



## North Central Soybean Research Program

### **Exploiting potential bio-control agents to manage seedling diseases of soybean**

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The goal of this project is to characterize the bio-control activity of a collection of fungal species isolated from soybean production fields and study the potential of the bio-control agents to improve management of soybean diseases caused by *Fusarium* spp., *Phytophthora sojae*, and *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.

#### **Project Objectives:**

- Test the effects of recently identified potential bio-control agents on soil-inhabiting fungal, oomycete, and nematode pathogens of soybean;
- Assess potential roles of these agents in eliciting defense mechanisms in soybean plants;
- Test the effect of fungicidal seed treatments on these organisms; and
- Follow the effect of commonly used management practices on the distribution and activity of these organisms in the soil.

#### **Results**

The in-vitro screening of the activity of fifty-eight potential bio-control agents (BCA) against several *Pythium* sp., *Fusarium* sp., *Macrophomina phaseolina*, and *Rhizoctonia solani* is complete. In many instances, several isolates of the BCA species were tested against several isolates of the pathogen. Bio-control agents with consistent high activity against the tested pathogens have been identified.

Greenhouse experiments have been conducted to test the effectiveness of the BCAs against *F. virguliforme*, *M. phaseolina*, and *R. solani*, and the preliminary data is promising.

Growth chamber evaluations of six of the most promising BCAs was done against four *Pythium* species: *Pythium 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. This experiment is being repeated using spore suspensions of one of two BCAs, and either treating the seed with the spore suspension, or using the spore suspension as an 'in-furrow' or 'drench' treatment. The experiment is inoculated with *P. sylvaticum*, which is the most prevalent species of *Pythium*

associated with soybean in the north central region.

A protocol to evaluate the BCAs in the field in 2016 was developed. Land to conduct the study was secured, and inoculum production is in progress. Seeds have been sent to SIU for BCA coating and will be shipped back to MSU and ISU to inoculate field trials. The field experiments will be conducted in IA, MI, and IL. The experiments will include five separate treatments of BCAs (individually or in mixture) that were selected based on the performance of the corresponding BCAs in lab, greenhouse or growth chamber assays. In these field experiments, BCAs will be assessed against oomycetes in IA, and MI, and against *F. virguliforme* and *M. phaseolina* in southern IL.

Bio-control agents can affect plant pathogens directly by affecting their growth, or indirectly by inducing defense mechanisms in the plants, thus enhancing the plants' tolerance to potential plant pathogens. At SIUC, in order to characterize the defense responses of soybean plants to fungal infection in the presence of the BCAs, soybean seeds were pre-treated with a spore suspension of BCAs. Three different BCAs combinations were used. The treated soybean seeds were planted in soils infested with *F. virguliforme* or *R. solani*. The experiment was carried out in the greenhouse, under a completely randomized design with three replications. The expression analysis of six plant defense-related genes was evaluated in both root and leaf tissue by quantitative RT-PCR. Our preliminary data indicates that the tested BCAs have the ability to induce plant defense both locally (in the roots) and distally (in the leaves) in treated plants. This indicates that pre-exposing soybean to the BCAs does enhance their tolerance to pathogens.

Fungicide sensitivity of 11 BCAs has been completed for the following fungicides: sedaxane, azoxystrobin, pyraclostrobin and trifloxystrobin. We have still to test fludioxonil, ipconazole, thiabendazole and fluopyram.

In the 2016 field season, BCA inoculum was produced at SIU, soybean seeds were coated with different BCA formulations and the coated seeds were shipped to MSU and ISU to conduct field trials. The different BCA treatments were selected based on the performance of the corresponding BCAs in lab, greenhouse or growth chamber assays.

Field experiments were conducted in three locations in IL (Carbondale, Shawneetown and Valmeyer), one location in IA and one location in MI. Treated soybean seeds were exposed to *Fusarium virguliforme* (SDS) and *Macrophomina phaseolina* (charcoal rot) in IL, and to oomycetes in IA and MI. Five different BCA treatments are being assessed in the IL locations and four different BCA treatments are being tested in MI and IA.

Stand counts were collected as well as disease symptoms for SDS and charcoal rot. Plant samples were also collected. The samples are currently being processed. DNA will be extracted and QPCR, using species specific probes, will be carried out to quantify the amount pathogen found in collected roots. This data will be compared and contrasted to other collected phenotypic data such as stand count and disease symptoms to determine if the BCAs were able

to provide protection against root colonization by the pathogens.

### **Benefit to Soybean Farmers**

The proposed research will result in the identification of bio-control agents that could effectively control major soybean pathogens. The generated data will also provide information regarding the mechanisms these agents use to affect soybean pathogens. This information is crucial to exploit the activity of these bio-control agents against plant pathogens by using them as soil or seed amendments or by identifying management practices that enhance the activity of bio-control agents already present in soybean production fields. The ultimate goal is to provide soybean growers with cost-effective tools to increase and sustain profitability by managing diseases.