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MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 78-2017 (YEAR 2)
2017 ANNUAL REPORT

Title: Characterization of soybean taproot decline; a new disease in Mississippi soybean production fields

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BACKGROUND AND OBJECTIVES

This research addresses the etiology of taproot decline (TRD) associated with soybean plants throughout Miss. production fields. The symptoms include foliar chlorosis, interveinal necrosis, stained vascular tissues, and signs of black stromata which is a black matrix of vegetative hyphae colonizing the lower stem and roots of affected plants. The disease is widespread throughout Mississippi and recurs in the same area within a field, particularly when soybean is planted in consecutive years. The pathogen over-winters as vegetative stromata on soybean stubble and roots which serves as primary inoculum for the next season's epidemic. Tillage practices, crop rotation, and soybean variety resistance are areas for future research to identify best management practices.

Objective 1. Confirmation of the taproot decline pathogen

Collections of soybean plants symptomatic for TRD were made throughout the 2017 growing season by Drs. Tom Allen, Billie Moore, and Tessie Wilkerson and delivered to our lab for fungal isolation. The TRD decline pathogen, *Xylaria* sp., was isolated and confirmed through ITS sequencing. This process consists of cleaning the roots free of soil and drying. Symptomatic roots are cut into 5 mm pieces, surface disinfected, and plated onto water agar medium. Following a 7-d incubation period, mycelium from colonies consistent with *Xylaria* sp. morphology are transferred to potato dextrose agar. The pure culture colonies are used for genomic DNA extraction, ITS PCR, and sequence analysis. The ITS sequence, GenBank Accession KY433853, is used to confirm *Xylaria* sp. isolates (Allen et al. 2017). The results of the 2017 TRD collection positively identified the disease in 29 Miss. Counties which were previously not reported with occurrence of TRD (Fig. 1). Results from the 2016 and 2017 TRD collections show TRD is widespread throughout Miss. and should be considered a primary disease of soybean (Fig. 2).

Objective 2. Characterize the life cycle of and disease cycle of the taproot decline pathogen

The diversity of *Xylaria* sp. isolates, supported by the identification of 15 haplotypes, may lead to differences in virulence or aggressiveness as well as optimum temperature for growth. To begin the characterization process, we conducted repeated greenhouse experiments to determine pathogenicity and virulence of 24 *Xylaria* sp. isolates collected in 2016 throughout Miss. 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. The data consisted of foliar and root disease severity ratings, plant height, and dry and fresh weights. 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; Fig. 3).

The optimal temperature for *Xylaria* sp. growth in pure 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).

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

Isolates from Miss. 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.

Objective 3. Phylogenetic analysis with isolates of the taproot decline pathogen throughout Mississippi soybean production fields

Collections from soybean fields expressing TRD symptoms were made throughout the growing season of 2016 and 2017 (Fig. 2). These samples represent 19 counties in Mississippi 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 the diversity, a distinctly divergent branch separates the TRD pathogen from its closest taxa, the *Xylaria arbuscular* aggregate (Fig. 4). A second analysis supports the concept that the TRD isolates in our collection have 15 sets of DNA variations, referred to as haplotypes. A haplotype map illustrates the diversity within the group of TRD isolates (Fig. 5). The *Xylaria* sp. isolates collected in 2017 will be shared with bioinformatics collaborators at LSU to continue in-depth phylogenetic analyses to confirm the fungus that is the causal agent of TRD is an undescribed species of *Xylaria*.

Deliverables

First Description of the Causal Agent of Taproot Decline of Soybean, an Emerging Disease in the Southeastern United States. 2017. Allen T., Bluhm B., Conner K., Doyle V., Price T., Sikora E., Singh R., Spurlock T., Tomaso-Peterson M., and Wilkerson T. Plant Health Progress. 18:35–40.

Potential varietal resistance to taproot decline of soybean. Allen T.W., Price T., Purvis M.A., Pruitt H., Tomaso-Peterson M., Wilkerson T. 2017. (Abstr.) Phytopathology 107:S3.9. http://dx.doi.org/10.1094/PHYTO-107-4-S3.9

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

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

The distribution of taproot decline in Mississippi soybean. H. Renfroe, T. Wilkerson, T. Allen, and M. Tomaso-Peterson. Southern Division-American Phytopathological Society Meeting. Fayetteville, AR. 17 February 2018. Oral presentation
Assessing pathogenicity and virulence of *Xylaria* sp. isolates from Mississippi soybean. H. Renfroe, T. Wilkerson, T. Allen, and M. Tomaso-Peterson. Southern Soybean Disease Workers Meeting. Pensacola Beach, FL. March 7, 2018. Oral presentation

Taproot decline of soybean is caused by an undescribed species in the genus *Xylaria*. 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. Southern Soybean Disease Workers Meeting. Pensacola Beach, FL. March 7, 2018. Oral presentation

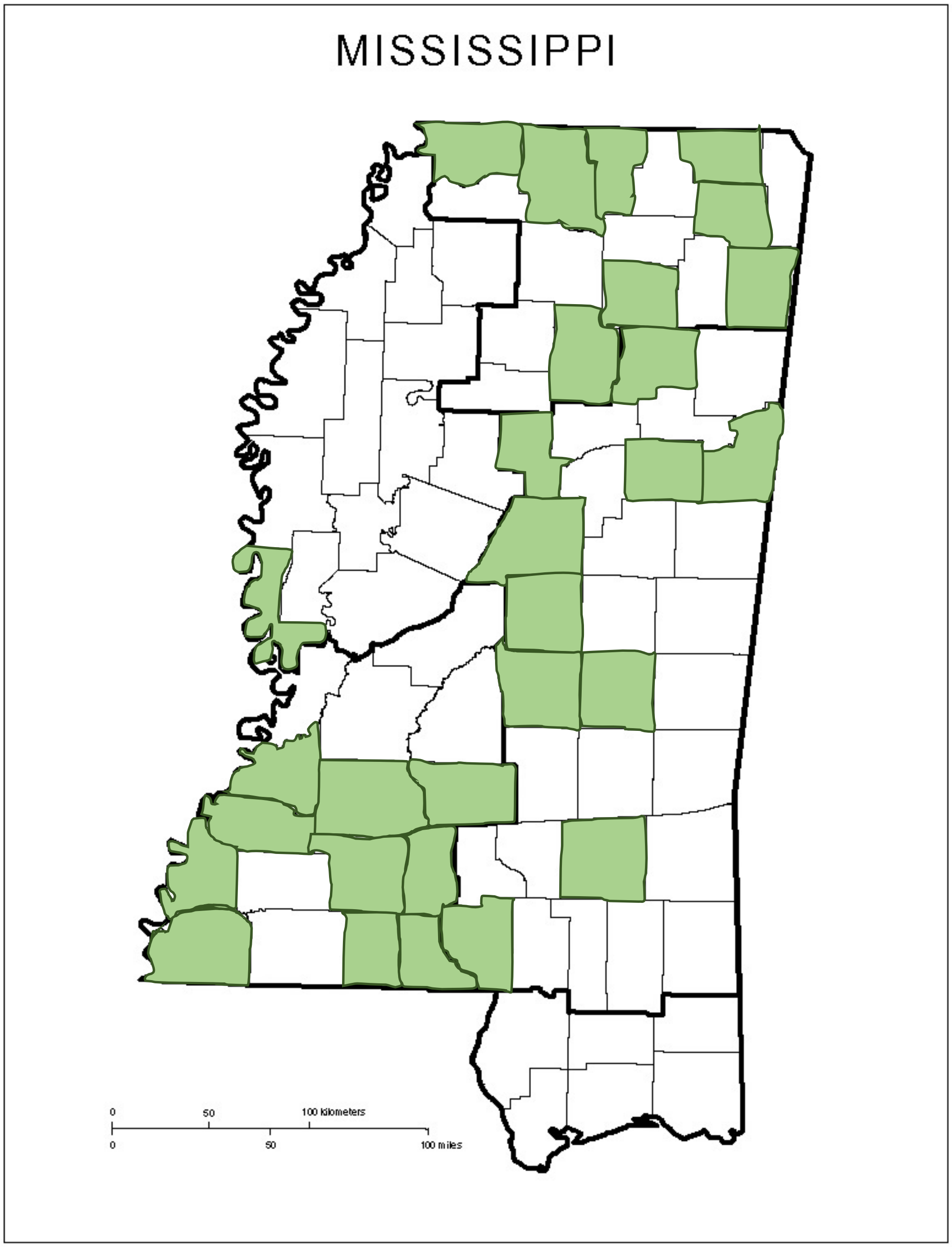

Figure 1. Mississippi counties (shaded) indicate the first occurrence of taproot decline of soybean. The identification was conducted during the growing season of 2017.

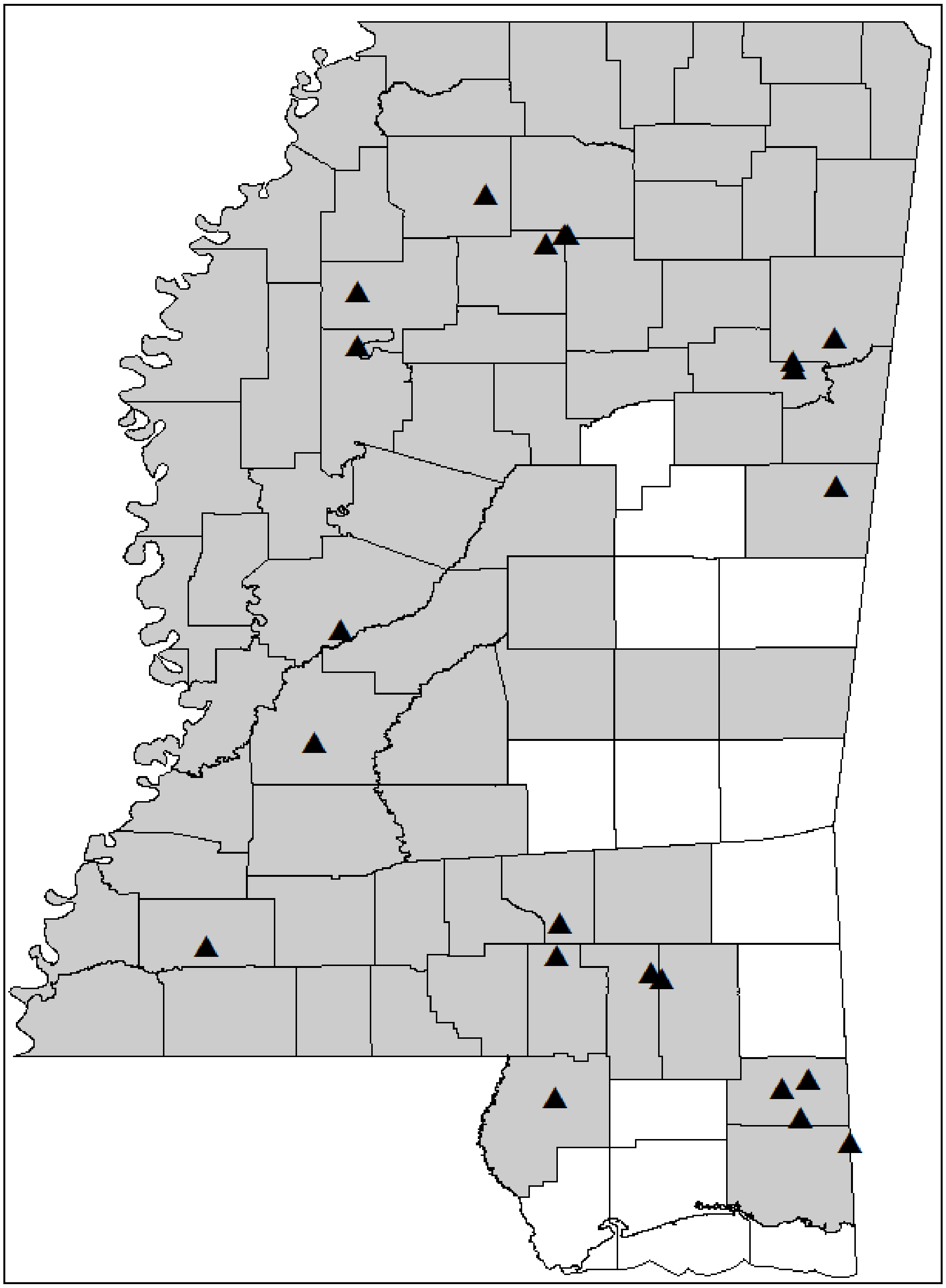

Figure 2. The distribution of taproot decline (TRD) in Mississippi. The counties shaded grey represent those in which TRD has been observed. The triangles represent the origin of *Xylaria* sp. isolates used in this study.

Figure 3. A 4-wk-old soybean seedling infected by George County *Xylaria* sp. isolate. The black stromata (matrix of vegetative hyphae) and the absence of taproot formation is consistent with the disease cycle of taproot decline.

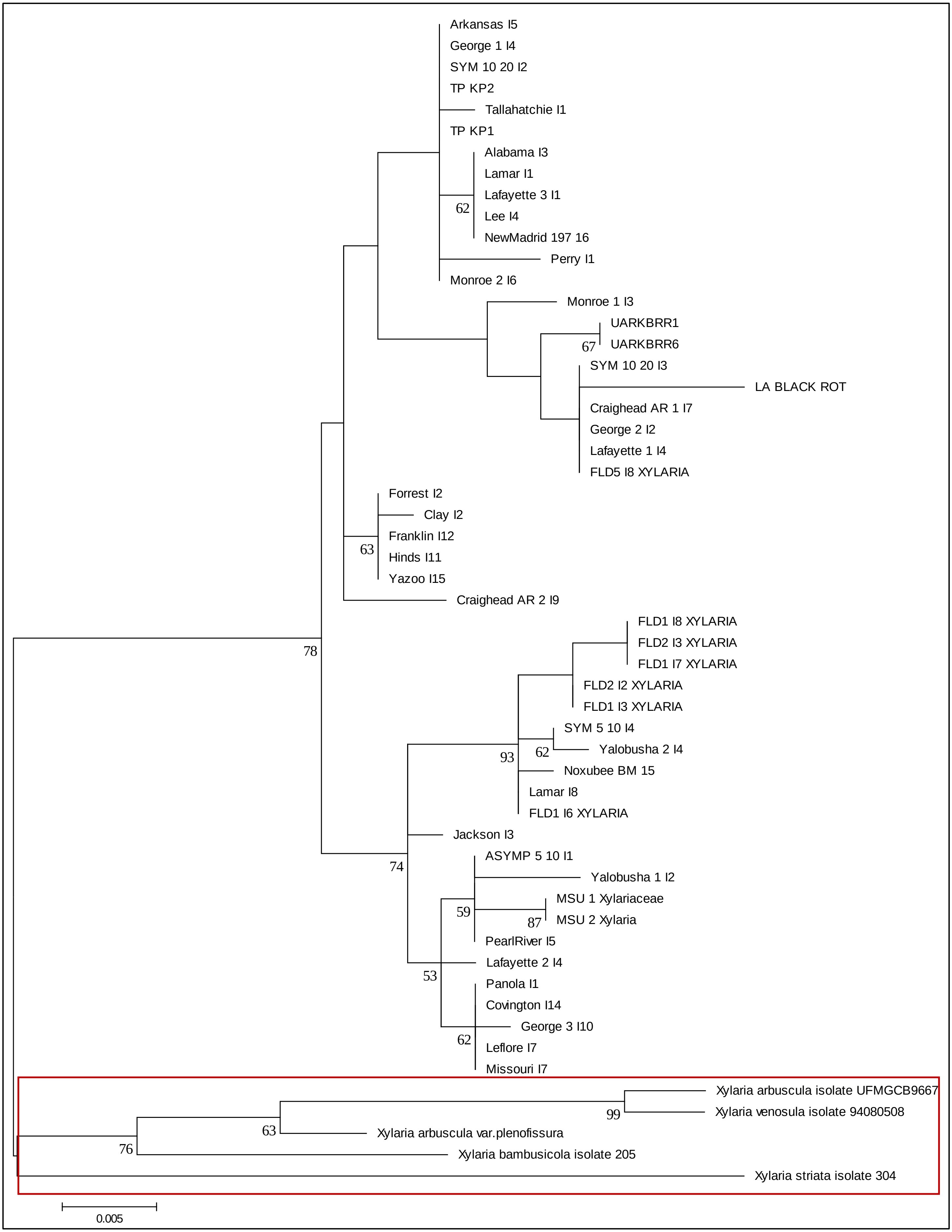
Arkansas I5
George 1 I4
SYM 10 20 I2
TP KP2
Tallahatchie I1
TP KP1
Alabama I3
Lamar I1
Lafayette 3 I1
62
Lee I4
NewMadrid 197 16
Perry I1
Monroe 2 I6
Monroe 1 I3
UARKBRR1
67
UARKBRR6
SYM 10 20 I3
LA BLACK ROT
Craighead AR 1 I7
George 2 I2
Lafayette 1 I4
FLD5 I8 XYLARIA
Forrest I2
Clay I2
Franklin I12
63
Hinds I11
Yazoo I15
Craighead AR 2 I9
FLD1 I8 XYLARIA
FLD2 I3 XYLARIA
78
FLD1 I7 XYLARIA
FLD2 I2 XYLARIA
FLD1 I3 XYLARIA
SYM 5 10 I4
62
Yalobusha 2 I4
93
Noxubee BM 15
Lamar I8
FLD1 I6 XYLARIA
Jackson I3
ASYMP 5 10 I1
74
Yalobusha 1 I2
MSU 1 Xylariaceae
59
87
MSU 2 Xylaria
PearlRiver I5
Lafayette 2 I4
53
Panola I1
Covington I14
George 3 I10
62
Leflore I7
Missouri I7
Xylaria arbuscula isolate UFMGCB9667
99
Xylaria venosula isolate 94080508
63
Xylaria arbuscula var.plenofissura
76
Xylaria bambusicola isolate 205
Xylaria striata isolate 304
0.005

Figure 4. A phylogenetic tree based on ITS showing the relatedness of *Xylaria* sp., the pathogen of taproot decline in soybean. The closest taxa is the *Xylaria* arbuscular aggregate shown in the bottom box.

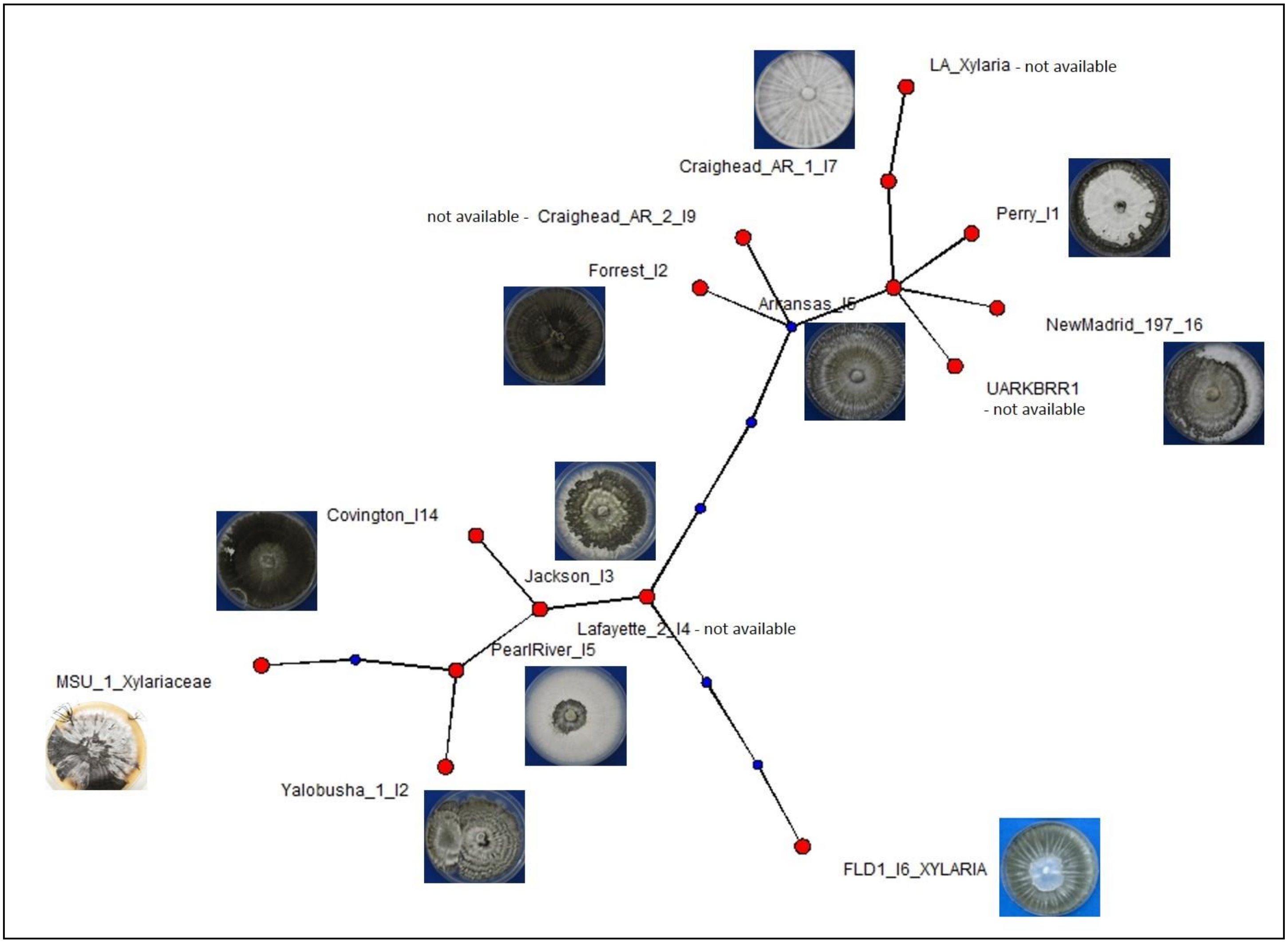

Figure 5. A haplotype map showing the diversity within the collection of taproot decline isolates (*Xylaria* sp.) collected from several Mississippi counties, Arkansas, Louisiana, and Missouri. The diversity is evident in the colony morph