MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 78-2014 NCE (YEAR 2)
2015 Final Report

Title: Identification and characterization of "mystery disease" pathogen that is prevalent in Mississippi soybean fields in 2014

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EXECUTIVE SUMMARY

An undescribed fungus has been isolated from infected soybean taproots. A colleague at LSU was successful in completing Koch's postulates, thus supporting the undescribed fungus is the etiological agent of taproot decline.

Collaborative efforts between plant pathologists from Arkansas, Louisiana, and Mississippi are working towards the identity of the suspected pathogen, which includes sequencing the genome of the fungus. If successful, the information generated from the fungal genome witL provide a foundation for complete characterization of the type fungus and other fungal isolates collected throughout Mississippi soybean fields expressing symptoms of taproot decline.

Results support the hypothesis that the undescribed taproot decline pathogen may be infecting the plant in the vegetative stage of soybean growth, but can only be isolated from the stroma that develops at the soil line of soybean in the reproductive stages in the field.

We are developing a hypothesis that as a weak competitor the pathogen must first develop stroma for successful re-isolation from inoculated soybean plants.

This work is ongoing as MSPB Project No. 78-2016 in order to definitively identify the causal pathogen so that potential pathogenicity and yield loss can be determined. Only after these steps can treatment strategies be developed if needed.

BACKGROUND AND OBJECTIVES

This research addresses the etiology of the "mystery disease" referred to as taproot decline associated with soybean plants throughout MS production fields. The symptoms include foliar chlorosis, interveinal necrosis, stained vascular tissues, and black stroma, a matrix of vegetative, mycelial hyphae observed on the stem close to the soil line. The disease is widespread throughout Mississippi and recurs in the same area of a field if soybean is planted in consecutive years. An undescribed fungus has been isolated from infected soybean taproots. A colleague at LSU was successful in completing Koch's postulates thus supporting the undescribed fungus is the etiological agent of taproot decline. Collaborative efforts between plant pathologists from Arkansas, Louisiana, and Mississippi are working towards the identity of the suspected pathogen which includes sequencing the genome of the fungus. If successful, the information generated from the fungal genome with provide a foundation for complete

characterization of the type fungus and other fungal isolates collected throughout Mississippi soybean fields expressing symptoms of taproot decline.

Objectives:

1. **Identification of the fungal pathogen(s) associated with taproot decline of soybean**
2. **Assess pathogenicity of fungal pathogen(s) through completion of Koch's Postulates**
3. **Determine the potential yield loss associated with taproot decline**

2015 PROGRESS

In June, soybean plants displaying symptoms of soybean taproot decline and its associated soil from fields in LeFlore, Humphreys, and Sunflower counties were collected. The soil was dried and prepared in clay pots for planting soybean in the greenhouse. A destructive sampling of soybean (four plants; two per pot) was made from each soil sample at growth stages VC, V1, V3, V6, R2, R3, and R4. The lateral roots were separated from the primary root of each plant and surface disinfested. Twenty-five random tissue pieces from each root section were plated onto ¼-strength potato dextrose agar and incubated for five to seven days on the lab bench. Fungal colonies associated with root pieces were identified to genus from which a fungal frequency of occurrence was determined. Axenic cultures of suspected causal agent of taproot decline and other unidentifiable fungal colonies were used for genomic DNA extraction. The internal transcribed spacer region (ITS) was amplified using ITS4 and ITS5 primers in PCR. Partial sequences were developed and compared to ITS sequences in the GenBank via Basic Local Alignment Search Tool (BLAST) for identification purposes. The undescribed fungus was not identified on soybean roots (primary or lateral) grown in field soil where soybean plants symptomatic for taproot decline were collected. Overall, *Fusarium* and *Macrophomina* spp. were the predominate genera isolated from roots of greenhouse-grown soybean cultured in field soils. Fungal occurrence was variable across fields. The frequency of *Fusarium* spp. from soils collected in LeFlore fields 1 and 2 and Humphreys was high with *Macrophomina* sp. resident at a high frequency in the soil from Sunflower County.

These results further support the hypothesis that the undescribed taproot decline pathogen may be infecting the plant in the vegetative stage of soybean growth but can only be isolated from the stroma that develops at the soil line of soybean in the reproductive stages in the field. This may be the reasoning behind the difficulty in recovering this pathogen in Koch's postulates. The fungus grows very slowly and the ability for recovery from diseased tissue is not likely. Successful recovery from infected soybean plants has only been from stroma. Despite an unsuccessful baiting study, we have gained knowledge in the growth characteristics of this pathogen to better understand its biology.

Soybean plants symptomatic for taproot decline were collected throughout the 2015 growing season. The pathogen was isolated from soybean plants at each location increasing the fungal collection initiated in 2014. In axenic culture many isolates produce stromata that may contain sexual or asexual fruiting bodies. We are currently generating thin-section light microscopy samples to prepare a histological examination. If the stroma are not sterile, we can use these results to initiate a morphological characterization of the pathogen. A holotype isolate has been identified through successful completion of Koch's postulates in the laboratory of Dr. Trey

Price, Plant Pathologist at LSU. At Mississippi State, we are preparing fungal cultures of the holotype (dried specimen) for deposit in the U. S. National Fungus Collections and the living culture as the ex-type in the Centraalbureau voor Schimmelcultures in the Netherlands.

Soybean plants growing in the greenhouse were inoculated in November with the taproot decline pathogen and two additional groups were inoculated in February and April 2016. The soybean plants will undergo natural senescence in hopes that the fungus will produce stroma (resting structure) at the base of the stem. We are developing a hypothesis that as a weak competitor the pathogen must first develop stroma for successful re-isolation from inoculated soybean plants.

Oral Presentations:

MEA/MAPPAN Current & Future Challenges Affecting Entomology & Plant Pathology. Tomaso-Peterson, M. and T.W. Allen. The facts behind the "Mystery Disease" of soybean. MEA/MAPPAN Joint Meeting. Starkville, MS 19 October 2015.

T. Spurlock, M. Tomaso-Peterson, T. Allen, P. Price, and R. Singh. Characterization of taproot decline in southern soybean. Soilborne Disease Symposium. Southern Soybean Disease Workers. Pensacola Beach, FL. 9–10 March 2016.

Proceedings:

M. Tomaso-Peterson, T. Allen, P. Price, R. Singh, and T. Spurlock. Characterization of taproot decline in southern soybean. Southern Soybean Disease Workers.