Biochar seed coating as an approach for preventing root infection of soybean seedlings by *Macrophomina phaseolina* and other soil fungi. MSPB No. 18-2020
Annual 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 three field trials of biochar coated soybean seeds in this reporting period, two field trials in Stoneville, MS, using Asgrow 38X8 and Asgrow 46X6 soybean seeds and one field trial in Jackson, TN, using Asgrow 38X8. These field studies use a completely randomized design to compare the following treatments: (1) bare, untreated seed alone; (2) hardwood biochar-containing coated seed; (3) coconut shell biochar-containing coated seed; (4) bare, untreated seed with unattached hardwood biochar added to the furrow (as control for coating); and (5) bare, untreated seed with unattached coconut shell biochar added to the furrow (as control for biochar present in the 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 Sustainable Solutions, 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, while currently ongoing field trials in the 2021 growing season are using machine-coated seed produced using a newly acquired Hege 11 seed treater.

There were no significant effects of biochar seed coating on the germination rate of Asgrow 38X8 with either hardwood or coconut biochar. These studies were also carried out with Asgrow 46X6 with the result that biochar seed coating was associated with an about 10% reduction in germination for both hardwood or coconut biochar. In a field study 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, 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*. The Jackson field trials used a completely randomized design to compare the following treatments: (1) uncoated, untreated seed alone; (2) hardwood biochar-containing coated seed; (3) coconut shell biochar-containing coated seed; (4) uncoated, untreated seed with unattached hardwood biochar added to the furrow as a control for coating; (5) uncoated, untreated seed with unattached coconut shell biochar added to the furrow as a control for coating; and (6) seeds treated with Syngenta's Apron MAXX fungicide-containing coating. 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. Soil sampling in Jackson indicated a high incidence of *M. phaseolina* in most plots. The efficacy evaluation available at this time was a comparison of soybean plant samples according to a charcoal rot test of severity rating scale in which plants from both seed coating treatments (coated with hardwood biochar and seeds coated with coconut biochar) were slightly lower in average charcoal rot severity rating than plants from either of the two control seed treatments, but the differences were not statistically significant.

Laboratory experiments were conducted to determine if the hardwood and coconut shell biochars are capable of binding undefined toxins produced by *M. phaseolina* isolates in culture. Culture filtrates were prepared in triplicate from four pathogenic *M. phaseolina* isolates (two known botryodiplodin producers and two known botryodiplodin non-producers) and an *A. verrucaria* isolate from soybean as positive control. Biochar binding was tested for both hardwood and coconut shell biochars as 10% (w/v) biochar suspensions in water mixed with an equal volume of culture filtrate, shaken 30 minutes on an orbital shaker and then filtered. Biochar-treated filtrates and controls were evaluated for root toxicity using quadruplicate soybean seedlings in hydroponic culture by adding culture filtrates or Czapek-Dox broth as a negative control to the hydroponic medium. Toxicity was measured as (i) reduced increase in stem length, (ii) reduced medium uptake, (iii) reduced increase in dry weight, and (iv) enhanced disease rating. Both hardwood biochar and coconut biochar significantly reduced toxicity compared to the controls, but hardwood biochar reduced toxicity significantly better than coconut biochar. The conclusions were the same whether or not the *M. phaseolina* isolate was known to produce botryodiplodin.

Major activities in the 2021 growing season have been design of field and laboratory trials and procurement of needed materials. Field trial space has been secured in SIMRU farm near Stoneville and in Jackson TN for randomized complete block design trials to begin when the fields dry out. The experimental group will use untreated soybean seed coated with two types of finely ground biochar embedded in a matrix of commercial cornstarch-based bioplastic. Procurement of the untreated Asgrow seed needed for this type of trial has been accomplished. Sufficient commercial bioplastic (Mater-Bi®) has been procured as well as seed coating machine time. Grinding of biochar in sufficient quantity has been arranged as a contribution from the hardwood biochar manufacturer, Rockwood Sustainable Solutions, Lebanon, TN. A major effort has been put into developing adequate controls, specifically procuring biochar in a form suitable to be added to the root zone not associated with the seed using standard field implements. The effort has focused on design and procurement of a form of pelletized biochar that could be introduced into the root zone by standard seed drill equipment. Rockwood Sustainable Solutions has purchased pelletizing equipment at their expense to work with us to develop a suitable binder and pelletizing technology that would (i) bind finely divided biochar into pellets stable enough for general handling and delivery to the root zone with standard seed drill equipment; (ii) disperse the biochar effectively in the soil when exposed to soil water;

(iii) serve as a source of fertilizer when decomposition of the binder occurs; and (iv) serve as a vehicle for additives, including other fertilizer components such as essential micronutrients, particularly the two most commonly deficient for soybeans, iron and manganese.

A study was conducted to investigate possible contamination with known mycotoxins of harvested soybean seed grown from biochar-coated seed (Stoneville field 17 with Dr. Rusty Smith), particularly mycotoxins known to be associated with the charcoal rot fungus *Macrophomina phaseolina*. Previous studies had indicated that this fungus uses one or more mycotoxins to facilitate initial infection of soybean plants through the roots, resulting in low levels of root contamination, but no detectable contamination of seeds. Seed was collected from 12 rows of Stoneville field 17, ground, extracted with 50% methanol and extracts sent for mycotoxin analysis by LC-MS/MS. The results are presented in the following table.

	Mycotoxin levels (ng/g seed)											
Row Number	DPR-467	DPR-471	DPR-475	DPR-479	DPR-482	DPR-486	DPR-490	DPR-495	DPR-499	DPR-503	DPR-507	DPR-511
3-Nitropropionic acid	240.3	15.6	0	93.7	0	2.6	12.4	7.8	28.0	3.0	20.6	0
Abscisic acid	740	710	713	1017	858	434	505	632	981	666	674	672
(-)-Botryodiplodin	0	0	0	0	0	0	0	0	0	0	0	0
Brevianamid F	2.24	2.03	2.03	2.73	2.40	1.86	2.08	1.96	2.66	2.09	2.21	2.09
Cercosporin	44.9	32.9	34.9	24.2	23.6	85.9	11.9	18.2	8.8	20.3	75.7	35.1
Cordycepin	100.6	103.2	99.4	119.9	93.7	111.2	110.3	130.5	109.1	132.4	129.6	110.4
cyclo(L-Pro-L-Tyr)	16.2	17.6	15.0	20.9	18.4	11.0	12.6	12.7	17.8	17.6	17.5	15.0
Kojic acid	0	0	0	0	0	0	0	0	0	0	0	0
Mellein	0	0	0	0	0	0	0	0	0	0	0	0
Moniliformin	0	0	13.7	0	0	0	36.6	94.0	0	0	0	260
Penicinoline	156	164	152	196	158	196	186	191	116	141	159	163
Phaseolinone	0	0	0	0	0	0	0	0	0	0	0	0

No detectable levels were found of the known *M. phaseolina* mycotoxins (-)-botryodiplodin, kojic acid, mellein and phaseolinone, as well as none of the following mycotoxins associated with other fungi: 5-methylmellein, 7-hydroxypestalotin, chrysogin, cyclo(L-Pro-L-Val), emodin, ethylorsellinic acid, gliocladic acid, heptelidic acid, hydroxyroridin A, infectopyron, iso-rhodoptilometrin, methylorsellinic acid, monocerin, orsellinic acid, pestalotin, questiomycin derivative, roridin A, roridin L2, satratoxin G, trichoverrin A, or verrucarin A. Sporadic contamination with low levels of moniliformin, a mycotoxin associated with *Fusarium* spp. and *M. phaseolina*, was observed.

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. Enriching soils with biochar is a way to sequester carbon for thousands of years. Addressing climate change by carbon sequestration may receive support from the current federal administration. If that support extends to carbon credits, Mississippi farmers may get paid to enrich their soil with biochar at some point in the future. A benefit of the proposed research would be experience with biochar and knowledge of how to use it under Mississippi cropping conditions.

End Products

Biochar toxin binding experiments were conducted by a graduate student, Mr. Vivek Hemant Khambhati, 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, to be awarded in August, 2021.