Determining Management-related Factors that Impact the Severity and Incidence of Soybean Taproot Decline (TRD) (Project 15-2020)

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Summary: Soybean taproot decline (TRD), an important soilborne root disease, is shaping up to become one of the major diseases facing soybean producers in Mississippi, and beyond. TRD has been increasing in Mississippi since the first reports more than 12 years ago but yield losses and economic damages have yet to be accurately quantified. The causal agent falls within the *Xylaria arbuscula* species aggregate, many of which are saprophytic fungi (decomposers), which is consistent with anecdotal observations linking TRD and prior soybean crop, or other residues. The composition and function of these microbial assemblages can be altered by agronomic practices in association with key soil characteristics. For instance, tillage, residue management, and cover crops have been shown to promote residue decomposition by increasing the population and efficiency of decomposers and improving aeration by promoting soil aggregation. Relatedly, keystone microbial taxa that exploit the same resources as *Xylaria* spp. can potentially exert considerable antagonistic influence on TRD. With an increased understanding of the relationships involved, there is an opportunity for increased field suppression due to more informed selection of management practices and scientific monitoring of the results. Our aim is to understand how management practices in Mississippi soybean production systems (e.g., early-planting, precision seed placement, irrigation systems, residue management) relate to the occurrence of TRD in order to verify the effects of altering management practices, and to determine the potential for enhancing the disease suppressive ability of these endemic soils. The aim is to broaden the range of cost-effective practices for TRD suppression that are amenable to incorporation into Mississippi soybean production systems.

Objectives

1. Survey of fields for TRD incidence and severity as well as distribution within agricultural fields that differ in farming system classification.
2. Evaluation of the ecology of *Xylaria* spp. based on pathogen population in soil and microbiome structure and function.

Report of Progress/Activity

Objective 1. Survey of fields for TRD incidence and severity as well as distribution within agricultural fields that differ in farming system classification.

Activities and Findings: Field survey of soybean producer farms were conducted and assessed for the intensity of TRD using a systematic rating system developed based on the percent of infected plants along 10 linear feet of row (0% = asymptomatic, 1-25% = light, 26-50% = moderate, and > 50% =

severe). 158 survey transects and a total of 20 soybean fields across the Mississippi delta farms were exploited to study the rate of TRD severity and distribution. Each field was divided into quadrants for assessment (Fig 1). Surveys were completed within two observation areas (10 m radius) per quadrant. From the field survey several parameters that rate the TRD disease intensity were recorded, namely, hot spots of TRD infected locations within the area, number of TRD infected plants per 100 plant transect length, surface residue coverage (%) and occurrence (yes/no) of buried residue within upper root zone of infected plants (two plants per transect). In addition, plant growth stage and residue ratings were documented at each survey transect. Percent coverage of each intensity category was visually estimated for each field. Field distribution characteristic of TRD was determined on a 0-5 scale (0 = No disease and 5 = Diseased plants observed in 10+ locations involving multiple rows and variable row length.). This assessment covers an array of factors such as soils, rotation, tillage, irrigation, variety, fertility regime, crop protection practices, etc. The data variables collected was organized within a geodatabase and classification methodologies were applied to generate a range of groupings across farms.

Based on the field survey and analysis, TRD infection rate ranged from 0-62 percent and there was no trend for the average infection rate across the 20 surveyed farms (Fig.2). Characterization of the TRD distribution in the field showed that higher TRD infection rates were associated with distribution patterns where the diseased plants were concentrated in 5-10 areas in the observation area (sub-areas) (Fig. 3a). The distribution of TRD in infected fields exhibits significant complexity with no clear patterns based on the survey instrument. Poor relationship (R^2=0.25, data not shown) was observed between filed distribution rate and TRD infection (%). Overall TRD rating ranged from 0-15, indication of the light incidence of the disease across the survey fields. Surface residue cover (%) was determined as the percent coverage in an area (3 m length x 1 m width) along the same row assessed for infection rate. Surface residue cover (%) ranged from 0-9 (Fig. 3b), and non-significant relationship was observed for TRD infection rate with the percent residue cover. Besides 97% of TRD infected plants had buried residue present in upper root zone. These results indicate the need for careful design of soil sampling schemes aimed at explaining the ecology of *Xylaria necrophora*, as it is recently identified as a TRD casual organism. And the impact on the incidence and severity and of cultural practices, i.e., crop rotation, tillage, residue management and/or increasing the disease suppressiveness of soils.

Objective 2. Evaluation of the ecology of *Xylaria* spp. based on pathogen population in soil and microbiome structure and function.

Activities and Findings: Based on the survey results, infection period and infection percent of TRD, seven (7) farms were selected for quantifying the microbial ecology of residue associated *Xylaria* spp. and TRD. Sampling was done at GPS-marked locations which account for soybean row position. Within a single soil type (soil map unit) in each field, a known volume of soil and residue was collected to a depth of 7.5 cm (approx. 3 inches). Three of these samples were collected close to one another at each of two separate locations within the soil unit. Sampling scheme in the selected farms covering three crop rotations, soybean-soybean, corn-soybean and cotton-soybean with a tillage/residue management combinations, minimum till-surface residue, conventional till-buried residue and cover crop-high residue. Sampling of bulk and residue (detritusphere) soil was done in November 2020 and April 2021 of the latent period the year from the selected TRD infected farms. The soil samples were placed and transported on the ice and stored in the -80 freezer in the lab until the analysis. The soil will be analyzed for macro- and micro-nutrients, pH, particle size, bioactive carbon, microbial biomass, fungal protein and metabolic diversity.

Soil microbial DNA extraction and amplicon sequencing: From the first latent period sampling (11 November 2020), soil microbial community DNA was extracted from the bulk and detritusphere soil from seven TRD infected farms. DNA quantity and quality were checked to carry out the amplicon sequencing.

16S and ITS fragment amplicon sequencing was done at the Institute for Genomics, Biocomputing & Biotechnology, Mississippi State University using Illumina MiSeq sequencing. Sequence analysis was carried out using QIIME 2 pipeline (an open-source bioinformatics pipeline for performing microbiome analysis from raw DNA sequencing data). Soil microbial DNA was extracted from the second latent period (7 April 2021) soil samples. 16S and ITS library preparation for the amplicon sequencing is underway.

Preliminary Results: Amplicons targeting bacterial 16S and fungal (ITS2) genes were sequenced using Illumina MiSeq sequencing platform. The distribution of the 500 most abundant OTUs (operational taxonomic units- bacterial species) in the soil samples from the two locations indicated two primary patterns of soil bacterial community structure. The location has a significant effect on the bacterial community structure (Fig 4). The important part of the structural variability was related to differences associated with the sampling dates. There was no significant difference in the pattern of bacterial community structure with mid and late sampling dates ($p=0.618$ and 0.529, respectively) indicating that the disease severity had a significant effect on the soil bacterial community composition.

Quantitative PCR for estimating gene copy numbers of *Xylaria* in soil: Based on pathogen tree of homology, we designed a primer set to target RNA polymerase II (RPB1) and BTUB region sequences of both *Xyleria arbuscula* and *Xylaria striata* species. PCR was performed for the *Xylaria* species to quantify the pathogen load of TRD in soil. Currently we are working on the standardization of the protocol (annealing temperature) for the *Xylaria* species.

Rhizosphere soil and soybean seedling sampling in the latent period of the disease: Rhizosphere soil sampling was done at the latent period during May 2021 in the seven TRD infected farms. In addition, TRD infected soybean seedlings were collected to culture the *Xylaria* spp., Plate culturing to pick *Xylaria* spp colonies is underway. These pure culture colonies will be used for standardizing quantitative PCR and greenhouse experiments.

Impacts and benefits to Mississippi Soybean Producers

Outcomes and information from this project will help soybean producers in both understanding and managing taproot decline disease and to develop effective management strategies for stakeholders. The survey and classification of farming systems in relation to TRD occurrence and severity is anticipated to provide direct benefits to soybean growers by identifying practical management decisions that may reduce the impact of TRD.

End Products-completed or Forthcoming

- Preliminary result was presented in ASM (American Society for Microbiology) annual meeting 2021.
- The results will be written as manuscripts for publication in peer-reviewed journals.

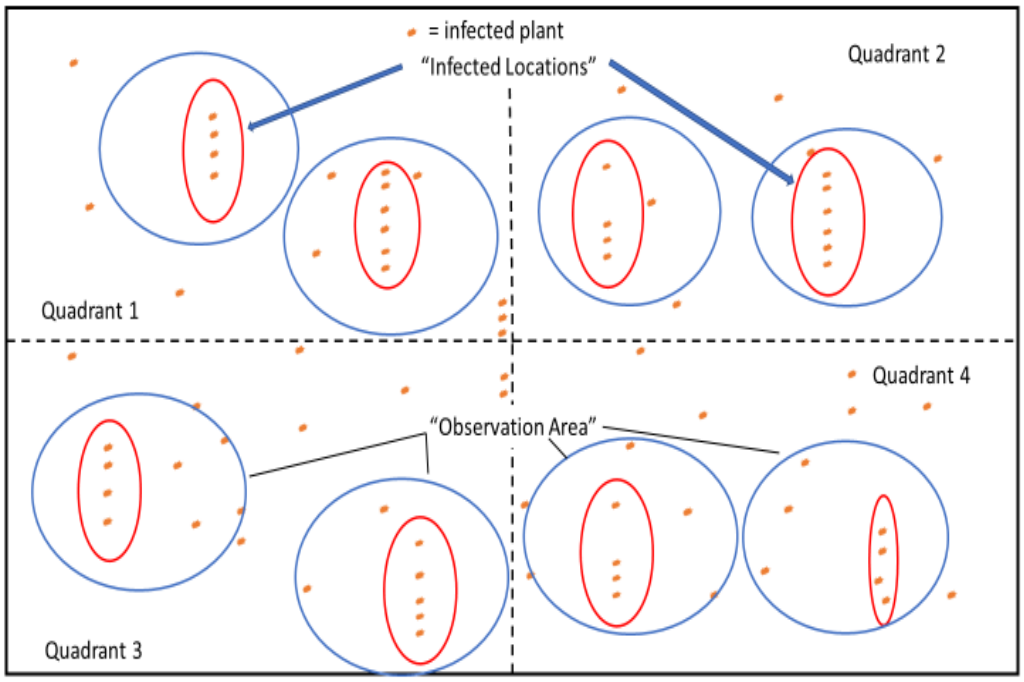

Fig. 1: TRD field survey method. Field was divided to into quadrants for the survey/observations.

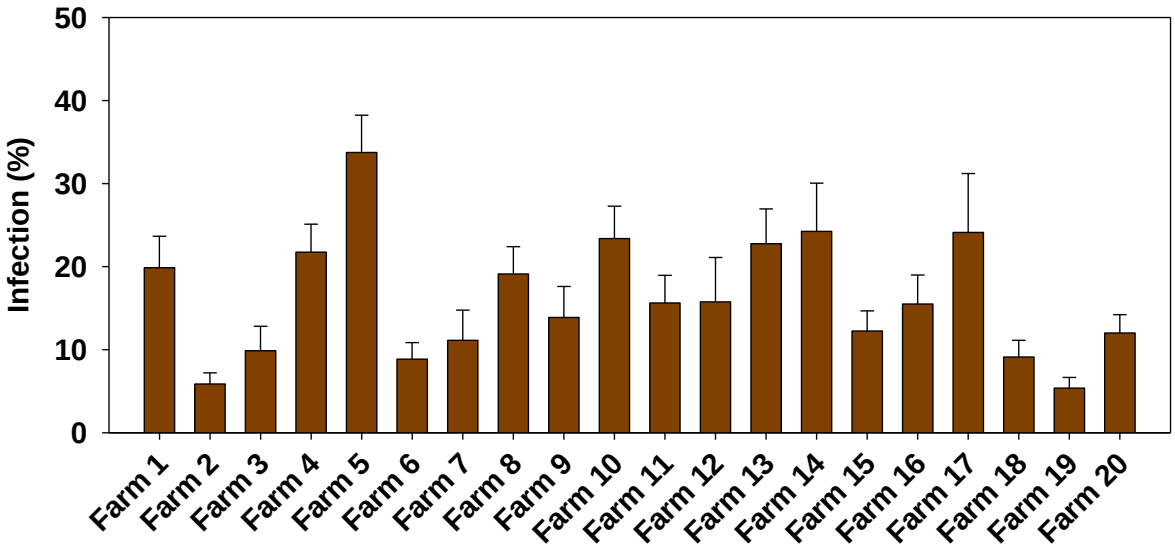

Fig.2: Average TRD infection rate for the 20 Mississippi Delta farms surveyed.

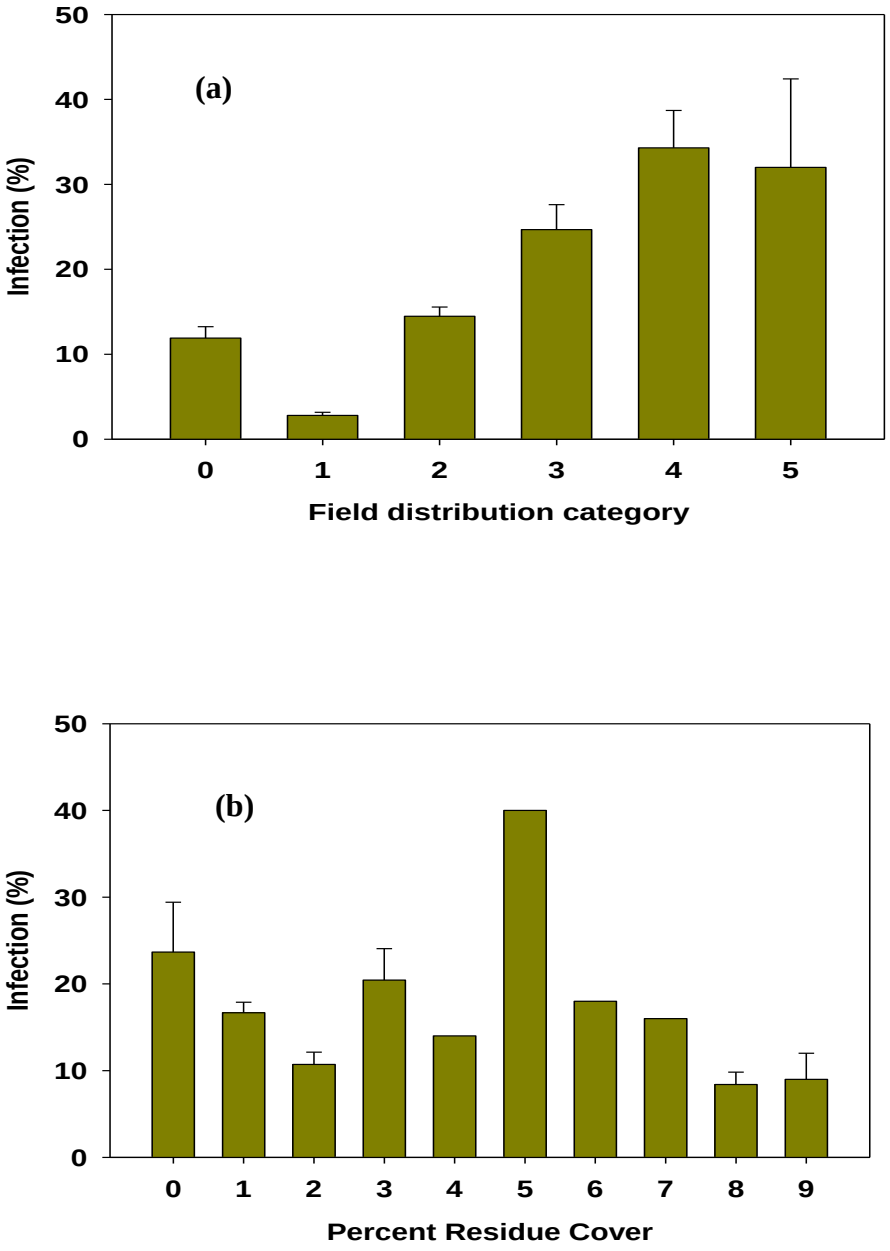

Fig. 3: Average TRD infection rate for the (a) field distribution categories describing TRD distribution and (b) different levels of percent surface residue cover within observation areas of the 20 surveyed farms.

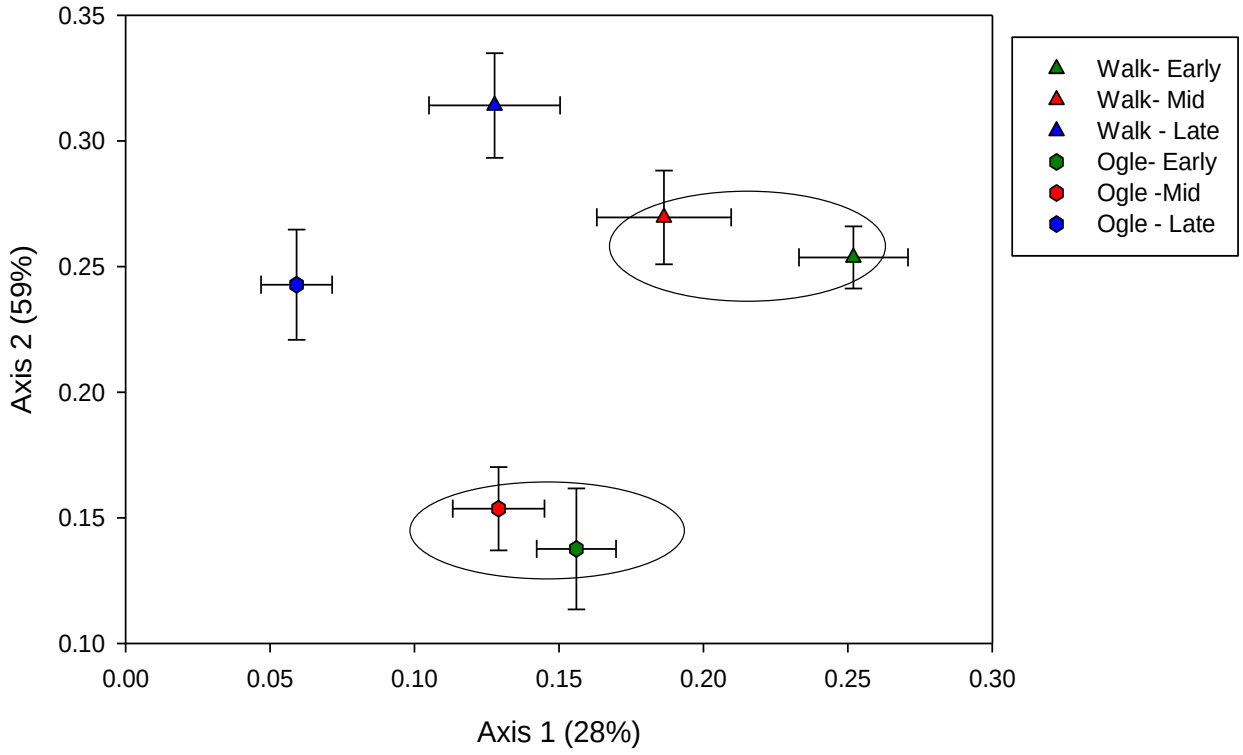

Fig 4: Bray-Curtis ordination plots showing the relationship between sampling dates, location (Walker and Oglesby farms) and bacterial community composition.