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TITLE: Mechanism of soybean root infection by *Macrophomina phaseolina*

INVESTIGATORS: Hamed K. Abbas, Hamed.Abbas@ars.usda.gov, and W. Thomas Shier

SUMMARY

The fungus *Macrophomina phaseolina* (Mp) causes charcoal rot disease by infecting soybeans from the soil reservoir. At the beginning of these studies, it was not understood (i) how the fungus locates soybean roots in the soil; (ii) how the fungus penetrates the root to enter the vascular system and spread throughout the plant; (iii) how the fungus locates seeds and establishes an endophyte relationship there; and (iv) how the fungus exits the root to spread to adjacent plants.

The studies summarized here have allowed the creation of a model for soybean root infection by *M. phaseolina* that suggests strategies for control of charcoal rot disease in soybeans, and should provide the basis for future studies of the infection process at the molecular level. These studies have sought, but failed to find, any evidence that *M. phaseolina* hyphae use chemotaxis toward soybean root-released substances in soil. Rather, these results suggest that *M. phaseolina* hyphae grow in all directions from a central nutrient source in soil, creating a discoid search pattern limited on top by the soil surface, on the bottom by lack of oxygen, and on the perimeter by nutrient availability.

These studies also indicate that when an advancing *M. phaseolina* hypha by chance nears a soybean root tip, polysaccharides on the surfaces of cells sloughed off the advancing root cap trigger a series of responses that play key roles in the infection mechanism. It is known that cells are sloughed off the advancing root cap into the surrounding soil, where they survive for 3 to 5 days while synthesizing mucilage that lubricates passage of the root through soil.

These studies demonstrated that certain polysaccharides induce in *M. phaseolina* hyphae the following three responses: (i) production of profuse branching in many directions by the responding hyphae that serves to slow its advance through the soil; (ii) release of the mycotoxin (-)-botryodiplodin, which kills rapidly dividing meristematic tissue in the nearby root tip, causing loss of the meristematic tissue and root cap, and (iii) producing necrotic tissue through which the fungus can readily propagate to enter the plant's vascular system.

Better understanding of the role of the polysaccharide trigger agent(s) in the normal life of soybean plants may allow plant breeders to develop non-triggering soybean cultivars that would be expected to be charcoal rot disease-resistant.

OBJECTIVES

Objective 1: Examine the mechanism by which *M. phaseolina* hyphae locate soybean root tips in soil.

Objective 2: Examine how *M. phaseolina* hyphae knows it is near a soybean root tip so that it releases a toxin such as (-)-botryodiplodin to facilitate root entry.

Objective 3. Understand the mechanism by which *M. phaseolina* hyphae enter the soybean root tip and establish an infection in the soybean plant.

REPORT OF PROGRESS/ACTIVITY

Objective 1: Examine the mechanism by which *M. phaseolina* hyphae locate soybean root tips in soil.

In culture assay systems were developed to search for evidence of chemotaxis exhibited by *M. phaseolina* isolates growing from a nutrient source toward a substance reported to be released from plant roots (Fig. 1). Chemotaxis is identified by the formation of a fungal colony that is not perfectly round due to preferential growth towards or away from the test substance. Long range detection of the presence of roots in soil could be most efficiently achieved by sensing a volatile released substance, whereas shorter range detection would be expected to involve diffusible non-volatile substances.

More than 80 substances have been reported to be released from the roots of various species of plants, including plant hormones, chelators of nutrient minerals, and a large number of other plant components for which there is no clear rationale for non-pathological release (Table 1). No chemotaxis was observed toward or away from any root-released substance tested (Table 2). This is consistent with chemotaxis playing no role in soybean root location by *M. phaseolina*, although it does not exclude the possibility that an untested substance(s) might play this role. *M. phaseolina* in plant debris in soil appears to seek soybean root tips by extending hyphae in every direction. Limitations imposed by the soil surface and the need for oxygen by the strict aerobe result in a discoid-shaped search pattern with an infected piece of plant debris or soybean root at the center.

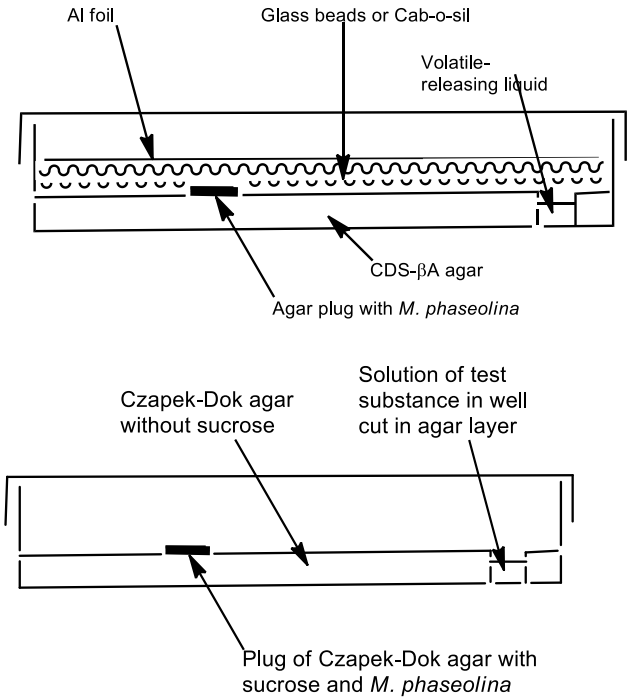


Figure 1. In culture assay systems for the detection of chemotaxis by *M. phaseolina* isolates from Mississippi soybean fields towards volatile (left) or diffusible (right) substances that have been reported to be released from plant roots.

Table 1. Substances reported to be released from plant roots that were shown to not induce any detectable chemotaxis by *M. phaseolina* isolates from Mississippi soybean fields.

A. Volatile Organic Compounds Tested: Isoprene, methyl salicylate, spermidine, spermine
B. Amino acids tested: Casein hydrolysate (Asp, Glu, Ser, Gly, His, Arg, The, Ala, Pro, Tyr, Val, Met, Cys, Ile, Leu, Try, Phe, Lys)
C. Organic acids: Citric, malic
D. Sugars: Glucose, sucrose, oligosaccharides
E. Vitamins: Biotin, thiamin, niacin, riboflavin, calcium pantothenate , pyridoxine, folic acid, biotin, α-lipoic acid, *myo*-inositol
F. Nucleosides: Cytidine, guanosine, thymidine, hypoxanthine, adenosine, guanine, uracil, uridine

Table 2. Substances reported to be released from plant roots that were shown to not induce any detectable (-)-botryodiplodin release by *M. phaseolina* isolates from Mississippi soybean fields.

A. Volatile Organic Compounds Tested: Isoprene, methyl salicylate, spermidine, spermine
B. Amino acids tested: Casein hydrolysate (Asp, Glu, Ser, Gly, His, Arg, The, Ala, Pro, Tyr, Val, Met, Cys, Ile, Leu, Try, Phe, Lys)
C. Organic acids: Citric, malic
D. Vitamins: Biotin, thiamin, niacin, riboflavin, calcium pantothenate , pyridoxine, folic acid, biotin, α-lipoic acid, *myo*-inositol
E. Nucleosides: Cytidine, guanosine, thymidine, hypoxanthine, adenosine, guanine, uracil, uridine

Objective 2: To examine how *M. phaseolina* hyphae knows it is near a soybean root tip so that it releases a toxin such as (-)-botryodiplodin to facilitate root entry.

*M. phaseolina h*yphae propagating at random in every direction from a piece of infected plant debris or an infected root need to have a signal that they have encountered a soybean root tip. The signal should induce the rapidly advancing hyphae to stop advancing and begin secreting a phytotoxin such as (-)-botryodiplodin. About half or more of *M. phaseolina* isolates from soybeans with charcoal rot disease in Mississippi are able to release (-)-botryodiplodin in culture.

We have developed an in-culture assay system to detect the secretion of (-)-botryodiplodin that has proven to be an extraordinarily powerful tool for understanding the signals received and the responses of *M. phaseolina* to them in this part of the soybean root infection process. It has been known since the 1960's that (-)-botryodiplodin reacts with tissues to produce a red pigment. We have shown that the reaction is with amines with glycine and β-alanine giving strong coloration. Incorporating either of them into culture medium such as Czapek-Dox agar provides a method for rapid visualization of (-)-botryodiplodin release from actively growing *M. phaseolina* cultures.

The in-culture model system has *M. phaseolina* growing out from a plug of Czapek-Dox agar containing sucrose across a plate of Czapek-Dox agar without sucrose toward a well containing a root-released substance being tested for ability to induce (-)-botryodiplodin release (Fig. 1). Two types of (-)-botryodiplodin release inducers have been identified in these studies. The first are high concentrations of the simple sugars, sucrose and to a lesser extent glucose, which are proposed to represent an exit

mechanism for *M. phaseolina* leaving root tips of infected soybean plants to spread to plants with interdigitating roots. The second are polysaccharides that may be similar or identical to ones associated with root tips. The discovery of polysaccharide inducers of (-)-botryodiplodin release was made as a result of the serendipitous observation that yeast extract was a potent inducer of (-)-botryodiplodin release, when it was tested as a convenient source of multiple B vitamins.

Subsequent bioassay-guided fractionation of yeast extract by reverse phase chromatography showed that any vitamins present were not (-)-botryodiplodin release inducers, whereas the activity was present in high molecular weight polysaccharide fractions. A screen of 23 polysaccharides (Table 3) identified two strong activators: locust bean gum and guar gum, both manno-galactans (Figure 2). These polysaccharides are structurally similar to a unique polysaccharide reported to be found on the surface of plant cells that get sloughed off root caps and grow in suspension culture (Willats et al., 2004). Sloughable cells are released from root caps on root tips, where they are shed into the soil and live independently for 3 to 5 days, synthesizing mucilage that acts as a lubricant enabling the soft root tip to penetrate soil (Gunawardena and Hawes, 2002; Gunawardena et al., 2005). Both the sloughed cell surface and the secreted mucilage are found in soil in exactly the right place to signal the presence of meristematic tissue nearby.

The polysaccharide specific to sloughable cell has been purified from pea hull by a literature method and shown to be an inducer of hyperbranching and (-)-botryodiplodin release from *M. phaseolina*. The mucilage secreted by the roots of ~100 soybean (Saline) seedlings growing hydroponically was collected and shown to not induce *M. phaseolina* hyperbranching and (-)-botryodiplodin release. This observation is consistent with *M. phaseolina* hyphae recognizing their proximity to the root tip meristematic tissue by interacting with cell surface polysaccharides on sloughed off root cap cells, not the mucilage they secrete. Similar conclusions were reached by Gunawardena et al. (2002; 2005).

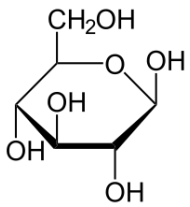
In order for a polysaccharide to stimulate *M. phaseolina* to initiate the three observed activities, (i) hyperbranching; (ii) (-)-botryodiplodin release, and (iii) microschlerotia formation, it must bind to a receptor. Proteins that bind to carbohydrate moieties are called lectins and contain one or many carbohydrate-binding domains in their structure. Because polysaccharides cannot pass through membranes, *M. phaseolina* must have a cell surface receptor protein containing at least one carbohydrate-binding domain, a transmembrane domain, and an intracellular signaling domain capable of activating multiple genes. Thus, this regulation appears to be different from that of aflatoxin release, for which regulation by small lipophilic molecules has been reported.
Because the genome sequence of an *M. phaseolina* isolate has been determined (Islam et al., 2012) and published in GenBank as AHHD00000000, searching the genome with UniProt identified 13 proteins coding for a lectin-like domain and a transmembrane domain identified the following genes. Table 4 describes the results and identifies just two genes as candidates for the receptor.

Table 3. Polysaccharides that were shown to not induce any detectable (-)-botryodiplodin release by *M. phaseolina* isolates from soybean plants with charcoal rot disease in Mississippi fields.

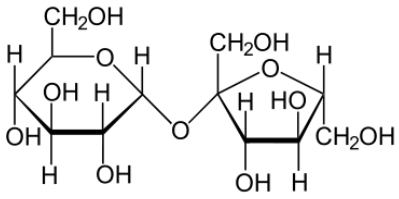
Carrageenan, Carboxymethylcellulose, Na Dextran sulfate, Corn starch, Dextrans, Na Polygalacturonate, Gum Ghatti, Yeast Mannan, Gum Arabic, Xanthum gum, Gum storax, Cellobiose, Chitosan hydrochloride, γ-Cyclodextrin.

Figure 2. Structures of saccharides shown to induce (i) hyperbranching, (ii) (-)-botryodiplodin release and (iii) microschlerotia formation by *M. phaseolina* isolates from Mississippi soybean fields.

a) Simple sugars that may play a role in the exit of fungal hyphae from infected roots.

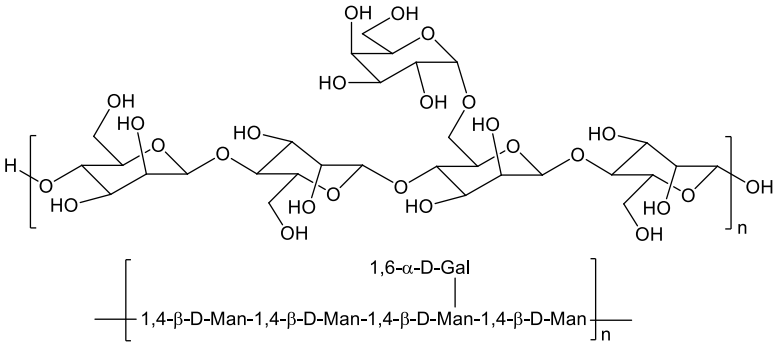


D-Glucose

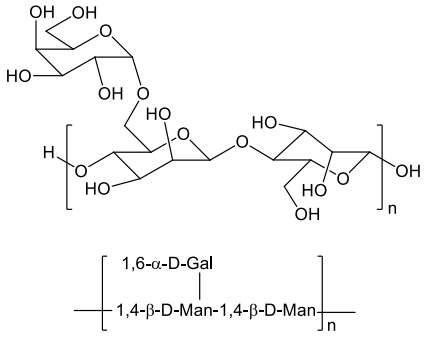


Sucrose

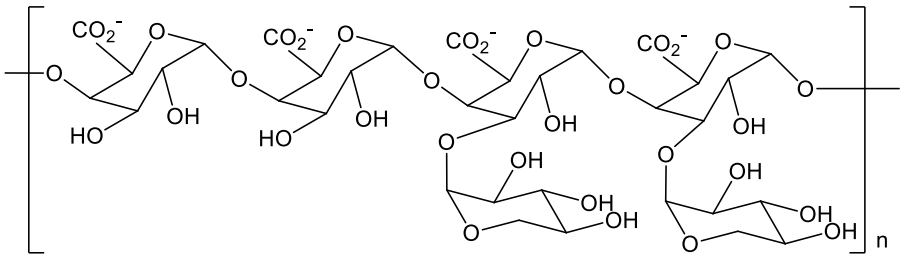
b) Polysaccharides that may signal a close encounter with a soybean root tip in soil

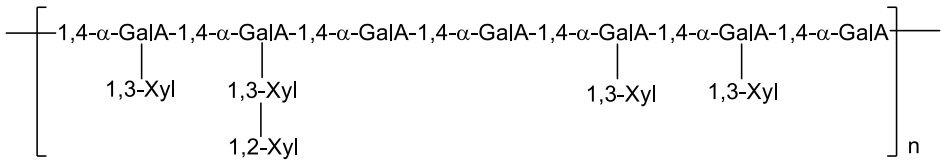


Locust (Carob) Bean Gum



Guar Gum





Xylogalacturonin purified from pea hulls

Table 4. Bioinformatic analysis of the *Macrophomina phaseolina* genome

	Gene	Protein type	Length	Comment
1.	K2SBT9	Calreticulin/calnexin	563	Activity needed for normal metabolism; transmembrane region is at the end with little or no room for a signaling domain.
2.	K2QXM4	Glycoside hydrolase family 16	1,031	Nine transmembrane regions consistent with being a transporter; needed for feeding.
3.	K2R3C1	Glycoside hydrolase family 16	365	Transmembrane region is at the end with little or no room for a signaling domain; needed for feeding.
4.	K2R8W9	Legume-like lectin	322	Transmembrane region is at the end with little or no room for a signaling domain; it probably holds carbohydrate molecules in place while hydrolases digest them.
5.	K2S384	Galactosyl transferase	344	Transmembrane region is at the end with little or no room for a signaling domain; activity needed for normal metabolism.
6.	K2QHS1	Legume-like lectin	359	Transmembrane region is at the end with little or no room for a signaling domain; it probably holds carbohydrate molecules in place while hydrolases digest them.
7.	K2QVF2	Glycoside hydrolase family 16	460	Intracellular enzyme needed for normal metabolism.
8.	K2RPC8	Glycoside hydrolase family 16	412	Fragment; intracellular enzyme needed for normal metabolism.
9.	K2RMF1	Uncharacterized protein	377	2/3 of protein is extracellular and 1/3 intracellular; coiled coil inside.
10.	K2RE07	Glycoside hydrolase family 16	451	Intracellular enzyme needed for normal metabolism; insufficient extracellular domain to bind anything.
11.	K2RHV2	Uncharacterized protein	470	Cytoplasmic carbohydrate-binding domain; insufficient extracellular domain to bind anything.
12.	K2RGU8	Beta-glucan synthesis-associated	654	¼ external, ¾ internal; enzyme needed for normal metabolism.
13.	K2S694	Uncharacterized protein	317	60% external, 40% internal.

Conclusion:
There are two candidate genes for the receptor(s) as follows:

K2RMF1:
MTTIVPVQAASSAAAASTAAAASTTLATSTTAASTGASTSSSFSSPSASTVALTTTFTPPPSCTEHRLSILSSPYYNLW
LNEPSPVPGSLVTDCYPSQFINGYTSLFNQTSSIAPVMSPLVCPDAWTAVNTFSSNYIACCPSGFLLHIPTTSLVDSNR
PAYGGTCYSTFTVGQTITVTGYNASTVTATQQWVASTSLDQAYAHVIDGFALAEASTSSSASAASSSSSNSLSGGAIAG
IVIGVVAFVGILAGFALWFFRRRRQNAAARSASHEVDGGDAFHEKAGDNDFRKELPGAGAATTAELESPDPVHELDSST
PAELDGGWKGAEVHTPGTEKEAERRREMLEMEDREARRLQGEDEGTVKVGSGERRTPPPSY

K2S694:
MWTASVLAQGTSNKDDSRNCFFPNGIQSSGSPCFPDQAVSPCCGPSFICLSNGLCQPGPDTRRTYHYTVYRSSCTDRTW
NSTDCPRICLGSDDNLDSGQGLATCGTGGSYCCGRGYDCCSNATDIFNYGTAEVVTTIPVESSTISTASSTISSVTSAS
PPSKSSSSSSSSATAIGVGVGVGVGGFAVLLASILLFLLFRRRRKQEGKQNHRQSGPNDVEGNQRCGSELPTHEMKHQ
GAGPENGGMPPLPTYHSSNLHNDVNSPSGDGGGDMVPGAPLNPAEFPSGPERERFEMDGEQHETWKDASLRRDRQRHEL
E

Stimulation of hyperbranching as a strategy for blocking the advance of *M. phaseolina* hyphae toward the root tips of germinating soybean seeds.

In principle, it may be possible to utilize the hyperbranching response to a potent polysaccharide inducer to block the approach of *M. phaseolina* hyphae toward the root tips of germinating soybean seeds. Locust bean gum is potentially useful in this role, and indeed this role may be the reason why the locust bean tree makes the gum. Figure 3 shows how effective locust bean gum can be when conditions are correct. At present, not enough is known about the conditions that stop hyphal advance to use it as a protective additive in seed coatings. The presence of sucrose in the medium prevents blockage.

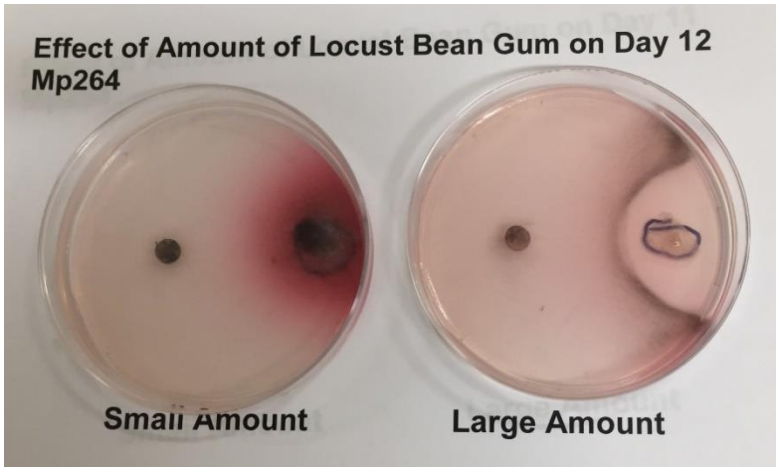


Figure 3. Addition of a small (left) or large (right) amount of solid locust bean gum to the surface of Czapek-Dox agar medium prepared without sucrose but adding β-alanine (20 mg/mL), which reacts with secreted (-)-botryodiplodin to give a red pigment. On the left, the fungus grows on top of the locust bean gum and forms abundant microschlerotia. On the right, the advance of the fungal hyphae across the agar surface is blocked.

Other considerations relating to the role of (-)-botryodiplodin in soybean root infection by *M. phaseolina*.

As indicated above, about half of *M. phaseolina* isolates from soybeans with charcoal rot disease in Mississippi do not release (-)-botryodiplodin in culture. There are several explanations for how they can cause charcoal rot disease without having (-)-botryodiplodin to infect roots. One plausible explanation is that they are endophytes that cause insufficient disease to kill the plant. This explanation was investigated by isolating ~200 endophytes from soybean cultivars (Manokin, Saline, Pharaoh and Egyptian) grown in Mississippi and determining the species of the 10 isolates with culture characteristics most closely related to *M. phaseolina*. Species identification was by DNA sequencing of the ribosomal RNA internal transcribed spacer (ITS-1) region, the beta-tubulin (tub2) region, and the translation elongation factor 1-alpha (tef1) region. Despite using three DNA regions, it was not possible to unequivocally assign species identification to all isolates (see Table 5), but it was possible to unequivocally establish that there was no endophytic *M. phaseolina* isolated from these four soybean cultivars. None of the ten isolates produced (-)-botryodiplodin in culture, with or without stimulation by locust bean gum, although proliferation and microschlerotia formation were stimulated in Saline-1, M-2 and M-4. The results of this study suggest that endophyte formation by *M. phaseolina* isolates lacking a root infection mechanism is not an important cause of charcoal rot disease in soybeans in Mississippi.

Table 5. Species identification of endophytes isolated from soybean cultivars

Cultivar/Isolate	Region	Species Identified by BLAST Search	Identity (%)
Pharaoh			
P	ITS1	*Aspergillus ruber*	100
		Aspergillus amstelodami	100
		Aspergillus rubrum	100
		Eurotium rubrum	100
	Tub2	*Aspergillus ruber*	100
		Eurotium rubrum	100
	Tef1	*Aspergillus uvarum*	89
Manokin			
Manokin	ITS1	*Aspergillus ruber*	100
		Eurotium rubrum	100
		Fungal endophyte sp	100
	Tub2	*Eurotium rubrum*	100
		Aspergillus ruber	100
	Ef1	*Aspergillus terreus*	97
M-1	ITS1	*Fusarium equiseti*	100
		Fusarium incarnatum	100
		Fusarium oxysporum	100
		Fungal endophyte sp	100
	Tub2	*Fusarium equiseti*	99
		Fusarium incarnatum	99
		Fusarium solani	99
		Fusarium langsethiae	99
	Ef1	*Fusarium equiseti*	100
		Fusarium incarnatum	100
M-2	ITS1	*Peyronellaea sp*	100
		Peyronellaea prosopidis	99
		Peyronellaea glomerata	99
		Peyronellaea pomorum	99
		Phoma destructiva	99
		Phoma gardeniae	99
		Phoma eupyrena	99
	Tub2	*Setosphaeria pedicellata*	88
M-4	ITS1	*Setosphaeria rostrata*	100
	Tub2	none	
	Ef1	none	
M-5	ITS1	*Acremonium sp*	100
		Sarocladium kiliense	100
		Sarocladium strictum	100
		Fungal endophyte	100
		Nectria mauritiicola	100
	Tub2	*Sarocladium kiliense*	100
		Acremonium sp	100
		Cercospora piaropi	100

		Fusarium sp	100
	Ef1	*Acremonium* sp	99
		Sarocladium kiliense	99
M-6	ITS1	*Sarocladium kiliense*	100
		Fungal endophyte	100
		Sarocladium strictum	100
		Nectria mauritiicola	100
	Tub2	*Sarocladium kiliense*	100
		Cercospora piaropi	100
		Sarocladium sp	100
		Acremonium sp	100
		Fusarium sp	100
	Ef1	*Acremonium* sp	99
		Sarocladium kiliense	99
Saline			
Saline-1	ITS1	*Phoma eupyrena*	100
		Didymella americana	100
		Peyronellaea glomerata	100
		Ascomycota	100
	Tub2	*Setosphaeria pedicellata*	88
	Ef1	*Setosphaeria pedicellata*	88
Saline-2	ITS1	*Fusarium equiseti*	100
		Fusarium oxysporum	100
		Fusarium chlamydosporum	100
		Fusarium longipes	100
		Fusarium incarnatum	100
		Fusarium culmorum	100
		Ascomycota sp	100
		Colletotrichum capsici	100
	Tub2	*Fusarium incarnatum*	100
		Fusarium equiseti	100
		Fusarium solani	100
	Ef1	*Fusarium incarnatum*	100
		Fusarium equiseti	100
Saline-3	ITS1	*Didymella americana*	100
		Peyronellaea glomerata	100
		Peyronellaea pomorum	100
		Phoma medicaginis	100
		Scytalidium thermophilum	100
	Tub2	*Sarocladium kiliense*	100
		Cercospora piaropi	100
		Acremonium sp	100
	Ef1	*Acremonium* sp	99
		Sarocladium kiliense	99

Another plausible explanation for *M. phaseolina* causing charcoal rot disease in soybeans without having (-)-botryodiplodin to infect roots is that they are using a different toxin that also targets dividing meristematic cells at root tips. This explanation was investigated by examining soybean seedling root toxicity of culture filtrates from *M. phaseolina* isolates that do not produce (-)-botryodiplodin. Two isolates that did not produce (-)-botryodiplodin in culture media but still blocked lateral root growth by soybean seedlings in hydroponic culture as is observed with *M. phaseolina* were examined further and ethyl acetate extracts were shown to be toxic to cultured mouse cells (Table 6) and bacteria (*Bacillus subtilis*). HPLC analysis (Figure 4) confirmed that the two isolates produced many different secondary metabolites, but neither produced (-)-botryodiplodin or patulin, a cytotoxic mycotoxin for which Islam et al. (2012) found the biosynthetic genes in the isolate on which they did genome sequencing. Thin layer chromatography with bioautographic detection using *B. subtilis* found the same Rf value for the antibiotic activity, so a minor peak in the tracings in Figure 4 may be the same for both isolates.

Table 6. Cytotoxicity of Ethyl Acetate Extracts of two *M. phaseolina* isolates (*Mp* 00003 and *Mp* 0205) with NIH3T3 Mouse Fibroblasts

Sample	~IC_{50} dilution	Init. Conc. (mg/ml)	~IC_{50} (µg/ml)
Mp00003-CD	1:4,000	211	52.8
Mp00003-PDA	1:1,000	121	121
Mp0205-CD	1:10,000	862	86.2
Mp0205-PDA	1:7000	94.4	13.5

CD = grown on Czapek-Dox agar medium; PDA = grown on potato dextrose agar medium

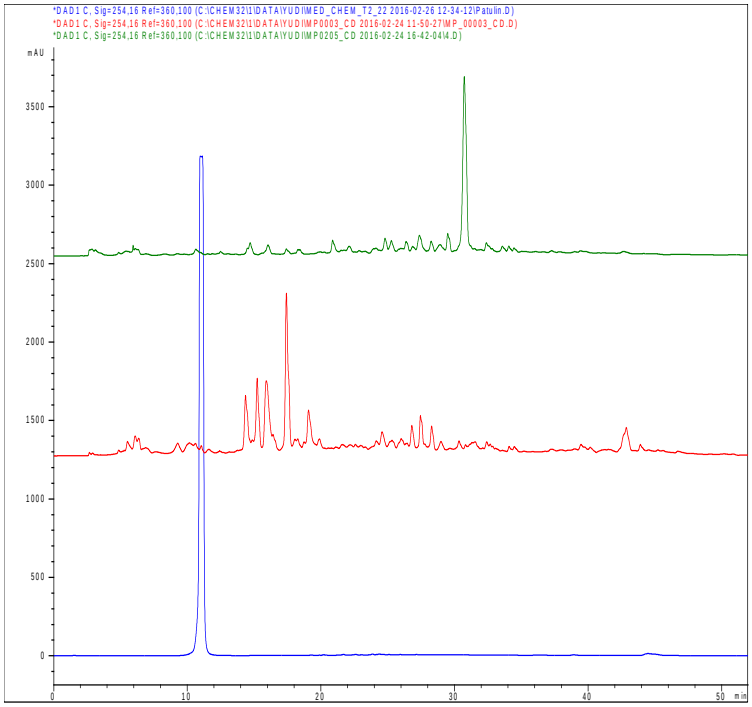


Figure 4. HPLC analysis of ethyl acetate extracts of *M. phaseolina* Isolates that produce no (-)-botryodiplodin or patulin, but still prevent lateral root growth**.** Elution OD at 254 nm for ethyl acetate extracts of cultures grown on Czapek-Dox agar medium. Top panel: *M. phaseolina* isolate *Mp* 0205; middle panel: *M. phaseolina* isolate *Mp* 00003 and bottom panel: patulin standard.

Objective 3. To understand the mechanism by which *M. phaseolina* hyphae enter the soybean root tip and establish an infection in the soybean plant.

The mechanism of entry of *M. phaseolina* hyphae into soybean root tips was investigated in soybean seedlings in hydroponic culture. Addition of (±)-botryodiplodin to the hydroponic culture medium at a concentration that blocks lateral root growth results in the destruction and loss of the root cap and meristematic tissue, leaving the root's vascular system unprotected (Figure 5). The most useful tool for studying the entry of *M. phaseolina* hyphae into the vascular system are mutant lines that express enhanced green fluorescent protein (EGFP) in their hyphae, because the fluorescent label allows the use of confocal microscopy to investigate entry into the vascular system as well as other aspects of root infection, endophyte formation and plant-to-plant spread through soil. Initial studies on mutant preparation used plasmid DNA, pPd-EGFP, obtained from Dr. Angus Dawe, Department of Biological Sciences, Mississippi State University. The plasmid includes genes for ampicillin resistance, EGFP and hygromycin B resistance. The plasmid was used to transform competent *Escherichia coli* BL21(DE3) (New England BioLabs). Plasmid-producing clones selected on ampicillin agar plates were grown in LB broth with ampicillin. Plasmid DNA was purified from the bacterial lysate using a Qiagen Plasmid Midi Kit and quantified on a nanodrop instrument. The DNA was used to transfect *M. phaseolina* 264 using the procedure of Mukherjee et al. (2010), in which protoplasts are prepared and the DNA shocked in with polyethyleneglycol 4000. EGFP-expressing mutants were selected on agar medium containing hygromycin B (20 µg/ml). Clones of transformants were selected as recommended,

but none were stable transformants. Additional handling and selection methods will be needed. A similar procedure has been selected for the preparation of *M. phaseolina* 264 knockout mutants with one of the two potential polysaccharide receptor genes inactivated using commercially-available CRISPR/Cas9 kits.

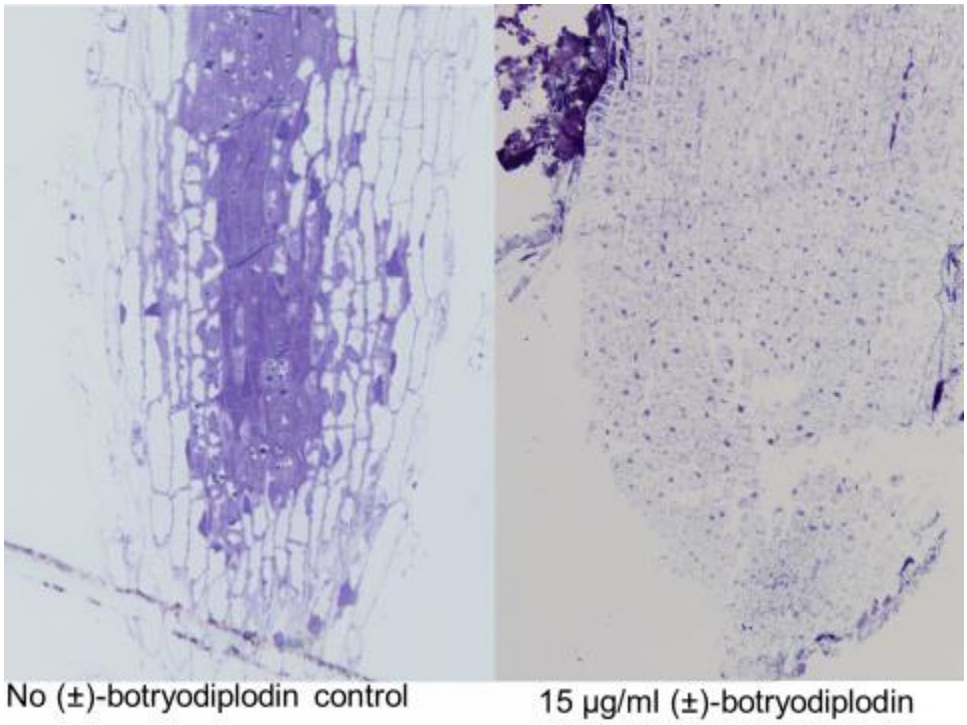


Figure 5. Light micrographs of soybean lateral root tips from hydroponically grown seedlings exposed to no (±)-botryodiplodin (left) or 15 µg/ml (±)-botryodiplodin (right) in the hydroponic medium.

IMPACTS AND BENEFITS TO MISSISSIPPI SOYBEAN PRODUCERS

The observation that certain polysaccharides can block the advance of fungal hyphae may result in the development of seed coating components that reduce root infection in seedlings. The identification of polysaccharides involved in the infection mechanism by *M. phaseolina* offers a strategy for developing resistance to charcoal rot disease in soybeans by developing cultivars that either do not express the polysaccharide at all, do not express it on sloughed off cells, or mask it in some functional way.

END PRODUCTS–COMPLETED OR FORTHCOMING

The studies conducted in this project have greatly advanced understanding of how root infection by *M. phaseolina* occurs. The results are expected to result in at least three publications in peer-reviewed research journals.

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