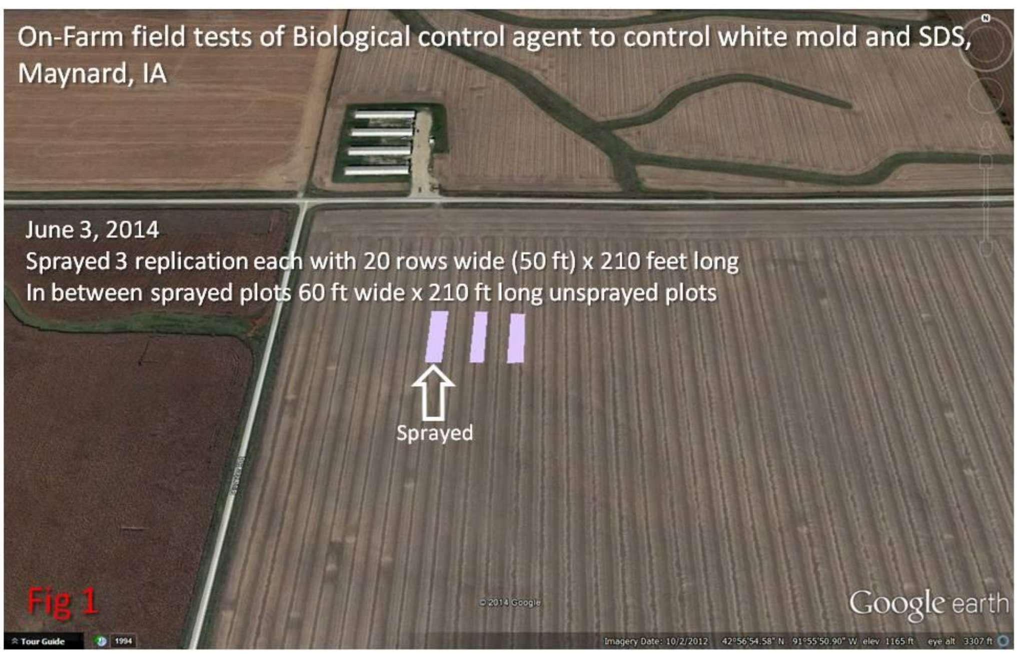
Location: Iowa State University, Ames, IA 50011

Treatments	Stand count 25 dap	Seedling stage SDS Inc%	Seedling stage SDS Sev%	Vigor 1 to 9	DX	Yield Bu/Ac
1. BCA-2 Plus ST	295.5ba	0.434ba	25.0a	6.0ba	12.22a	50.28a
2. BCA-2 Plus ST 5ml/kg and spray	267.5b	0.172b	10.0a	6.8ba	14.63a	52.16a
3. HeadsUp alone ST 1g/L	297.5ba	0.094b	7.5a	6.5ba	11.68a	51.51a
4. BCA seed treat @ 5ml/kg	311.3ba	0.132b	10.0a	6.0ba	7.12a	53.38a
5. BCA ST @ 5ml/kg and spray	345.0a	0.175b	15.0a	6.8ba	10.65a	55.09a
6. T4 + BCA colonized milo at planting @ 4 cc/ft	320.5ba	0.811a	27.5a	6.0ba	15.98a	55.24a
7. CMV spray at flowering	299.5ba	0.171b	5.0a	7.0a	9.39a	53.13a
8. Control1-inoculated	321.5ba	0.239ba	20.0a	5.5b	7.22a	53.22a
9. Control 2-Not inoculated	317.8ba	0.000b	0.0a	7.0a	9.61a	57.22a
10. TRA 1	356.0a	0.127b	7.5a	6.8ba	11.43a	54.04a
11. TRA 2	315.3ba	0.077b	5.0a	7.3ba	9.26a	53.40a
12. TRA 3	302.812	0.217b	10.0	6.8a	7.51a	50.06a

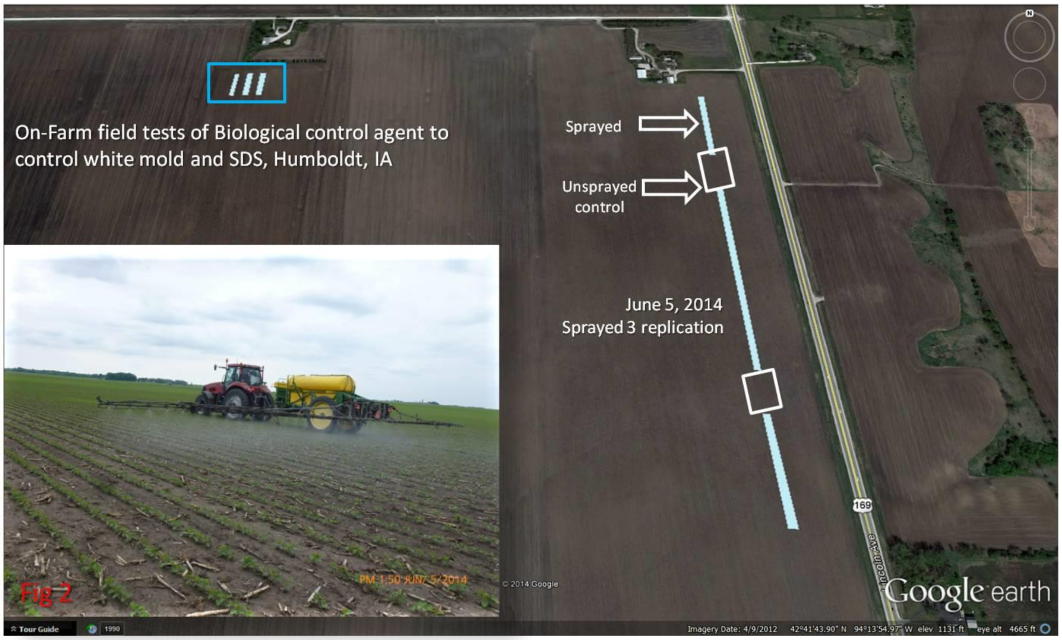
Means with the same letter are not significantly different ($P<0.05$).

[1]Values are mean of four plots, each plot with 87.5 sq ft

Two-row plots comparison between different seed treatments on the effect of biological control agents on the occurrence of soybean sudden death syndrome from our Ames test at ISU Hinds Farm. Photo was taken in later reproductive stage. Some treatments preformed superior to others or to non-treated control in terms of suppression of foliar symptom expression of SDS.

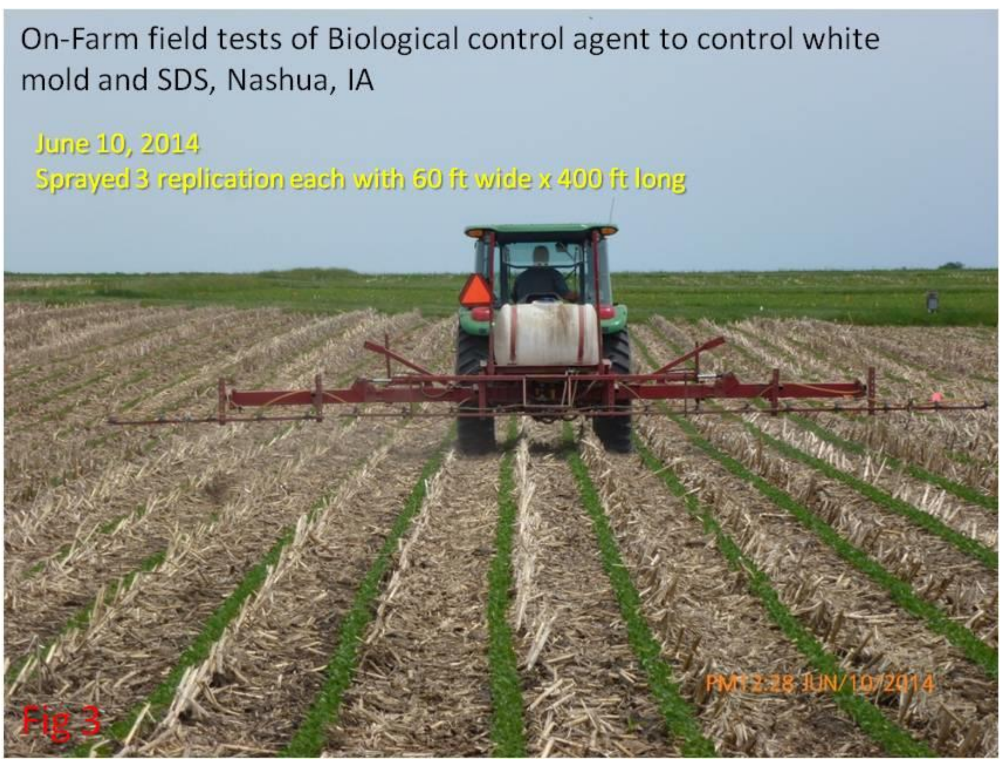


Field test map for Maynard location. The application was made after seedling emergence in areas with previous history of severe white mold infestation. In 2014 season, only SDS was found in this field however and was widely spread in the entire field but our test area. Aerial photo and observations show that the disease was free in sprayed area and around of our applied area. We are yet to determine if dispersal effect led to the suppression of SDS in our test area.



Field test map for Humboldt location 1. The application was made after seedling emergence with production equipment as indicated in the graph over a large area in a field with occurrence of SDS (upper). Field visit showed sign of suppression with more green area compared with unsprayed (lower). The field has been harvested and yield data were collected by producer and yet to be provided to us for analysis.

Field test map for Humboldt location 2. The application was made after seedling emergence in areas with previous history of severe white mold infestation. No significant disease was observed in this location this year.



Field test map in Nashua location 2014. The application was made after seedling emergence with production equipment as indicated in the graph over a large area in a field with occurrence of SDS. The field has been harvested and yield data were collected by our farm manager. Data are under analysis.

*2015 season field trials***.**

In 2015 growing season, field trials for seed treatment with different biological control products were conducted in ISU research farm using RCBD. 2015 season was ideal to the occurrence to SDS and the disease was very severe in Iowa, providing a good condition for our field test. Field trials were conducted at different locations across Iowa.

Our results further demonstrated that biological control products current available on the market or under developed can deliver a yield benefit compared with non control. At Ames location where SDS was very severe, seed treatment with Headsup had 5 bushel yield increase (Fig. 2). The seed treatment by BCA had a yield increase over 6 bushel per acre. If added with milo at planting, the yield increase was over 10 bushel.

At Nahsul trial (Fig. 3) where both SDS and white mold occurred in the season, yield increase of 4 bushel per acre was found after seed treatment.

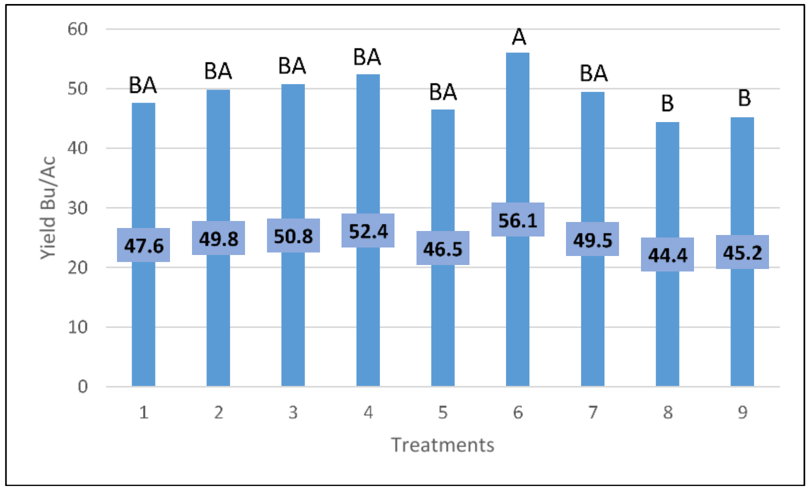


Fig. 2 Results of yield for 2015 field trials of seed treatments with different biological control products at Ames Iowa. 1= BCA-2; 2= BCA-2 + sprayed v4; 3 =Headsup; 4= BCA-1; 5=BCA-1 + spray @v4 ; 6 = BCA-1 + mio; 7 =CMV; 8 = check 1; 9 = check 2.

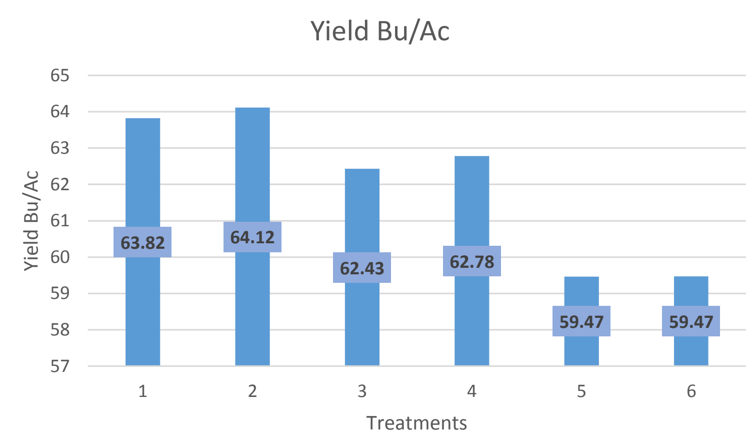


Fig. 3. Results of yield for 2015 field trials of seed treatments with different biological control products at Nashua, Iowa. 1= BCA-2; 2= BCA-2 + sprayed v4; 3 =Headsup; 4= BCA-1; 5 =CMV; 6 = check.

Other measurements consistently showed the control effect from seed treatment with these biological control products. With the stand counts, we found several treatment had higher number of seedling stand compared with control (Fig. 4). In a field infested with both SDS and white mold in northwestern Iowa, the disease incidence for white mold was reduced by using seed treatment (Fig. 5).

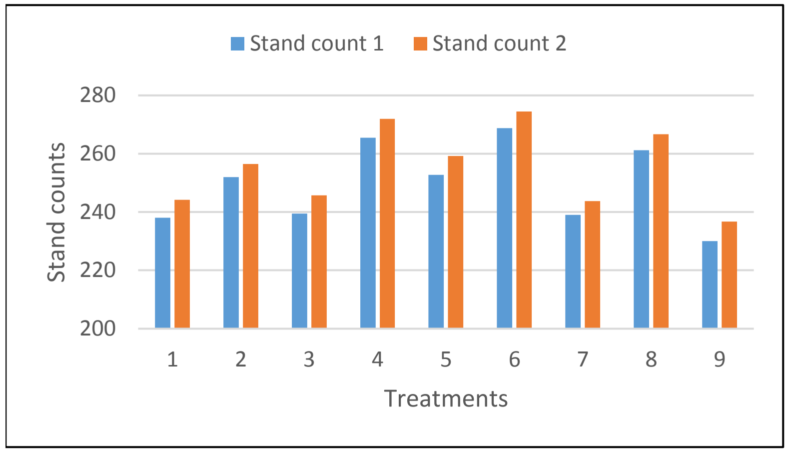


Fig. 4. Seedling stand count for 2015 field trials of seed treatments with different biological control products at Ames Iowa. 1= BCA-2 Plus; 2= BCA-2 + sprayed v4; 3 =Headsup; 4= BCA; 5=BCA + spray @v4 ; 6 = BCA-1 + mio; 7 =CMV; 8 = check 1; 9 = check 2.

In Illinois, field trials for control of *Sclerotinia* stem rot (white mold, Dekalb, IL), sudden death syndrome (SDS, Urbana, IL), and frogeye leaf spot (Dixon Springs, IL) were conducted using soil drench, seed-treatment and foliar spray, respectively. For SDS, we used RCBD microplots with four replicates and 10 seeds each. For white mold and frogeye leaf spot, we used RCBS row plots with 10 plants each. Disease ratings for SDS were recorded at 28, 38, 49, 80 and 96 days post inoculation; and disease ratings for white mold and frogeye spot were recorded on two days each (9/4/2014 and 9/19/2014; 8/30/2014 and 9/12/2014, respectively). Unfortunately, the results were not as expected, possibly due to that we used a single application of BAC, weather conditions were not favorable for the survival of the BAC, and possibly lack of formulation. Future studies will be focused on repeating field trials and development of new formulation or fermentation methodology, as well as delivery methodology.

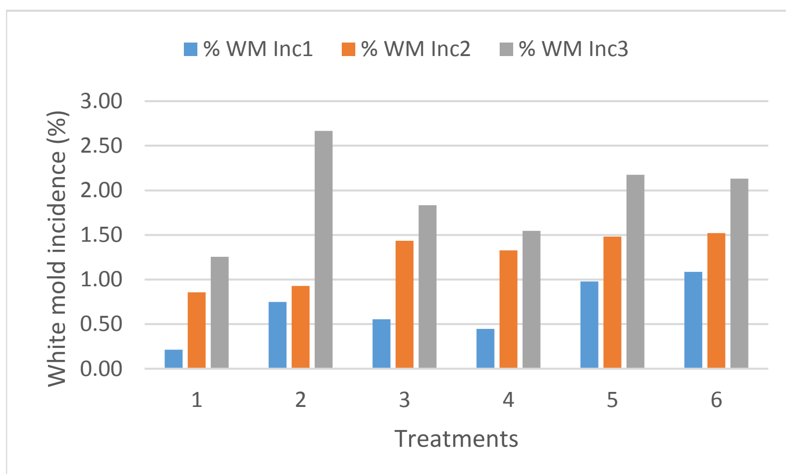


Fig. 5. Results of white mold control for 2015 field trials of seed treatments with different biological control products at Nashua, Iowa. 1= BCA-2 Plus; 2= BCA-2 + sprayed v4; 3 =Headsup; 4= BCA-1; 5 =CMV; 6 = check.

Phase II.

Experiments have been established in both university research farms and commercial research farms. In university research farms, design was for comparison of multiple treatments. Field trials for control of *Sclerotinia* stem rot (white mold, Dekalb, IL), sudden death syndrome (SDS, Urbana, IL), and frogeye leaf spot (Dixon Springs, IL) were conducted in 2016 using soil drench, seed-treatment and foliar spray, respectively. For SDS, we used RCBD microplots with four replicates and 10 seeds each. For white mold and frogeye leaf spot, we used RCBS row plots with 10 plants each. Disease ratings for SDS were recorded periodically post inoculation; and disease ratings for white mold were recorded.

Table. Treatments for 2016 plot experiment at Iowa State University field trial.

1	BCA-1 seed treatment @5ml/kg (dissolve 0.5g in 100ml)(use commercial UT seed P22T61R)
2	BCA-1 seed treatment 5ml/kg and spray @ 26 g in 1.4 gal water for 4000 sq feet at v3 or v4
3	BCA-2 seed treatment alone ST 1g/L (use commercial UT seed P22T61R)
4	BCA seed treat @ 5ml/kg ($1x10^{10}$/ml) (use commercial UT seed P22T61R)
5	BCA ST @ 5ml/kg ($1x10^{10}$/ml) + spray @ 0.68 gal per 2000 sq feet at V3/V4 stage
6	T4 + BCA colonized milo at planting @ 4 cc/ft
7	CMV spray at flowering (upon initial SDS symptoms seen) @ (UT seed + Inoculum).
8	CONTROL 1: No seed treatment (Use commercial UT seed and inoculate with SDS at Ames)

Results from University Research Farms

We have had experiments in multiple locations of university farms. All plots have been harvested and yield data were collected. So far as this report, we have analyzed data from Hinds Farm of Iowa State University. In this farm, SDS was observed in the growing season with moderate level of severity. Seed treatment with biological control agents increased soybean yield by as much as 3 bu/ac compared with control.

Table . NCSRP: 2016 BCA seed treatment/spray efficacy tests --- *Fusarium virguliforme* (Sudden death syndrome)

Tr #	Treatments	stct 1	stct 2	vigor	sDX	DX 1	DX2	DX3	DX4	DX5	DX6	Bu/ac
1	BCA -1 seed treatment	261 a	303 a	7.3a	2.68 a	0a	0.06 a	0.14 a	0.71 a	1.65 a	3.84b a	60.14b
2	Tr# 1 + spray at V3/V4	269 a	310 a	7.5a	2.70 a	0a	0.02 a	0.02 a	0.28 a	0.53 a	1.78b	67.74a
3	BCA-2 seed treatment	262 a	314 a	7.3a	2.09 a	0a	0.06 a	0.11 a	0.64 a	1.56 a	3.51b a	62.58b a
4	BCA seed treatment	284 a	300 a	7.4a	3.47 a	0a	0.09 a	0.15 a	0.49 a	2.00 a	3.78b a	65.25b a
5	Tr# 4 + BCA spray at V3/V4	260 a	364 a	7.3a	3.58 a	0a	0.05 a	0.05 a	0.25 a	0.78 a	3.46b a	64.40b a
6	Tr# 4 + BCA on white milo at planting	269 a	297 a	6.8a	2.50 a	0a	0.08 a	0.16 a	0.63 a	4.15 a	6.93a	65.08b a
7	CMV spray at flowering	290 a	301 a	7.3a	3.99 a	0a	0.09 a	0.23 a	1.03 a	2.34 a	5.31b a	65.49a
8	Control (no seed treatment/spray)	272 a	307 a	7.5a	1.83 a	0a	0.09 a	0.11 a	0.55 a	1.31 a	2.66b a	64.44b a

Treats: 6, Reps: 4, Plot size: 87.5 sq ft/rep, Row spacing: 30 inch, Treat#= treatment numbers, stct1 and stct2= stand counts 1 and 2, vigor= vigor on 1-9 scale (1=poor, 9=excellent), SDX= seedling stage sudden death syndrome disease index (SDS incidence x SDS severity)/9), Similarly DX1 to DX6 are SDS disease index in reproductive growth stages, yldbuac= Yield Bu/Ac at 13% grain moisture. Means with the same letter are not significantly different in each column at 5% level of significance.

Table: 2016 plant based and or BCA seed treatment study against SDS, Ames, Iowa

			Sudden death syndrome				Yield Bu/Ac
Treatments	StCt2	Vigor	S-DX	DX4	DX5	DX6	
Untreated control	362.3a	7.8a	6.0ba	9.08a	71.8a	83.01a	52.02a
HeadsUp @ 1g/L	338.8a	8.0a	4.2ba	6.85ba	71.1a	81.92a	51.97a
HeadsUp @ 2g/L	343.0a	7.8a	6.5a	2.76b	68.6a	82.21a	53.47a
HeadsUp @ 1g/L+BCA-1	374.5a	8.1a	3.5ba	4.25ba	69.1a	78.21a	53.98a
HeadsUp @ 2g/L+BCA-1	344.0a	8.3a	1.4b	2.26b	67.7a	75.50a	53.84a
CGE	372.3a	8.0a	4.4ba	2.14b	64.9a	75.00a	48.64a

Planted Pioneer 22T61RR, June 1: Stct1, June 15: Stct2; SDS counts June 15, July 28, Aug 7, Aug 24, Aug 30. S-DX=seedling stage SDS disease index, DX1-DX6: reproductive stage disease index. Harvested: Sept 30, 2016.

Results from Commercial field trials.

Experiments for field trials to control SDS and white mold have been conducted in farmer's fields in northern Iowa at Sioux Center and Humboldt. Biocontrol agents were sprayed in early vegetative stage V3-V4 and yield harvest have been completed. Each treatment was made according to standard commercial production, each 5 acres/treatment.

Fig. Field Trial application of biological control agent in a soybean fields infested with soybean white mold in spring 2016. Each treatment had a treated area of five acres. (Owner, Bruce Zomermaand, at Sioux Center).

Yield Increase in farm trials

Yields have been harvest and recorded. Yield maps are yet to be provided. The field trials conducted have shown promising results in control SDS or white mold. Yield improvement was found in treated areas due to reduction of disease damage. At Sioux Center, producer reports good control to white mold with BCA and an increase of 9.9 bu/acre compared with no check area. Second type of biological control agent BCA-1 also had 7 bu/acre yield increase. Specifically, the yield were 72 bu/ac for control check, 81.9/bu/ac for BCA, 78.5 bu/ac for BCA-1, 81.5 bu/ac for Cobra.

Observations made with satellite images in the treated crop demonstrates healthy crop of BCA application indicated by higher NDVI values for areas treated with BCA. Using satellite remote sensing to quantify disease damage is a new technique and we are working to improve this method.

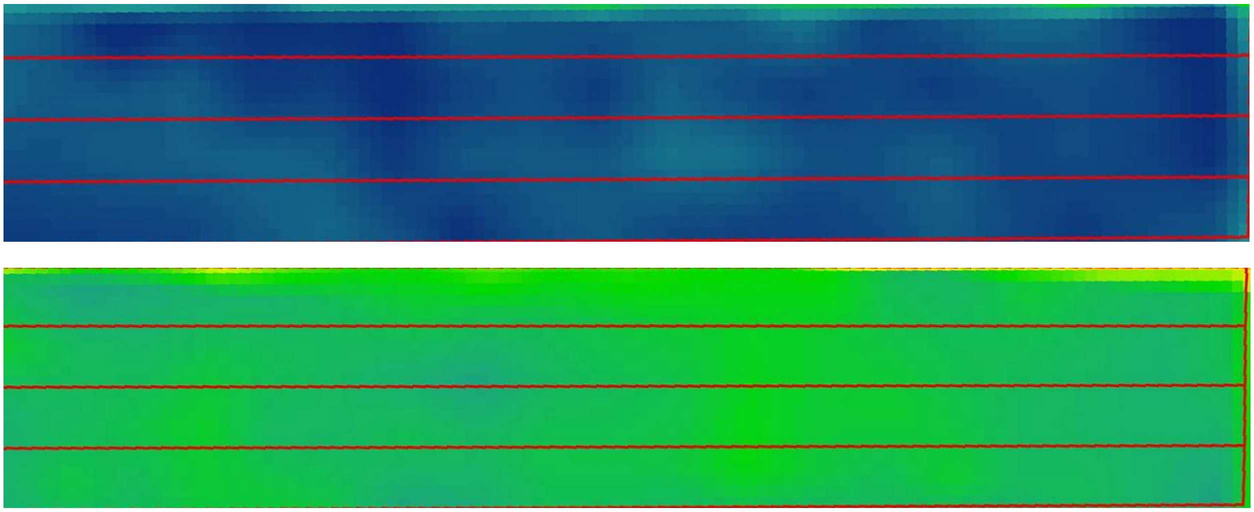


Figure. Satellite image NDVI for four stripe plot (5 acre per plot) in a soybean field in Sioux Center. Field was infested with white mold. Upper stripe with darker band shows healthy growth of soybean plants for BCA plots compared with other treatments.

Farmer's response after field test

Below is the text messages from Bruce Zomermaand, a soybean grower at Sioux Center who conducted BCA tests in his field severely infested with white mold fungus.

"Im looking forward to using bca on all corn acres when it becomes available."

"We haul liquid swine manure in the fall. Would it be possible to add to that tank when we spread and would it stay in suspension without agitation? Bruce Zomermaand"

"I would like to test it on corn if its ok this coming year. Is this do somewhat the same thing as heads up or does it work differently? Do recommend treating corn and soybean seed with heads up?"

In first year of the test in his land, he got very good yield and the results were excellent (see satellite figure). He was obviously happy with the result.

Report from University of Illinois

Progress in Phase I

In this project, we mainly focused on Objective 2, i. e. investigate a wide-host-range bacterial bio-control agent (BCA) that is effective in killing SDS and white mold pathogens for management of SDS and white mold in Illinois. We also extended our work to six other important soybean fungal /oomycete pathogens. We collected pathogen samples from fields in Illinois to be tested against the BCA, including 10 isolates each of *Phytophthora, Rhizoctonia* and sudden death syndrome (SDS), 11 isolates of *Pythium*, 3 isolates of charcoal rot, and 5 isolates of white mold, 9 isolates of Septoria brown spot, and 8 isolates of frogeye leaf spot. Using dual-culture assay, we screened them against the BCA and found that the BCA inhibited mycelia growth and spore germination for all of them. The results suggest that the BCA exhibits broad spectrum inhibition activities against fungi/oomycete pathogens.

We determined the efficacy of the BCA in controlling white mold using cut-petiole and detached leaf spray methods, in controlling *Rhizoctonia*, SDS, *Pythium* and *Phytophthora* using seed-treatment/soil drench treatment techniques, and in controlling frogeye leaf spot and *septoria* brown spot using detached leaf spray method. We estimated that the percentage of leaf lesions of white mold disease was reduced more than 50% to 100% as compared to controls in detached leaf assays; and dead plants were 42 to 54% in BAC-treated plants as compared to 70% in controls using cut-petiole inoculation method. We showed both seed treatment and soil drench treatment effectively reduced SDS disease severity and root lesions, and BCA treatment reduced frogeye leaf spots to 7.5% to 17.5% as compared to 80% in control plants. These results indicated that BCA could effectively suppress root, stem, and foliar soybean fungal diseases in both lab and greenhouse conditions. However, the results for control of white mold, SDS, and frogeye leaf spot in field trials using soil drench, seed-treatment and foliar spray, respectively, were not successful, partly due to conditions to induce BCA antifungal activities were not favorable and lack of proper delivery methodology.

In order to improve this, we determined the conditions that will increase the antifungal activities of BCA by changing various growth conditions and bacterial inoculum concentration, which is important for future fermentation experiments. We found that strongest inhibition activity was observed for PDA concentration less than 25% with $OD_{600} > 0.1$ at suitable temperature with certain shaking speed. In order to determine what genes may be responsible for BCA's antifungal activity, we further employed a molecular approach by generating a mutant library with more than 4000 mutants and screened for loss of antifungal activities. We screened the mutant library against white mold/SDS pathogens and obtained 16 mutants with no antifungal activities against both white mold and SDS. We then determined the insertion site of the mutants and identified the genes that have been mutated, which include diverse functions. Further characterization of these mutants will determine what aspects of antifungal activities affected. The results will provide insights into what controls the antifungal activities of the BCA and will help improve the biocontrol efficacy of the BCA.

The bacterial biocontrol agent (BCA) inhibited mycelia growth and spore germination of eight soybean root, stem and foliar fungal pathogens, including 10 isolates of *Phytophthora sojae*, 10 isolates of *Rhizoctonia solani*, 11 isolates of *Pythium* spp (which included isolates from 4 species: *P. ultimum*, *P. dissoticum*, *P. torulosum*, and *P. sylvaticum*), 3 isolates of *Macrophomina phaseolina*, and 5 isolates of *Sclerotinia sclerotium*; 10 isolates of *Fusarium virguliforme*; 9 isolates of *Septoria glycines*; and 8 isolates of *Cercospora sojina* (**Fig. 1**), suggesting that BCA exhibits broad spectrum inhibition activities.

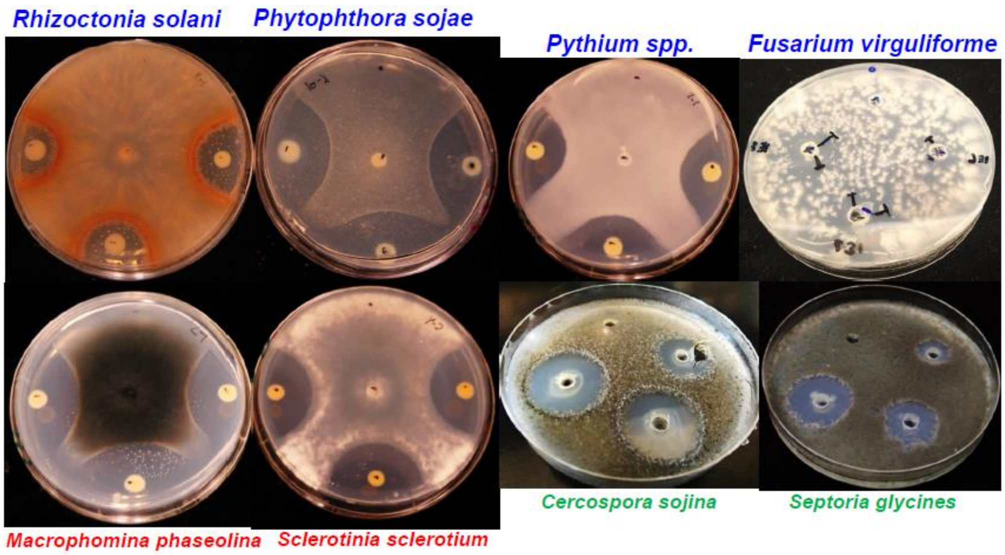


Fig. 1. BCA inhibits spore/mycelia growth. Blue: root pathogens; Red: stem pathogens; Green: Foliar pathogens.

Progress in Phase II

1) *Control white mold.* Experiments were conducted and repeated in lab and greenhouse conditions to determine the efficacy of the bacterial biocontrol agent (BCA) in controlling *Sclerotinia* stem rot (White mold, *Sclerotinia sclerotium*) using cut-petiole and detached leaf spray methods (**Figs. 1 and 2**). Results showed that the percentage of leaf lesions of white mold disease was reduced more than 50% to 100% as compared to controls in detached leaf assays (**Fig. 1**); and dead plants were 42 to 54% in BAC-treated plants as compared to 70% in controls using cut-petiole inoculation method (**Fig. 2**).

2) *Control root rot diseases.* Experiments were conducted and repeated in lab and greenhouse conditions to determine the efficacy of the BCA in controlling root and stem blight (*Rhizoctonia solani*), SDS (*Fusarium virguliforme*), Pythium and Phytophthora root rot (*Pythium ultimum* and *Phytophthora sojae*) using seed-treatment/soil drench treatment of BCA. Results showed that both seed treatment and soil drench treatment effectively reduced SDS disease severity and root lesions.

3) *Control foliar disease*. Experiments were conducted and repeated in lab and greenhouse conditions to determine the efficacy of the BCA in controlling foliar pathogens (frogeye leaf spot, *Cercospora sojina* and *septoria* brown spot, *Septoria glycines*) using detached leaf spray method. Results showed that BAC treated plants reduced frogeye leaf spots to 7.5% to 17.5% as compared to 80% in control plants.

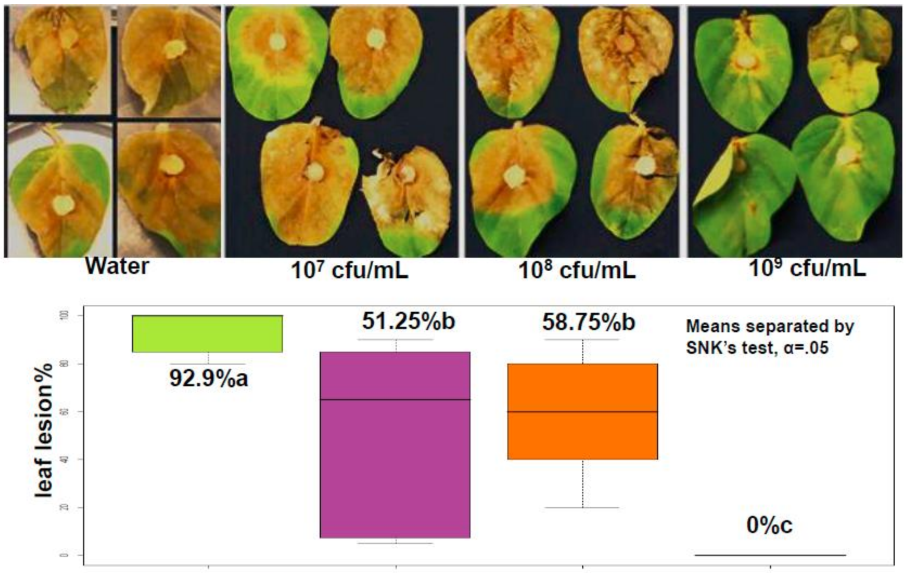


Fig. 1. BCA spray application reduces lesions from *S. sclerotium* in a detached leave assay

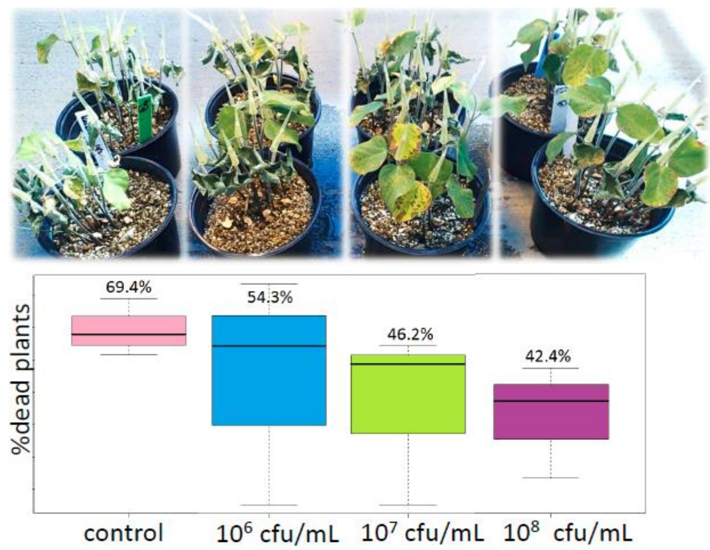


Fig. 2. BCA-spray treatment suppresses white mold caused by *S. sclerotium* in greenhouse

Publications by University of Illinois:

1. Nian, J., Bradley, C., and Zhao, Y. F. 2016. Evaluation of *Lysobacter enzymogenes* C3 for control of soybean fungal diseases. Phytopathology 106:S1.6
2. Thesis: Jeffrey Nian. 2015 (MSc). Evaluation of *Lysobacter enzymogenes* C3 for control of soybean fungal diseases. Pp99

3. Yu, M., Zhang, G., and Zhao. Y. F. 2017. Identification and characterization of genes in antagonism against fungal pathogens by the biocontrol agent *Lysobacer enzymogenes* strain C3. APS North Central Division Meeting, Urbana, IL

Results from University of Minnesota

Research plot tests

1. Rosemount, MN: SDS control experiment with two bio-fungicides, an uninfecting inoculum control and an untreated control treatment laid out in RCB with four reps (each plot 150 sq ft) planted on May 21 and June 11. At planting, each treatment and the untreated control was inoculated with SDS inoculum consisting of sorghum infested with *Fusarium virguliforme*. Plots were to be managed with irrigation; however frequent rains made irrigation unnecessary. Stand count and seedling stage SDS foliar disease observations were made on 23 July followed by observations at 15 day intervals. The late season observation for SDS and defoliation percentage was conducted on Sept 4.

Observations: no differences were observed among the treatments in emergence (Table 1), early or late season foliar symptoms. The 21 May planting was harvested on 16 October and 11 June planting harvested on 27 October but yields were not available on 29 October since samples were still being threshed and weighed.

Rosemount, MN: White mold control experiment with two bio-fungicides and an untreated/unsprayed control treatment were established in RCB with four reps (each plot 150 sq ft) on May 21 in field previously inoculated with white mold, irrigated, and planted at 200,000 ppa to induce white mold. Plots were treated with biocontrol treatments as spray treatment at R1 stage on 23 July. Plots were then evaluated at three week interval disease incidence and presence of apothecia.
Observations: White mold was present at a low level of incidence unrelated to plot treatment. We did not observe apothecia.

2. Becker, MN: White mold control experiments with two bio-fungicides and an untreated control were laid out in RCB design with four reps (each plot 150 sq ft). The plots were planted in an area that has had severe white mold previously. Plots were irrigated. Plots were treated with biocontrol treatments as spray treatment at R1 stage on 23 July. Plots were then evaluated at three week interval disease incidence and presence of apothecia. The plots were harvested on 8 October but yields were not available on 29 October since samples were still being threshed and weighed. White mold was present at very low levels of incidence unrelated to plot treatments.

Observations: Apothecia numbers appeared to have been affected by the treatments; however, the differences were not significant because of high variability. An average of 6.25 apothecia/15 foot of row was observed in untreated plots, 1.75 apothecia/15 foot of row in BCA-2 treated plots and 3.5 apothecia /15 foot of row in HeadsUp treated plots.

Table. Stand count (plants per acre) in biocontrol treated plots observed four weeks after planting. The differences among treatments observed within planting dates were not significant.

Treatment	21 May Planting	11 June Planting
Inoculated,Headsup	142115	90931
Inoculated, BCA-2	132313	89298
Inoculated, Untreated	151371	113800
Uninoculated	119790	88209
Mean	136397	95832

Future plan

The BCA improves soil health by reducing pathogenic fungi in the soil. Therefore application BCA should reduce a number of diseases not only on soybean but also corn. If farmers rotate with corn next year, an application in soybean or corn should help reduce stalk rot. This provide a new tool for sustainable disease control and we should continue to work toward to that direction. As to apply the BCA in fall, the best time is October when soil temperatures are good for biological control agents.

This project has proven through field trials that the BCA has a great potential for commercialization. It is reliable to protect soybean from multiple diseases (SDS, white mold, and seedling diseases). It can increase yield as high as 9.9 bu/ac and farmers liked the product. The next step is to put this product into commercial soybean production.

Because BCA control disease by increasing soil health and improving structure of soil microbial community, an application in one season can take care of disease problems for a few years, as it has been shown in literature and it is yet to prove in our case. However, this type of soil health improvement treatment will generate less profits than annual spray. Commercialization of soil health product can be challenging.

A producer who collaborated with us was interested in testing mix application of BCA with liquid manure in fall and wanted to test it with us. We are studying the possibility.