# Characterization of the soybean taproot decline pathogen *Xylaria* sp.; a new disease and pathogen in Mississippi soybean production fields

**78– 2014-2020**

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## Background and Objectives:

A mystery disease of soybean gained attention by growers and consultants in the late 2000s. Interest among Mississippi State University Plant Pathologists soon followed. What we discovered was an emerging soybean disease caused by an undescribed fungus. The symptoms of the disease consisted of foliar chlorosis and interveinal necrosis, like many documented root diseases. As a result, we believe the disease was prevalent in MS soybean production but may have been mis-diagnosed in the field. The symptom unique to this disease is the brittle taproot, which results in the plant breaking at the soil line. The presence of black stroma is also observed on the taproot of affected soybean. By 2017, the soybean disease, now referred to as taproot decline (TRD), was identified in 70 MS Counties. In the interim, we also focused on the identification of the pathogen which was an undescribed *Xylaria* sp. Fungi within *Xylaria* are characterized as non-pathogenic wood-rot fungi. The characterization of an emerging disease and undescribed pathogen required in-depth studies to which we applied the following objectives over the course of this research.

1. **Identification and confirmation of the fungal pathogen(s) associated with taproot decline of soybean**
2. **Characterize the life cycle and disease cycle of the taproot decline pathogen**
3. **Phylogenetic analysis with isolates of the taproot decline pathogen throughout MS soybean production fields**
4. **Evaluate pathogenicity of *Xylaria* sp. isolates to soybean and define the optimal temperature for *Xylaria* sp.**
5. **Determine host range of *Xylaria* sp.**
6. **Evaluate *Xylaria* sp. for fungicide sensitivity**
7. **Determine the effect of continuous soybean and rotational crops have on residual *Xylaria* sp. inoculum**
8. **Analysis of the expression of defense-related gene transcripts of soybean in response to infection by *Xylaria* sp.**

## Progress/Activity:

### Objective: Identification and confirmation of the fungal pathogen(s) associated with taproot decline of soybean

In June 2015, 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 as bate to recover the pathogen. A destructive sampling of soybean (four plants; two per pot) was made from soil collected from each county at growth stages VC, V1, V3, V6, R2, R3, and R4. The lateral roots were separated from the taproot 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 on the lab bench for five to seven days. Fungal colonies associated with root pieces were identified to genus from which a fungal frequency of occurrence was determined. Axenic (pure) cultures of the 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. We were not successful in the isolation of the unknown pathogen. Soybean symptomatic for TRD were collected in 2015 and isolation of the pathogen was successful. Axenic cultures were

obtained from infected plants and the pathogen was confirmed by sequencing the internal transcribed spacer (ITS) region and compared to other TRD isolates in our database at MSU.

**Objective: Characterize the life cycle and disease cycle of the taproot decline pathogen**

Initial life/disease cycle characterization of the TRD pathogen was conducted in the summer of 2016 using an ultra-mini rhizotron prototype developed in our laboratory. The plexiglass design allows for the observation of root development with no disturbance. Destructive sampling occurred 4-weeks post germination. The intent of this preliminary study was to develop a procedure to readily examine the infection process of the pathogen. The hypocotyl appeared to be the site of initial infection and subsequently infecting the developing taproot (Fig. 1A–B). As infection and colonization advanced on the soybean seedlings, the taproot and lateral roots became colonized, initiating root damage which produced foliar chlorosis and necrotic foliar symptoms. Symptomatic and asymptomatic taproots were divided into eight, 1-cm sections. The majority of TRD isolations came from top, 1-cm portion of symptomatic taproots. TRD isolations became less frequent in the distal portion of symptomatic taproots. This may indicate the pathogen's preference for root material closer to the soil surface. With advanced infection, extensive colonization of the taproot and lateral roots was observed.

The damage incurred to the overall root system leads to subsequent foliar decline presented as foliar chlorosis and necrosis. Infected root tissues were fixed in paraffin, stained with toluidine blue, and thin section for microscopic viewing. Bright-field microscopy was used as a tool to facilitate the study of the infection process and the extent of fungal colonization. The results indicated the TRD pathogen forms a layer of stroma which results in the degradation of epidermal cells associated with the taproot and lateral roots. Further evidence from infected root samples of both the tap and lateral roots showed a structural breakdown of the cortical parenchyma and endodermis. Fungal colonization within the xylem and phloem was also observed in infected roots (Fig. 2).

**Objective: Phylogenetic analysis with isolates of the taproot decline pathogen throughout MS soybean production fields**

Collections from soybean fields expressing TRD symptoms were made throughout the growing season of 2016. These samples represented 19 Mississippi Counties where we recovered 38 TRD isolates and extracted genomic DNA for sequencing. A phylogenetic analysis based on ITS, showed diversity among the Mississippi TRD isolates; however, despite that diversity, a distinctly divergent branch separated the TRD pathogen from its closest taxa, the *Xylaria* arbuscular aggregate. The *Xylaria* sp. isolates collected in 2017 were shared with bioinformatics collaborators at LSU to continue in-depth phylogenetic analyses to characterize the causal agent of TRD, an undescribed species of *Xylaria*.

**Objective: Evaluate pathogenicity of *Xylaria* sp. isolates to soybean and define the optimal temperature for *Xylaria* sp.**

Greenhouse experiments were conducted to determine pathogenicity and virulence of 24 *Xylaria* sp. isolates collected in 2016 throughout MS. Soybean variety, ASGROW 4632, was used as the host. Soybean seed were inoculated with *Xylaria*-infested corn cob grit and planted in a soilless potting medium in a 10-cm diameter clay pot. The experiment (repeated twice) lasted 12 weeks. All *Xylaria* sp. isolates were pathogenic to soybean resulting in the ability to infect and produce stromata. Virulence, the degree of pathogenicity, was variable among *Xylaria* sp. isolates. Based on the results of the foliar and root disease severity ratings, only 17% of *Xylaria* sp. isolates were determined to be highly virulent with the majority identified as moderately virulent to soybean in greenhouse studies (Table 1).

The optimal temperature for *Xylaria* sp. growth in axenic culture was determined using temperature-regulated incubation chambers. *Xylaria* sp. isolates were exposed to five temperatures (18, 22, 26, 30 and 34°C) and incubated at each for 10-d. The colony growth data were subjected to quadratic regression analysis and fit to the quadratic regression equation for optimal temperature. The study was repeated twice. A narrow difference of 3°C separated the lowest to highest optimal temperature. The growth temperature of 26°C was found to be optimal for the majority (45%) of *Xylaria* sp. isolates (Table 1).

**Objective: Determine host range of *Xylaria* sp.**

*In vitro* studies – Five selected *Xylaria* sp. isolates were used to evaluate *in vitro* pathogenicity against soybean, corn, and cotton. 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).

The host crops that are commonly associated with rotation or double cropping of soybean were evaluated following inoculation with *Xylaria* sp. in greenhouse studies. These included 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 randomized complete block (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 crops, used in rotation with soybean, contribute to the increase in *Xylaria* sp. inoculum in the field.

**Objective: 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 26°C in the dark, four radial measurements were recorded. 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 ioslate 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).

**Objective: Determine the effect of continuous soybean and rotational crops have on residual *Xylaria* sp. inoculum**

A greenhouse study, experiment 1, was initiated 14 June and the second, experiment 2, was initiated 21 June 2019. For each experiment, the first planting of a four-rotation cycle was completed. The first rotation was planted to 1) continuous soybean; 2) soybean–cotton–soybean–cotton; 3) soybean–corn–soybean–corn; and 4) wheat–soybean–wheat–soybean. The seed were placed in direct contact with infested *Xylaria* sp. corn cob grit (CCG) consisting of equal aliquots of *Xylaria* sp. previously isolated from soybean, cotton, corn, and wheat, all originating from the 78-2018 agronomic host study. The non-inoculated checks include sterile CCG. Soil cores were taken to determine the baseline quantification of *Xylaria* sp. five days post planting. Meanwhile in the laboratory, a specific primer for *Xylaria* sp. was developed using the RPB2 gene sequence. The primer was tested against other *Xylaria* spp. and no amplification was detected, validating the species-specific primer. A standard curve was developed to test the RPB2 primer efficiency and DNA detection. Total DNA was extracted from the soil samples and tested using the *Xylaria*-specific RPB2 primers. Positive results were obtained indicating the presence of *Xylaria* sp. in soil from inoculated treatments. No *Xylaria* sp. was detected in the non-inoculated checks. *Xylaria* sp. DNA copies were quantified from the initial soil cores taken five days post inoculation/planting for experiments 1 and 2 using qPCR protocol. At 12 weeks post inoculation/planting, the soybean and wheat plants were removed at the soil line leaving the root material in place as substrate for *Xylaria* sp. Soil cores (12-wk) were removed from the area adjacent to the furrow where the *Xylaria* sp. inoculum was originally placed in the pot. To prepare soil cores for total DNA extraction, cores were placed in aluminum tins and dried overnight in the biosafety cabinet. The dried cores were ground and total genomic DNA (gDNA) extracted using a power soil kit. The gDNA from each soil core was added to a defined master mix for the qPCR analysis. The average baseline inoculum density of *Xylaria* sp. DNA in the soil at five days post inoculation/planting was 19,864 DNA copies.

The second rotation was planted using surface disinfested seed for each rotation treatment; 1) continuous **soybean**; 2) soybean–**cotton**–soybean–cotton; 3) soybean–**corn**–soybean–corn; and 4) wheat–soybean–wheat–soybean. These methods were repeated for each rotation. The inoculum density of *Xylaria* sp. monitored under four crop rotations was completed after 48 weeks.

The wheat–soybean–wheat–soybean rotation resulted in the least amount of *Xylaria* sp. inoculum density compared to the other rotations (Figs. 3 & 4). This supports results of the host specificity evaluation where wheat had reduced disease severity and incidence. The soybean–corn–soybean–corn rotation produced the greatest accumulated inoculum density, close to 140,000 DNA copies. This may be due to the extensive root system of the corn, providing substrate for *Xylaria* sp. colonization. The soybean–cotton–soybean–cotton rotation had the second greatest inoculum density (124,807 DNA copies). We have observed the virulence of *Xylaria* sp. on cotton in previous greenhouse experiments. There may be some level of cotton infection in the field but based on the results of the inoculum density trial, *Xylaria* sp. is a colonizer of cotton roots. Interestingly, the continuous soybean had the second lowest inoculum density of 87,485 DNA copies (Fig. 4). This may be due to the reduced soybean root system translating into less substrate for *Xylaria* sp. to colonize. All soybean plants exposed to *Xylaria* sp. showed symptoms of TRD and never fully developed compared to the non-inoculated soybeans.

**Objective: Analysis of the expression of defense-related gene transcripts of soybean in response to infection by *Xylaria* sp.**

Soybean utilizes three signaling pathways for defense, jasmonic acid, ethylene, and salicylic acid of which, numerous genes are involved. Primers for pathogenic-related (PR) proteins were designed specifically for this project by Dr. A. Bronzato-Badial. Total gDNA was extracted from soybean seedlings grown in an aseptic environment and cDNA was synthesized. The soybean cDNA was used to amplify the selected genes and evaluate the gene expression of soybean plants in response to *Xylaria* sp. infection. The 19 PR genes associated with induced systemic resistance were validated with RNA/cDNA of 46X6 (TRD susceptible variety) extracted from the soybean root.

At 10 days post *Xylaria* sp. inoculation, root samples were collected at time zero, 6, 12, 24, 48, 72 hours and one week. The resistant Osage initiated a response within 24-hr of inoculation with *Xylaria* sp. and the susceptible soybean, 46X6, was delayed showing a response at 48-hr. Visible symptoms are not observed on Osage one-week post inoculation. Symptoms were visible on 46X6 within 24-hr post inoculation with *Xylaria* sp. (Fig. 5). Gene expression for jasmonic acid and ethylene was greater for Osage, whereas 46X6 has greater gene expression in the salicylic acid pathway (Table 5).

## Impacts and Benefits to Mississippi Soybean Producers:

Significant progress has been made in the characterization of the 'mystery disease' of soybean. At the onset of this research it was not known who/what was the causal agent nor the extent of the mystery disease throughout MS. Initial sightings of the soybean disease were in fields near Inverness and Isola MS. As of 2020, the soybean disease, taproot decline (TRD), has been observed in 73 MS counties (Fig. 6). The symptoms of TRD (official name of the disease in 2017), first appear as foliar chlorosis. As the disease progresses, plants become stunted. In the latter stages of disease development, interveinal chlorosis, foliar necrosis, and dry rot at the base of the plant is evident. The taproot is dry and brittle, often the stem breaks at the soil line. The affected taproot and lateral roots are covered with black, superficial stroma produced by the pathogen. In 2019, the estimated yield loss due to TRD in MS soybean production was 0.3%, rating seventh overall among soybean diseases.

Little information on the pathogen causing TRD was initially available. Koch's postulates (confirmation of the pathogen) was successfully carried out after 18 months of diligent attempts. Because the pathogen was sterile in culture, molecular techniques were used for the initial identification. The results of a fungal database search indicated the pathogen resided in the genus *Xylaria*. No results for species identification were obtained, meaning the species was most likely undescribed. Phylogenetic analyses supported the identification of the pathogen as *Xylaria* sp. Through collaborative research efforts with colleagues at LSU, the undescribed *Xylaria* pathogen was confirmed as a novel species and the accepted name will be published in the journal *Mycologia* in the near future. *Xylaria* spp. are generally characterized as saprophytes, referred to as wood-rot fungi; however, the *Xylaria* sp., a pathogen of soybean, is highly virulent on many soybean varieties.

No information was available on the host range or fungicide sensitivity of this pathogen. Therefore, studies were conducted to fill in the blanks. We evaluated the pathogenicity of *Xylaria* sp. on other agronomic crops routinely planted in rotation with soybean as well as the inoculum density associated with continuous soybean and rotations in greenhouse studies. Cotton and corn rotated with soybean did not result in a decrease in inoculum density. Only when soybean was rotated with wheat was there a significant decrease in *Xylaria* sp. inoculum density. In June 2020, soil samples collected from adjacent root systems of TRD-symptomatic soybean grown in a corn-cotton-corn-soybean rotation had significantly less *Xylaria* sp. inoculum density than soil sampled from TRD soybean grown in continuous soybean. Soybean rotation with a monocot crop may be beneficial in reducing the inoculum density of *Xylaria* sp., thus reducing TRD incidence in soybean.

Fungicide sensitivity evaluations resulted in *Xylaria* sp. insensitivity to QoI, SDHI, and DMI fungicides. Thiophanate-methyl (Topsin) was the only fungicide that *Xylaria* sp. showed sensitivity. These results show the importance of in-furrow fungicide trials to determine field efficacy of currently labeled soybean fungicides. Currently, the most promising management of TRD is planting resistant soybean varieties. *In vitro* studies showed differences in symptom expression of the resistant Osage variety as compared to the susceptible 46X6 soybean variety. The expression of defense-related gene transcripts in response to infection also differed between the two soybeans varieties, indicating genetic diversity in terms of TRD resistance. This discovery is promising for breeding taproot resistant soybean varieties.

## End Products – Completed and Forthcoming:

### 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. October 2015. Starkville, MS.

T. Spurlock, M. Tomaso-Peterson, T. Allen, P. Price, and R. Singh. Characterization of taproot decline in southern soybean. Soilborne Disease Symposium. 43rd Southern Soybean Disease Workers Meeting. March 2016. Pensacola Beach, FL.

Renfroe, H., Allen, T. W., Wilkerson, T. H., and Tomaso-Peterson, M., (2017). Investigations into the presences of taproot decline in Mississippi Soybean. Mississippi Association of Plant Pathologists and Nematologists Annual Meeting, MSU. Received 3rd place in graduate student oral competition.

H. Renfroe, T. Wilkerson, T. Allen, and M. Tomaso-Peterson. The distribution of taproot decline in Mississippi soybean. Southern Division-American Phytopathological Society Meeting. February 2018. Fayetteville, AR.

H. Renfroe, T. Wilkerson, T. Allen, and M. Tomaso-Peterson. Assessing pathogenicity and virulence of Xylaria sp. isolates from Mississippi soybean. 45th Southern Soybean Disease Workers Meeting. March 2018. Pensacola Beach, FL.

T. Garcia-Aroca, P. Price, M. Tomaso-Peterson, T. Spurlock, T.R. Faske, B. Bluhm, K. Conner, E.J. Sikora, R. Guyer, H. Kelly, T.W. Allen, and V.P. Doyle. Taproot decline of soybean is caused by an undescribed species in the genus *Xylaria*. 45th Southern Soybean Disease Workers Meeting. March 2018. Pensacola Beach, FL.

Renfroe, H., Wilkerson, T. H., Allen, T. W., and 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., and Tomaso-Peterson, M. Determining the sensitivity of *Xylaria* sp. to commercially available fungicide active ingredients. Southern Division-American Phytopathological Society Meeting. February 2019. Gainesville, FL.

Becton, H.R., Wilkerson, T., Allen, T.W., and Tomaso-Peterson, M. Identification of alternative hosts for *Xylaria* sp., the pathogen of taproot decline of soybean. 46th Southern Soybean Disease Workers Meeting. March 2019. Pensacola Beach, FL.

Bronzato-Badial, A.; Phillips K.; Wilkerson, T.; Popescu, S.; and Tomaso-Peterson, M. Determining inoculum density of *Xylaria* sp., the taproot decline pathogen, in soil under various crop rotation systems. 47th Southern Soybean Disease Workers Annual Meeting. March 2020. Pensacola Beach, FL.

### Proceedings/Abstracts:

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

Allen T.W., Price T., Purvis M.A., Pruitt H., Tomaso-Peterson M., Wilkerson T. Potential varietal resistance to taproot decline of soybean. 2017. Phytopathology. Vol. 107:S3.9.

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

Renfroe, H., Wilkerson, T., and Tomaso-Peterson, M. Determining the sensitivity of *Xylaria* sp. to commercially available fungicide active ingredients. Phytopathology. Vol. 109-9-S1-12.

Garcia, T.G., Prices, P., Tomaso-Peterson, M., Wilkerson, T., Spurlock, T., Faske, T.R., Bluhm, B.H., Conner, K.N., Sikora, E.J., Guyer, R., Kelly, H.M., Allen, T., Doyle, V. (2020). Taproot decline of soybean is caused by a novel *Xylaria* sp. that produces phytotoxins associated with foliar symptoms. Phytopathology. Vol. 110-7-S1.7.

Bronzato-Badial, A., Phillips, K., Wilkerson, T., and Tomaso-Peterson, M. (2020). Does crop rotation influence inoculum density of *Xylaria* sp., the pathogen of taproot decline of soybean? Phytopathology. Vol. 110-7-S1-19.

**Posters:**

P. Price, M. Purvis, H. Pruitt, T. Allen, M. Tomaso-Peterson, T. Wilkerson. Potential Varietal Resistance to Taproot Decline of Soybean. 2017. Southern Division-American Phytopathological Society Meeting. February 2017. College Station, TX.

Garcia, T.G., Prices, P., Tomaso-Peterson, M., Wilkerson, T., Spurlock, T., Faske, T.R., Bluhm, B.H., Conner, K.N., Sikora, E.J., Guyer, R., Kelly, H.M., Allen, T., Doyle, V. (2019). A novel *Xylaria* sp. is capable of infecting soybean roots and producing systemic secondary metabolites responsible for foliar symptoms. Mycological Society of America. Minneapolis, MN.

Badial, A., Phillips, K., Wilkerson, T., and Tomaso-Peterson, M. (2020). Does crop rotation influence inoculum density of *Xylaria* sp., the pathogen of taproot decline of soybean? Southern Division-American Phytopathological Society Meeting. February 2020. Charleston, SC.

**Peer-reviewed publications:**

First Description of the Causal Agent of Taproot Decline of Soybean, an Emerging Disease in the Southern United States. 2017. T. Allen, B. Bluhm, K. Conner, V. Doyle, T. Price, E. Sikora, R. Singh, T. Spurlock, M. Tomaso-Peterson, and T. Wilkerson. Plant Health Progress. 18:35−40. http://dx.doi.org/10.1094/PHP-01-17-0004-RS

Draft genome sequence of Xylaria sp., the causal agent of taproot decline of soybean in the southern United States. Sharma S., Zaccaron A. Z., Ridenour J. B., Allen T. W., Conner K., Doyle V. P., Price T., Sikora E., Singh R., Spurlock T., Tomaso-Peterson M., Wilkerson T. H., Bluhm B. H. 2018. Data in Brief. (17) 129-133. https://doi.org/10.1016/j.dib.2017.12.060

*Xylaria* sp. nov. is an emerging root-associated pathogen responsible for taproot decline of soybean in the southern United States. 2020. T. Garcia-Aroca, P.P. Price, M. Tomaso-Peterson, T. Allen, T. Wilkerson, T.N. Spurlock, T. Faske, B. Bluhm, K. Conner, E. Sikora, R. Guyer, H. Kelly, B.M. Squiers, and V.P. Doyle. Mycologia (*In Press*).

The host range of the taproot decline pathogen, *Xylaria* sp., and associated inoculum density. Becton, H.R., Bronzato-Badial, A., Wilkerson, T. and Tomaso-Peterson, M. *In preparation for Plant Disease.*

**Thesis:**

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.

**Figures and Tables:**

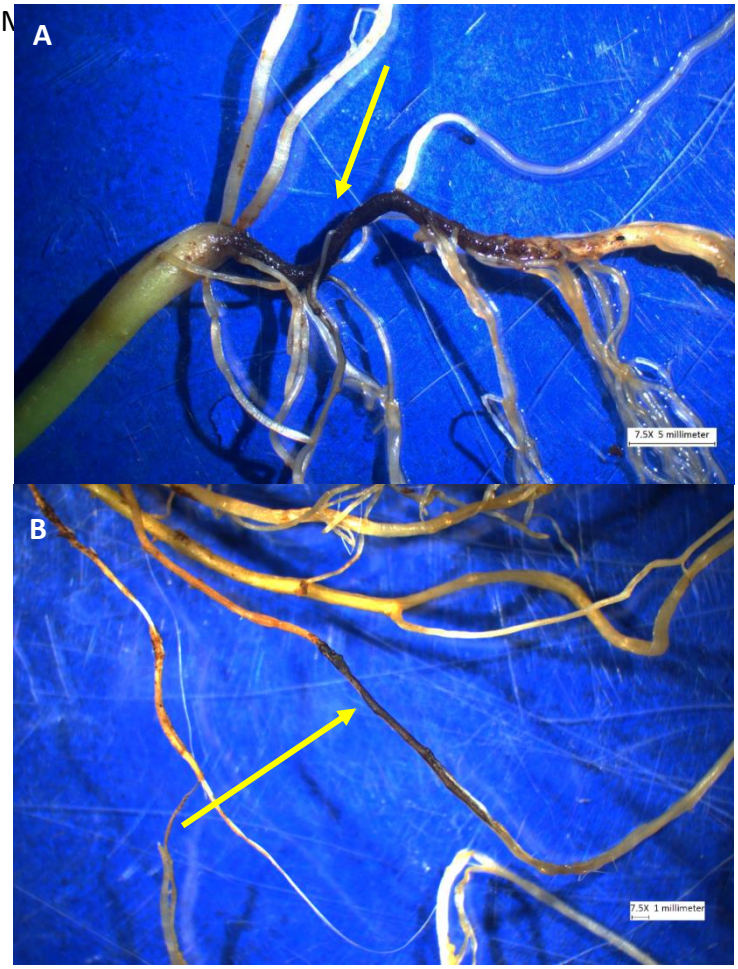


**Figure 1.** The presence of black stroma on the taproot (A) and lateral root (B) of soybean indicates infection by the taproot decline pathogen. Yellow arrows indicate site of infection and colonization.

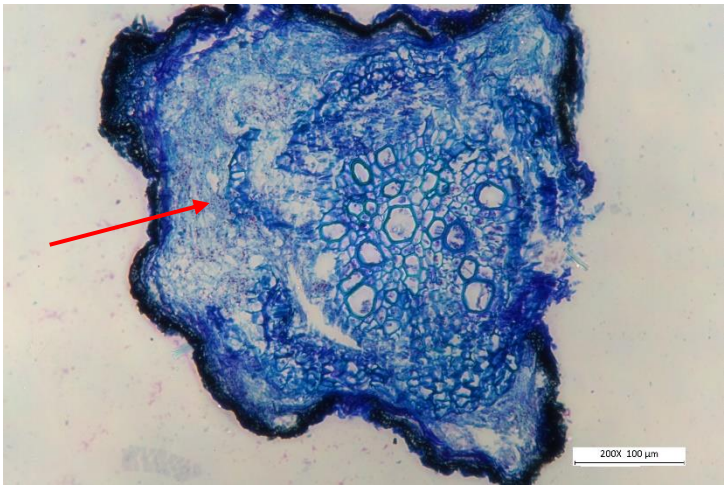


**Figure 2.** Thin section of a soybean taproot infected by *Xylaria* sp. Black stroma, produced by the pathogen, covers the outer surface of the root. Structural breakdown of the cortical parenchyma and endodermis (see arrow). Fungal colonization within the xylem and phloem was also observed in infected roots (blue/purple stain).

**Table 1.** Virulence ranking and defined optimal temperature for colony growth of *Xylaria* sp. isolates collected in 2016 from MS Counties.

| Isolates from MS Counties – 2016 | Virulence[z] | Optimum temperature (°C) |
|---|---|---|
| Clay Co. | Moderate | 25.4 |
| Covington Co. | High | 27.3 |
| Forrest Co. | Weak | 26.5 |
| Franklin Co. | Moderate | 27.9 |
| George Co. 1 | Moderate | 26.7 |
| George Co. 2 | Moderate | 26.1 |
| George Co. 3 | Moderate | 26.4 |
| Hinds Co. | Weak | 25.8 |
| Jackson Co. | Moderate | 26.7 |
| Lafayette Co. | Moderate | 28.0 |
| Lee Co. | Weak | 26.3 |
| Leflore Co. | High | 26.6 |
| Lamar Co. 1 | High | 26.2 |
| Lamar Co. 2 | High | 25.3 |
| Monroe Co. 1 | Moderate | 26.2 |
| Monroe Co. 2 | Weak | 25.8 |
| Noxubee Co. | Moderate | 25.5 |
| Panola Co. | Moderate | 27.3 |
| Pearl River Co. | Moderate | 26.9 |
| Perry Co. | Moderate | 27.4 |
| Tallahatchie Co. | Moderate | 27.6 |
| Yalobusha Co. 1 | Moderate | 26.3 |
| Yalobusha Co. 2 | Moderate | 28.0 |
| Yazoo Co. | Moderate | 27.7 |

[z] Virulence ranking determined for each *Xylaria* sp. isolate based on results of foliar and root disease severity ratings.

**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).

**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).

**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).

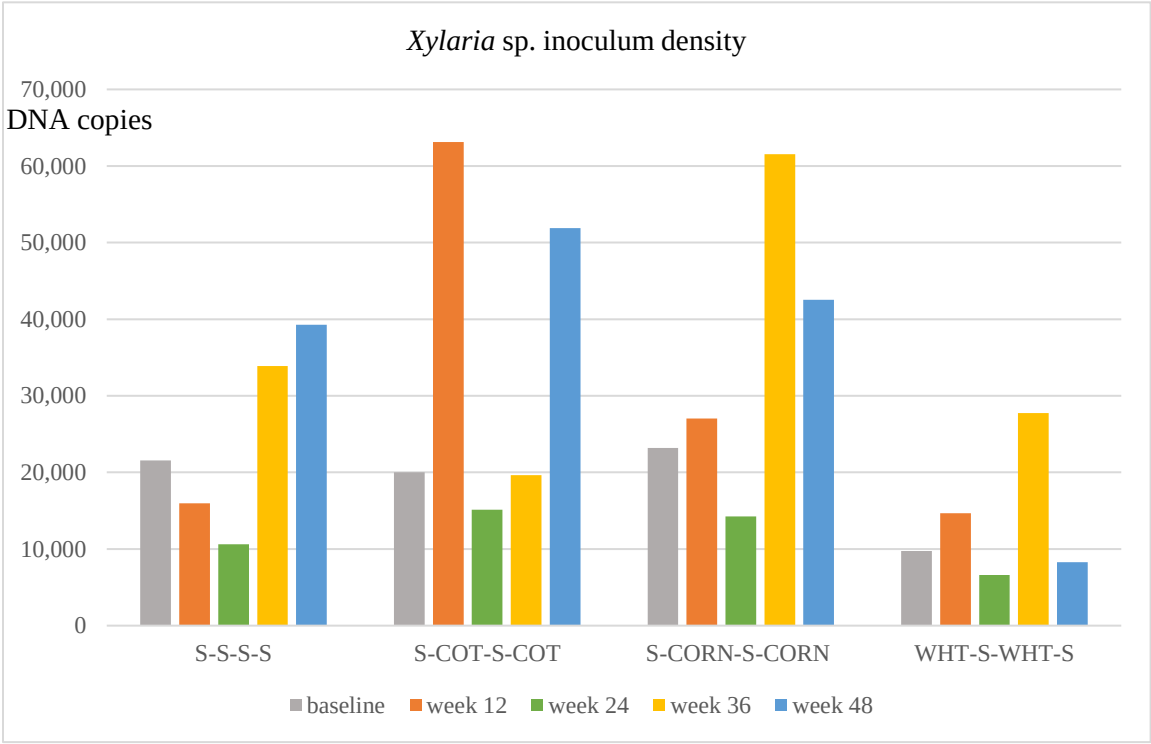


**Figure 3.** The effect of crop rotation on the inoculum density of *Xylaria* sp. in greenhouse trials. The root systems of each crop were not disturbed throughout the 48-week study.

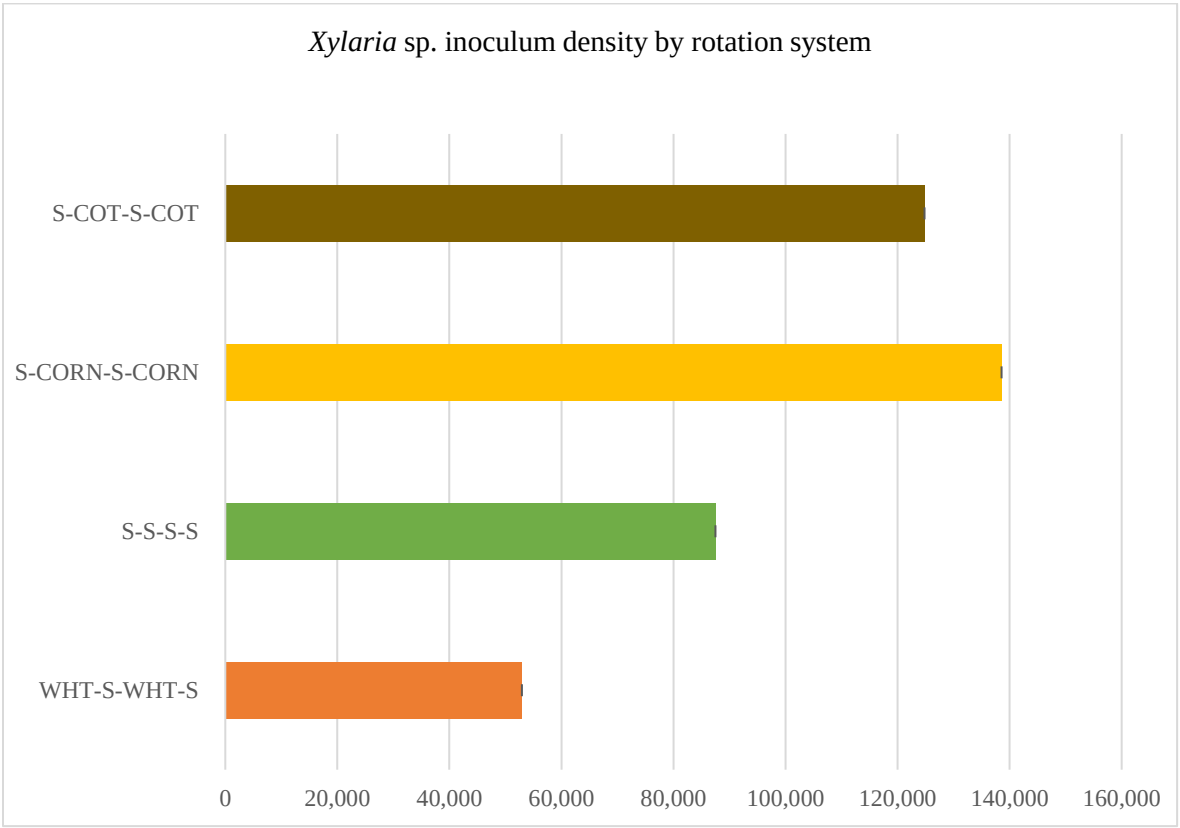


**Figure 4.** The accumulation of *Xylaria* sp. inoculum density (number of DNA copies) following 48 weeks of colonization under various crop rotation systems. Soybean-cotton-soybean-cotton (S-COT-S-COT), soybean-corn-soybean-corn (S-CORN-S-CORN), continuous soybean (S-S-S-S), and wheat-soybean-wheat-soybean (WHT-S-WHT-S).

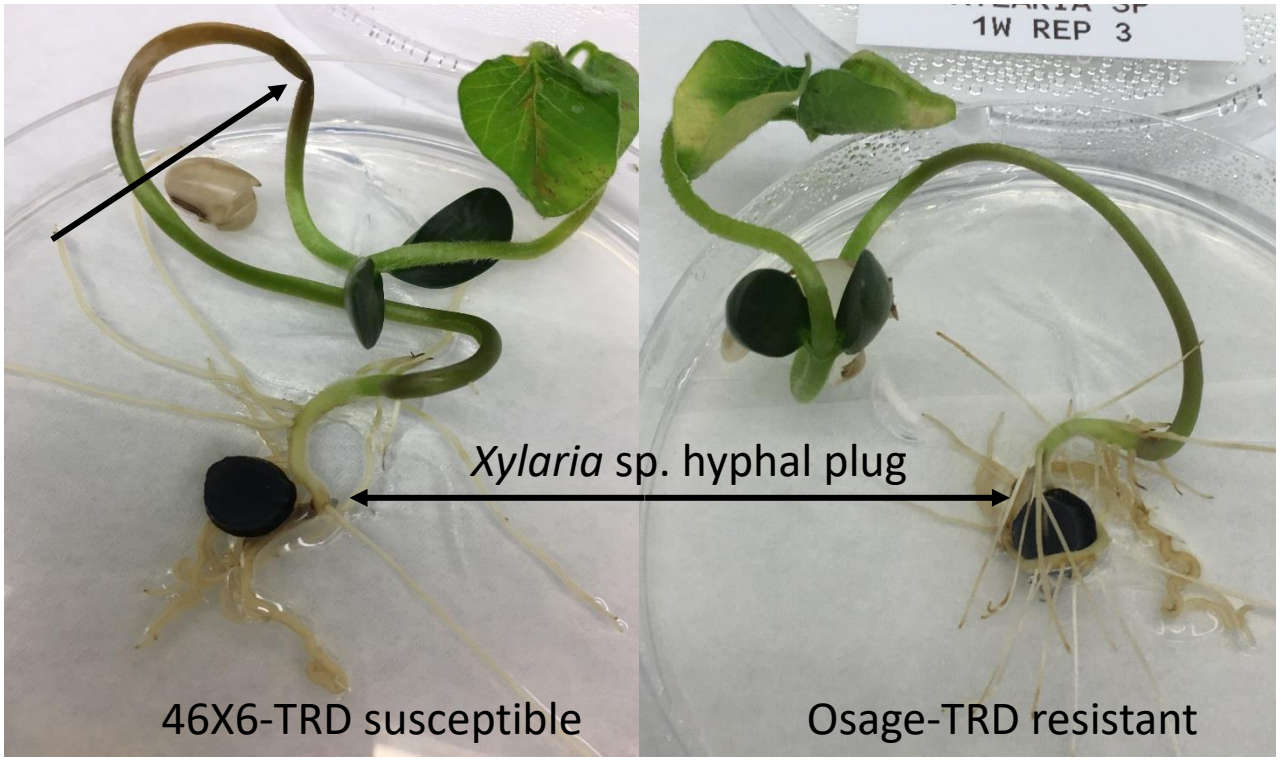


**Figure 5.** Soybean seedlings one-week post inoculation with *Xylaria* sp. Cultivar 46X6 is showing symptoms of stem necrosis (arrow) and foliar symptoms of taproot decline (TRD) in contrast to Osage which is asymptomatic.

**Table 5.** Signaling pathways used by resistant Osage and susceptible 46X6 soybean varieties in response to infection by *Xylaria* sp.

| Gene expression: | | |
|---|---|---|
| SIGNALING PATHWAYS | | |
| **Jasmonic acid (JA)** | **Ethylene (ET)** | **Salicylic acid (SA)** |
| PR10 | PR2 | PR5 |
| PR3 | PR3 | PAL |
| PR12 | PR4 | CHS |
| AOS | IPER | IPER |
| IPER | EBP1 | PR1 |
| MYB51 | ERF113 | |
| MYC2 | | |

| |
|---|
| OSAGE TRD resistant |
| 46X6 TRD susceptible |
| BOTH |

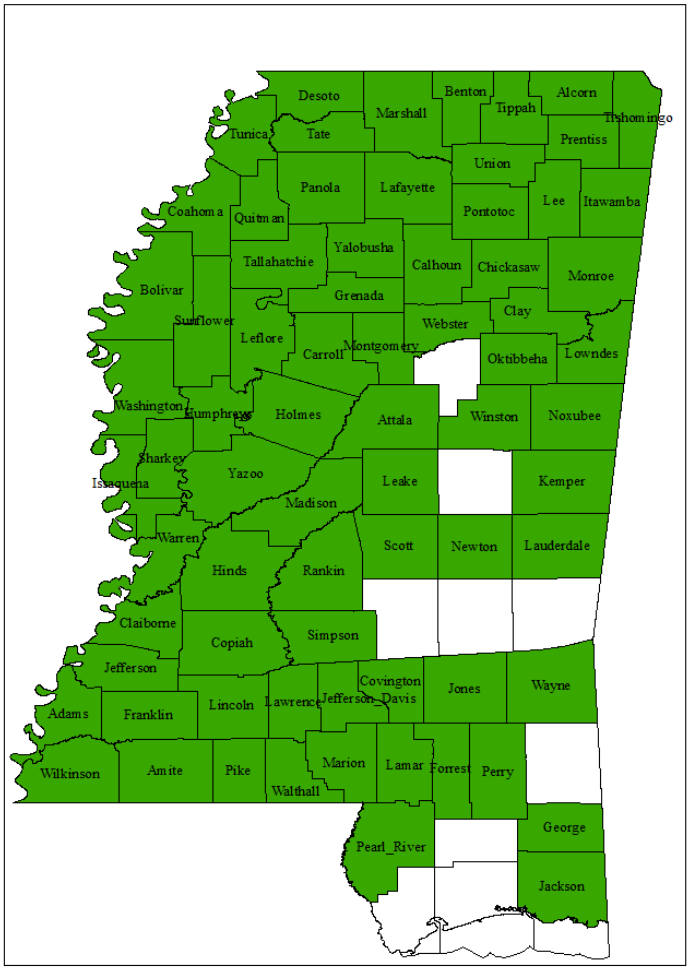


**Figure 6.** The current distribution of taproot decline of soybean in Mississippi soybean production fields. *Courtesy T.W. Allen*