Biochar seed coating as an approach for preventing root infection of soybean seedlings by *Macrophomina phaseolina* and other soil fungi. Project 18-2022

Final Report

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Background:
Prior studies in this laboratory on the mechanism of root infection by *Macrophomina phaseolina* in charcoal rot disease of soybean indicated that the fungus detects the proximity of meristematic tissue near root tips by binding to a unique polysaccharide on the surface of sloughed off root cap cells in the soil. Binding the polysaccharide triggers (i) (-)-botryodiplodin release, which kills dividing cells of the meristem causing loss of the root tip and exposure of the root's vascular system; and (ii) intense hyphal branching that results in hyphae growing into the root's vascular system. Early research on *M. phaseolina* toxins indicated that (-)-botryodiplodin binds tightly to charcoal. The proposed research is to determine if biochar, which is a type of charcoal produced as a soil amendment, can be used to protect germinating soybean seeds from root infection by fungi that use (-)-botryodiplodin or other charcoal-binding mycotoxins to facilitate root infection by killing meristematic tissue. Two approaches that can be envisaged for using biochar are (i) by addition to the soil around the seed at planting and (ii) by incorporating biochar into a bioplastic-based seed coatings. Of these approaches, biochar-containing seed coatings are considered the most practical approach. Successful application of biochar in seed coatings would be expected to significantly reduce seed losses for Mississippi soybean growers, thereby reducing costs and improving profitability.

Objective: The objective of the proposed research was to determine if bioplastic-based seed coatings can be used to protect germinating soybean seeds from root infection by fungi in the soil, including *M. phaseolina.*

Progress Report
Substantial progress has been made in the evaluation of biochar-containing seed coatings applied in a matrix of the commercially available cornstarch-based bioplastic, Mater-Bi. It has been possible to carry out field trials of biochar coated soybean seeds in three areas in each of the two growing seasons during the funding period. This involved two field trials in Stoneville (Washington County), MS, and one in Jackson, TN. The trials used four soybean varieties (Asgrow 46x6, Asgrow 38x8, Pioneer P40A47X, and germplasm line Y227-1). These field studies used a completely randomized block design to compare the following treatments: (1) bare, untreated seed alone; (2) coated seed containing hardwood biochar in a cornstarch-based bioplastic matrix; (3) coated seed containing coconut shell biochar in the same matrix; (4) bare, untreated seed with the same amount of hardwood biochar added to the furrow unattached or as a coating on autoclaved sorghum seed as control for coating; (5) bare, untreated seed with the same amount of coconut shell biochar added to the furrow unattached or as a coating on autoclaved sorghum seed as control for coating; and in Jackson (6) seed treated with seeds treated with Syngenta's Apron MAXX fungicide-containing (mefenoxam and fludioxonil) coating. These studies used 5% wt/v homogenized, heat-treated Mater-Bi to attach hardwood biochar (22.5 mg/seed) and coconut shell biochar (36.5 mg/seed). Hardwood biochar was obtained from Rockwood Recycling, LLC, Lebanon, TN, and coconut shell biochar was purchased from Cool Planet, Inc., Greenwood Village, CO. Hand-coating of soybean seeds was used for the 2020 growing season, whereas the 2021 growing season used machine-coated seed produced with a Hege 11 seed treater. The hardwood and coconut shell biochars were shown in laboratory studies to be capable of binding botryodiplodin and undefined toxins produced by *Macrophomina phaseolina* isolates in culture.

There were no significant effects of biochar seed coating on the germination rate of Asgrow 38X8 with either hardwood or coconut biochar. In a field study in 2020 carried out in collaboration with Dr. Nathan Little at SIMRU farm, all biochar treatments (seed coating and granule placement in furrow) gave insignificant increases in soybean germination rate except hardwood biochar seed coating, which gave a significant 7% reduction. However, soybean plants growing from hardwood biochar seed coated soybeans were noticeably bigger and healthier, although the observation could not be quantitated in the current study. The overall conclusion from pilot and field studies so far is that there is no consistent effect of biochar seed coating on the germination rate of soybean seed.

In the Stoneville (SIMRU farm) trials in 2020, yields with seed coatings containing hardwood biochar or coconut seed were higher than controls (4.4% higher for seed coating containing hardwood biochar and 2.6% higher for seed coating containing coconut biochar), but the differences were not statistically significant due to the way the data was collected. Soil sampling at SIMRU farm indicated a sporadic, but generally low incidence of *M. phaseolina*. In the Jackson field trials in 2020, the highest yields were observed with hardwood biochar added to the furrow as a control for coating (+8.5%, corresponding to 52.1 bushels per acre) and coconut biochar added to the furrow control (+4.6%, corresponding to 50.2 bushels per acre), whereas yields from biochar-coated seeds were lower. However, none of the yield differences were statistically significant in the Jackson trials due to the way the data was collected.

In the Stoneville (SIMRU farm) trials with Asgrow 46X6 seed in 2021, yields were compared between untreated seed and (i) seed coated with hardwood biochar in a bioplastic matrix, (ii) seed coated with coconut shell biochar in a bioplastic matrix, (iii) uncoated seed with the same amount of hardwood biochar added to the furrow at planting coated on autoclaved sorghum seed, (iv) uncoated seed with coconut shell biochar added to the furrow at planting, coated on autoclaved sorghum seed and (v) biochar added to the soil. No significant differences in yield were observed comparing all other treatments to untreated seed or comparing either type of biochar as a seed coating versus biochar added to the furrow at planting.

In the Stoneville (Washington County) Field 17 trials in 2021, two soybean hybrids were studied comparing untreated seed with seed coated with either hardwood biochar in a bioplastic matrix or coconut biochar in a bioplastic matrix. With the Y227-1 germplasm line there was no significant difference in the charcoal rot disease severity rating ($P>0.05$, paired two-sample *t*-test assuming equal variances). However, with Pioneer P40A47X soybean variety, the hardwood biochar containing seed coating resulted in a significantly lower charcoal rot disease severity rating ($P<0.02$, paired two-sample *t*-test assuming equal variances).

In the field trials in Jackson, TN in 2021, untreated soybean seed was compared to (i) seed coated with hardwood biochar in a bioplastic matrix, (ii) seed coated with coconut shell biochar in a bioplastic matrix, (iii) hardwood biochar added in the same amount to the furrow, (iv) coconut shell biochar added in the same amount to the furrow and (v) seed treated with Apron MAXX seed treatment with two chemical fungicides, mefenoxam and fludioxonil. No significant difference ($P>0.05$, paired two-sample *t*-test assuming equal variances) was observed between untreated seed and biochar-treated seed either as a seed coating or in the furrow for the charcoal rot disease severity rating, or for yield measured as either bushels per acre or weight per hectare. Studies culturing fungus from plants exhibiting symptoms of charcoal rot indicated contamination with *M. phaseolina* with and without production of botryodiplodin. The only significant difference observed was lower Spring and Fall stand counts with hardwood biochar-containing seed coating and lower Fall stand counts with coconut shell biochar-containing seed coating.

Additional studies are needed to explore alternate formulations for seed coating matrices and application processes.

Impacts and Benefits to Mississippi Soybean Producers

Successful development of a strategy for reducing root infection of soybean seedlings by fungi in the soil using biochar-coated seeds would reduce seed costs, improve land use and increase profitability. A secondary result would be the enrichment of soils with biochar as well as accomplishing carbon sequestration that may someday receive government support and create a new revenue source for Mississippi farmers. A benefit of this research is expanded experience with the use of seed coatings to deliver substances to the soil at the time of planting that can improve crop performance without increasing labor inputs and other input costs.

End Products

The following are the five most important outputs of the research on this project.

1. A graduate student, Mr. Vivek Hemant Khambhati, used biochar toxin binding experiments as part of his MS thesis research, which resulted in the thesis "Identification and evaluation of mycotoxins produced by *Macrophomina phaseolina.*" in partial fulfillment of the requirements for the Degree of Master of Science in Agricultural Life Sciences (Plant Pathology) from the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State, Mississippi, which was awarded in August, 2021.
2. The following publication included research on *Macrophomina phaseolina* and charcoal rot disease of soybean supported by the Mississippi Soybean Promotion Board:

   Khambhati V.N., Abbas H.K., Sulyok M., Tomaso-Peterson M., (2020) First report of the production of mycotoxins and other secondary metabolites by *Macrophomina phaseolina* (Tassi) Goid. isolates from soybeans (*Glycine max* L.) symptomatic with charcoal rot disease. *Journal of Fungi*, **6**, 332; doi:10.3390/jof6040332.
3. The following publication included research on *Macrophomina phaseolina* and charcoal rot disease of soybean supported by the Mississippi Soybean Promotion Board:

   Alam S., Abbas H.K., Sulyok M, Khambhati V.H., Okunowo W.O., Shier W.T. (2022) Pigment produced by glycine-stimulated *Macrophomina phaseolina* is a (-)-botryodiplodin reaction product and the basis for an in-culture assay for (-)-botryodiplodin production. *Pathogens*, 11, 280. https://doi.org/10.3390/pathogens11030280.
4. Field trials associated with these studies provided information supporting the release of soybean germplasm line DS31-243 with tolerance to mature seed damage.
5. Forthcoming publication of field trial results and a possible patent application relating to biochar in seed coatings.