**MISSISSIPPI SOYBEAN PROMOTION BOARD**
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**TITLE: Characterization of the soybean taproot decline pathogen *Xylaria* sp.; a new disease and pathogen in Mississippi soybean production fields**

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## BACKGROUND, OBJECTIVES, AND PROGRESS IN EACH OBJECTIVE

Over the past 4 years, taproot decline of soybean (TRD) has been identified as a widespread disease throughout Mississippi and is a concern to our producers. We are well underway in developing an understanding of TRD and the pathogen *Xylaria* sp. The pathogen is soilborne resulting in a root disease of soybean that recurs in the same area of a field in successive growing seasons. The black stroma produced by the pathogen is a long-term resting structure; therefore, crop rotation alone may not be sufficient to reduce TRD in future soybean plantings. The research we are conducting to characterize TRD will provide insight on the disease cycle, aggressiveness of the pathogen, *Xylaria* sp., host range, as well as sensitivity to commercial fungicides used in soybean management.

### Objectives and Progress

**1. Define optimal temperature for *Xylaria* sp.:** Twenty-four selected *Xylaria* sp. isolates from across Mississippi were evaluated to determine optimal growth temperature. The temperature range was fairly consistent with only a 3.0 C difference with the range of 25.3 to 28.0 C. This temperature range is also suitable soil temperatures for soybean germination. There may be an association with *Xylaria* sp. colonizing germinating soybean/seedlings which provides insight to the disease cycle of taproot decline.

**2. Evaluate pathogenicity of Xylaria sp. isolates to soybean:** *Greenhouse studies* – The 24 selected *Xylaria* sp. isolates were evaluated to determine pathogenicity and virulence (aggressiveness) to soybean, ASGROW 4632. The selected *Xylaria* sp. isolates were pathogenic to soybean; however, many differed in virulence. The more aggressive isolates caused significant taproot decline, characterized by the production of black stroma on the soybean taproot. These results provide insight on the variability of *Xylaria* sp. across the state of Mississippi.

*In vitro studies* – Five selected *Xylaria* sp. isolates were used to evaluate *in vitro* pathogenicity against soybean, corn, and cotton (**Table 1**). Five hyphal plugs of each isolate with a subsequent incubation of

7 days at 26 C in the dark. Surface disinfested seed of each host were placed in contact with the edge of a *Xylaria* sp. isolate colony (1 seed/colony/5 seeds/plate). Following a seven day host-pathogen incubation, seed germination and percentage of seed/seedling *Xylaria* sp. colonization was recorded. Three replicates per isolate/host were included and the experiment was repeated twice. Corn and cotton had significantly greater germination compared to soybean following inoculation with five *Xylaria* sp. isolates; however, no differences were noted among hosts for *Xylaria* sp. colonization (**Table 2**).

**Table 1.** *Xylaria* sp. isolates collected from various Mississippi (MS) Counties and selected for use in the *in vitro*, host range, and fungicide sensitivity studies based on their virulence on soybean.

| ***Xylaria* sp. isolate ID Code** | **MS County** | **Virulence[a]** |
|---|---|---|
| CI14 | Covington | High |
| GI16 | George | Moderate |
| LMI1 | Lamar | High |
| LFI7 | Leflore | High |
| TEI1 | Tallahatchie | Moderate |

[a] Virulence is based on results from Soybean ASGROW 4632 pathogenicity study.

**Table 2.** The *in vitro* effect of *Xylaria* sp. isolates on germination and colonization of corn, cotton, and soybean following a seven day incubation in the laboratory.

| **Host** | **Germination (%)** | **Colonization (%)** |
|---|---|---|
| **Corn** | 88 a[a] | 92 |
| **Cotton** | 93 a | 93 |
| **Soybean** | 73 b | 90 |

[a] Means (n=54) within columns followed by the same letter are not significantly different according to Fisher's protected least significant difference (α=0.05).

**3. Determine host range of *Xylaria* sp.:** The host crops that are commonly associated with rotation or double-cropping of soybean were evaluated following inoculation with *Xylaria* sp. and include corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), rice (*Oryza sativa* L.), sorghum (Sorghum bicolor L.) and wheat (*Triticum aestivum* L.). Soybean was included as the standard host. The five selected *Xylaria* sp. isolates previously evaluated *in vitro* were also used in this study. Infested corn-cob grit (CCG) served as the inoculum source for each isolate and sterile CCG served as the non-treated control. Four surface disinfested seeds of each species were planted with the 1.5 g of inoculum at a depth of 3.5 cm per 10 cm plastic pot and arranged in a RCB design with five blocks in the greenhouse. Each pot was thinned to three plants and each plant served as a sub-sample. The duration of the experiment was ten weeks and repeated twice. Plant height (cm) and fresh and dry weights (g) were recorded; however, no significant differences were observed among *Xylaria* sp. isolates and the non-treated control. A visual root disease severity rating scale of 0 to 4 was recorded based on percent stroma colonization where 0 = no stroma, 1 = 1 to 25% stroma colonization of the entire root, 2 = 26 to 50% stroma colonization, 3 = 51 to 75% stroma colonization, and 4 = 76 to 100% stroma colonization

of the root. Disease severity was analyzed by taking the midpoints of the percentage range of the disease severity scale. Disease incidence was determined using a binary assessment of 0 = no stroma and 1 = presence of stroma on roots. Data were subjected to the analysis of variance using PROC GLM. Lastly, the pathogen was confirmed through re-isolation and ITS.

The *Xylaria* sp. isolates colonized all hosts. Root disease severity ranged from 4.7 to 10.3% on alternate crops while soybean had the greatest taproot disease severity (**Table 3**). Sorghum and soybean had similar disease incidence which was greater than the remaining crops (**Table 3**). Based on these results additional research is needed to determine whether these alternate crops increase the inoculum potential of *Xylaria* sp.

**Table 3.** The effect of *Xylaria* sp. isolates on root disease severity and disease incidence of rotational and double-cropping hosts associated with soybean production in Mississippi following ten weeks in the greenhouse.

| Host | Root disease severity (%)[a] | Disease incidence (%)[b] |
|---|---|---|
| **Corn** | 5.5 de[c] | 50 cd |
| **Cotton** | 7.7 c | 66 b |
| **Rice** | 6.4 cd | 56 bc |
| **Sorghum** | 10.3 b | 86 a |
| **Wheat** | 4.7 e | 43 d |
| **Soybean** | 13.6 a | 79 a |

[a]Root disease severity is based on percent stroma colonization of *Xylaria* sp. root systems of hosts.
[b]Disease incidence is based on the number of plants (n=225) exhibiting stroma colonization on root systems.
[c]Means (n=225) within columns followed by the same letter are not significantly different according to Fisher's protected least significant difference (α=0.05).

**4. Evaluate *Xylaria* sp. for fungicide sensitivity:** Fungicide assays were carried out using thiophanate-methyl, prothioconazole, flutriafol, fluxapyroxad, and pyraclostrobin plus Salicylhydroxamic acid (SHAM inhibits alternative oxidase pathway in respiration). Final concentrations for all fungicides were 0.0001, 0.001, 0.01, 0.1, 1.0, 10.0, and 100.0 ppm. A 6 mm plug from the margin of an actively growing 7 day-old colony of a *Xylaria* sp. isolate was transferred to the fungicide-amended PDA. Each concentration per *Xylaria* sp. isolate was replicated three times. After 10 days at 25°C in the dark, four radial measurements were recorded and 6 mm was subtracted to exclude the initial plug. Relative growth was calculated by taking the average radial growth on amended media divided by the average colony growth on the non-amended control and multiplied by 100. Percent relative growth for each *Xylaria* sp. isolate at all concentrations of each fungicide was subjected to PROC REG in SAS. The effective concentration ($EC_{50}$), the dose required to inhibit colony growth by 50%, was calculated using linear regression using PROC REG in SAS.

*Xylaria* sp. isolates were evaluated separately with respect to each ai. *Xylaria* sp. isolates were sensitive to thiophanate-methyl with 0% relative growth at 100 µg/ml and a range of $EC_{50}$ values from 0.24 to 0.40 µg/ml with George 3 being less sensitive (**Table 4**). Thiophanate-methyl exhibited fungistatic properties based on the uninhibited growth of these isolates when hyphal plugs, inhibited at 100 µg/ml thiophanate-methyl, were transferred to non-amended PDA. *Xylaria* sp. isolates were not

sensitive to pyracolstrobin + SHAM, fluxapyroxad, flutriafol, or prothioconazole (Table 4). The range of relative growth at 100 µg/ml for *Xylaria* sp. isolates exposed to pyracolstrobin + SHAM-amended media was 64 to 90% resulting in an $EC_{50}$ value of >100 µg/ml. *Xylaria* sp. isolates exposed to fluxapyroxad-amended PDA exhibited relative growth ranging from 83 to 89% and $EC_{50}$ values >100 µg/ml. The relative growth of *Xylaria* sp. isolates when exposed to flutriafol-amended PDA ranged from 91 to 103% resulting in $EC_{50}$ values >100 µg/ml. Similarly, *Xylaria* sp. isolates exposed to prothioconazole-amended PDA had a range of relative growth from 41 to 68% with $EC_{50}$ values >100 µg/ml (**Table 4**).

**Table 4.** The effective concentration to inhibit colony growth by 50%, $EC_{50}$ (µg/ml), of each *Xylaria* sp. isolate when exposed to thiophanate-methyl, pyraclostrobin + SHAM, fluxapyroxad, flutriafol, and prothioconazole at 26 C for 10 days in the dark.

| ***Xylaria* sp.** | **Thiophanate-methyl[a]** | **Pyracolstrobin + SHAM** | **Fluxapyroxad** | **Flutraifol** | **Prothioconazole** |
|---|---|---|---|---|---|
| Covington | 0.24 bc[b] | > 100 | > 100 | > 100 | > 100 |
| George 3 | 0.40 a | > 100 | > 100 | > 100 | > 100 |
| Tallahatchie | 0.30 ab | > 100 | > 100 | > 100 | > 100 |
| LSD *P*=0.05 | 0.12 | – | – | – | – |

[a] Fungicide concentrations: 0.0001, 0.001, 0.01. 0.1, 1, 10, and 100 µg/ml.
[b] Means (n=15) within columns followed by the same letter are not significantly different based on Fisher's protected least significant difference (α=0.05).

**Deliverables:**

Becton, H.R. **Characterization of *Xylaria* sp., the causal agent of taproot decline in Mississippi soybean.** Master of Science Thesis. Mississippi State University. August 2019.

Renfroe, H., Wilkerson, T. H., Allen, T. W., Tomaso-Peterson, M. (2018). **The distribution of taproot decline of Mississippi soybean.** Vol. 108-12-S2.8. Phytopathology.

Renfroe, H., Wilkerson, T. H., Allen, T. W., Tomaso-Peterson, M. **Investigations into the host range of Xylaria sp., causal agent of taproot decline in soybean.** Mississippi Plant Pathologists and Nematologists, Mississippi Plant Pathologists and Nematologists, October 2018. Starkville, MS.

Renfroe, H., Wilkerson, T. H., Allen, T. W., Tomaso-Peterson, M. **Assessing pathogenicity and virulence of Xylaria sp. isolates from Mississippi soybean.** Southern Soybean Disease Workers. March 2018. Pensacola Beach, FL.

Garcia-Aroca, T. Price III, P. P., Tomaso-Peterson, M., Spurlock, T., Faske, T., Bluhm, B., Conner, K., Sikora, E., Guyer, R., Kelly, H., Allen, T. W., Doyle, V. P. **Taproot decline of soybean is caused by an undescribed species in the genus *Xylaria*.** Southern Soybean Disease Workers. March 2018. Pensacola Beach, FL.