Southern Region Small Fruit Consortium Proposal Final Report

Title: Distribution of Botryosphaeria stem blight in blueberry production of Alabama

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Name, Mailing and Email Address of Principal Investigator(s):

Sushan Ru (Principal Investigator), Assistant Professor, Department of Horticulture, Auburn University. 559 Devall Dr, CASIC building, Auburn, AL 36849. Email: szr0099@auburn.edu
Elina Coneva (Co-PI), Professor & Extension Specialist, Department of Horticulture, Auburn University. 101 Funchess Hall, Auburn University, AL 36849. Email: edc0001@auburn.edu
Kathy Lawrence (Co-PI), Professor, Department of Entomology & Plant Pathology, Auburn University. 559 Devall Dr, CASIC building, Auburn, AL 36849. Email: lawrekk@auburn.edu
Ebrahiem Babiker (Co-PI), Research Plant Geneticist, USDA-ARS Thad Cochran Southern Horticultural Laboratory. 810 HWY 26 West, P.O. Box 287, Poplarville, MS 39470-0287. Email: ebrahiem.babiker@usda.gov
Jonathan Oliver (Co-PI), Assistant Professor, Department of Plant Pathology, University of Georgia. 2360 Rainwater Road, Tifton, GA 31793. Email: jonathanoliver@uga.edu
Melba Salazar-Gutierrez (Co-PI), Assistant Professor, Department of Horticulture, Auburn University. 112 Funchess Hall, Auburn University, AL 36849. Email: mrs0146@auburn.edu

Cooperators:
Chip East, Regional Extension Agent, Clay County, Alabama Cooperative Extension System. **Arlie Powell**, Professor Emeritus, Department of Horticulture, Auburn University & Owner of Petals from the Past Farm in Jemison, Alabama. **Bisho Lawaju**, Postdoctoral Research Associate, Department of Entomology and Plant Pathology, Auburn University

Objectives:
This project aims to identify the distribution and causal pathogens of blueberry stem blight in Alabama and nearby regions while conducting outreach activities to help blueberry growers better identify and manage Botryosphaeria stem blight. Specifically, we aim to:

Objective I: assess the distribution and causal pathogens of blueberry stem blight in Alabama and neighboring regions such as Georgia and Mississippi.

Objective II: offer growers research-based education on management strategies to minimize risks of Botryoshpaeria stem blight in blueberry production.

Justification and Description:

Botryosphaeria stem blight: a top limiting factor for blueberry production

Global blueberry production has been constantly increasing at an average annual rate of 6.1% since 1970 (FAOSTAT, accessed on 2021-04-22). Despite rising crop values and an expanding market, blueberry production in Alabama remains marginal compared to major producers such as Georgia and Florida (USDA NASS, accessed on 2021-04-23). A top limiting factor for Alabama blueberry industry is the threat of blueberry stem blight or twig dieback caused by species of the Botryosphaeriaceae (East, 2019). Botryosphaeria stem blight can significantly reduce productivity or even cause plant death especially in young plantings (Smith 2004). In addition to Alabama, Botryosphaeria stem blight is also a major disease in other blueberry production areas in southeastern United States (Cline & Schilder, 2006; Milholland, 1972; Smith, 2004). Since the first reported incidence in North Carolina in 1959 (Milholland, 1972), Botryosphaeria stem blight is now found in states such as Alabama, Florida, Georgia, Mississippi and North Carolina (Creswell & Milholland, 1987; East, 2019; Smith, 2009; Wright & Harmon, 2010) and other countries such as Australia (Scarlett et al., 2018), China (Xu et al., 2015), Italy (Guarnaccia et al., 2020), and Peru (Rodríguez-Gálvez et al., 2020). As its occurrence continues to rise, Botryosphaeria stem blight is considered **the most damaging disease of blueberry in Alabama** (East, 2019) and **economically the most important blueberry disease in Florida** (Wright & Harmon, 2010).

Symptoms of blueberry stem blight

Fungi from the Botryosphaeriaceae family can infect blueberry plants through wounds or natural openings (e.g., lenticels, stomata) to cause drought-like symptoms such as wilting of twigs, reddening and necrosis of leaves, cane dieback, and eventually plant death (**Fig. 1**) (Caruso & Ramsdell, 1995; Flor et al., 2019). Two major types of blueberries grown in the southeast, southern highbush (*V. corymbosum* interspecific hybrid) and rabbiteye (*V. virgatum* Reade) blueberries, are both susceptible to Botryosphaeria stem blight to various degrees (Caruso & Ramsdell, 1995; Milholland, 1972). Although good horticultural and pest management practices are recommended to reduce the chance of severe outbreaks, no management practices or cultivars can effectively prevent or mitigate Botryosphaeria stem blight (Caruso & Ramsdell, 1995).

Figure 1. Typical symptoms of Botryosphaeria stem blight: twig dieback (left) and internal wood discoloration (right). Modified from Xu et al. 2015

Causal pathogens of blueberry stem blight

Botryosphaeria dothidea was considered the dominant species infecting blueberry plants based on morphological analysis in early studies (Smith, 2004; Smith, 2009). However, overlapping morphological characteristics among Botryosphaeriaceae species make it difficult to accurately differentiate similar species. As technologies improve, many new species have been identified

through a combination of DNA sequencing, phylogenetic analysis, and morphological analysis. Wright and Harmon (2010) identified two dominant species in Florida: *Lasiodiplodia theobromae* and *Neofusicoccum ribis*. Xu et al. (2015) identified three species, *L. theobromae*, *N. parvum*, and *Botryosphaeria dothidea*, in 20 blueberry sites across China. Additionally, eight species were found to cause blueberry stem blight in Australia, with *N. parvum* being the most common one, followed by *N. kwambonambiense*, *N. occulatum*, *L. theobromae*, *B. dothidea*, *N. australe*, *N. macroclavatum* and *L. pseudotheobromae* (Scarlett et al., 2018).

Challenges of controlling blueberry stem blight in Alabama

The majority of blueberry production in Alabama consists of rabbiteye blueberries grown on small U-Pick farms. Compared to larger farms, small farms lack the financial means and resources to mitigate crop losses caused by diseases like Botryosphaeria stem blight. Despite an increased incidence of blueberry stem blight across Alabama (personal communication with extension specialists Dr. Elina Coneva and Chip East), little data is available on the occurrence, distribution and causal pathogens for this destructive disease in Alabama. Virulence of the pathogen can vary greatly between species of fungi (Creswell & Milholland, 1987), which makes identification of the species the first step to conquer Botryosphaeria stem blight in Alabama and nearby regions. The overall goal of this project is to identify the distribution and causal pathogens of blueberry stem blight in Alabama and nearby states while conducting outreach activities to help blueberry growers better identify and manage Botryosphaeria stem blight.

Methods & Procedures

Sample collection and pathogen isolation

Stem blight samples collected from a total of 26 different cultivars and 8 locations were kept in respective plastic bags and brought back to the laboratory for pathogen isolation, using the procedure described by Xu et al. (2015) with slight modifications. Small pieces of twig approximately 1 inch were cut from each sample using a sterile scalpel and surface sterilized in 75% ethanol for 30 seconds followed by another 1 minute in 10% NaOCl solution then rinsed with sterile water. The cuttings were then placed in potato dextrose agar (PDA) plates acidified with 85% lactic acid at 1.0ml/L. The cultures were incubated at 28°C until fungal colonies were observed. Pure cultures were obtained by using a sterilized scalpel to transfer hyphal tips from actively growing mycelia mat into fresh APDA plates.

DNA extraction, PCR amplification, and phylogenetic analyses

Pure fungal cultures were kept at room temperature for 24 hours prior to DNA extraction. From each isolate, 75*mg* of fungal mycelium was harvested into lysing tubes. DNA extraction was conducted using the ZymoBiomics™ kit. DNA concentration was examined with Nanodrop 2000 spectro-photometer (ThermoFisher Sci., Waltham, MA). PCR and DNA sequencing followed Xu et al. (2015). Specifically, primers ITS1 and ITS4 will be used to amplify the ribosomal DNA ITS region (the ITS1,5.8S, and ITS2). Partial sequence of the β-tubulin (BT) gene, BT2, will be amplified using primers Bt2a and Bt2b. Part of the translation elongation-factor 1-alpha (EF) gene will be amplified using the primers EF1-728 F and EF1-986R. Phylogenetic analysis will be conducted by comparing sequences of representative Botryosphaeriaceae isolates with those in GenBank. ITS, β-tubulin, and EF1-α sequences of related Botryosphaeriaceae isolates from other hosts and regions will be retrieved from GenBank for phylogenetic analysis.

Results

Surveys of the occurrence of Botryosphaeria stem blight were conducted in eight locations of Alabama, Georgia, and Mississippi between September 2021 and May 2022 to identify common species of Botryosphaeriaceae. A total of 41 symptomatic samples were collected, from which 49 isolates have been cultured. DNA samples of the isolates were extracted, amplified, and shipped for sequencing for the ITS region to identify the family of the pathogens. Fungal samples confirmed to be in the family of Botryosphaeriaceae based on both DNA sequence of the ITS region and morphological characteristics (**Fig. 1**) will be further sequenced for the β-tubulin (BT) gene and 1-alpha (EF) gene to confirm their genera and species. We expect to receive sequencing results for the ITS region in early December and results for other genomic regions in mid-December. Data analysis will be conducted following the receipt of sequencing data to identify the genus and species of surveyed Botryosphaeriaceae samples. Meanwhile, a review paper titled "A review of Botryosphaeria stem blight of blueberry from the perspective of plant breeding" is under review for the journal *Agriculture*.

Table 1. Summary of disease samples collected between August 2021 and July 2022

Location	Time of collection	No. of plant samples	No. of isolates
Auburn, AL	03/11/22 – 05/20/22	11	12
Chilton, AL	03/01/22 – 06/10/22	6	8
Fairhope, AL	05/20/22	3	4
Jemison, AL	09/07/21	3	3
Shorter, AL	04/22/22 – 06/12/22	7	9
Hahira, GA	09/10/21	1	2
Lake Park, GA	04/15/22	7	7
Poplarville, MS	05/09/22	3	4
		Total: 41	**Total: 49**

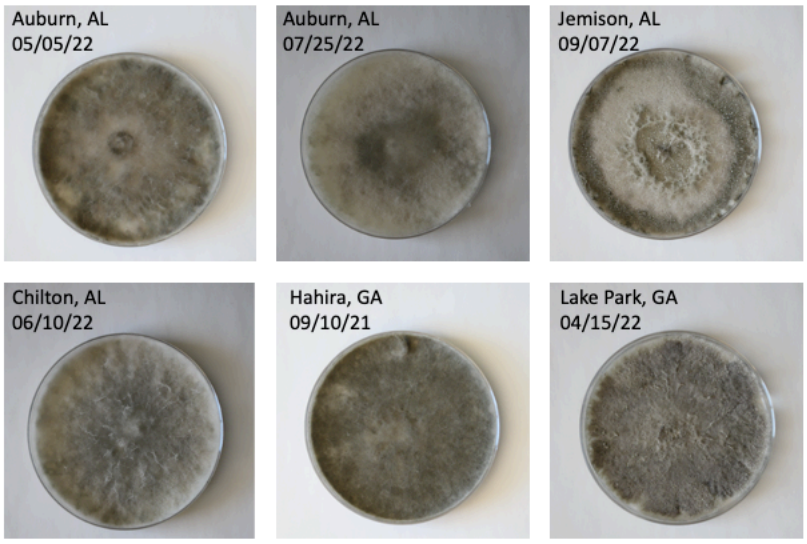

Figure 1. Colonial morphologies of selected fungal isolates

Conclusion

This study surveyed multiple regions of Alabama and adjacent areas in Georgia and Mississippi for the occurrence and causal pathogens of Botryosphaeria stem blight. Common species of Botryosphaeriaceae identified in this study will be used for the screening blueberry cultivars for resistance against Botryosphaeria stem blight. Information and fungal isolates obtained from this study will serve as the foundation for the development of resistant cultivars and more effective management strategies.

References:

Caruso F and Ramsdell D (Eds.) 1995. Compendium of Blueberry and Cranberry Diseases. APS Press, St. Paul, MN

Cline WO, Schilder A (2006) Identification and control of blueberry diseases. In: Childers NF and Lyrene PM (eds) Blueberries for Growers, Gardeners and Promoters. Dr. Norman F. Childers Publications, Gainesville, Florida, pp. 115-138

Creswell TC, Milholland RD (1987) Responses of blueberry genotypes to infection by Botryosphaeria dothidea. Plant Dis. 71(8):710-713

East C (2019) Blueberry disease management recommendations. Alabama Cooperative Extension System, www.aces.edu/blog/topics/crop-production/blueberry-disease-management-recommendations/, accessed 2021-07-15

FAOSTAT. 2021. Production: Crops: Blueberry. Food and Agriculture Organization of the United Nations, Rome, Italy. Accessed 2021-04-22

Flor NC, Phillips DA, Harmon PF (2019). Botryosphaeria stem blight on southern highbush blueberry in Florida. UF/IFAS Extension. 347

Guarnaccia V, Martino I, Tabone G, Brondino L, Gullino ML (2020) Fungal pathogens associated with stem blight and dieback of blueberry in northern Italy. Phytopathol Mediterr 59(2): 229-245. DOI:10.14601/Phyto-11278

Rodríguez-Gálvez E, Hilário S, Lopes A (2020) Diversity and pathogenicity of *Lasiodiplodia* and *Neopestalotiopsis* species associated with stem blight and dieback of blueberry plants in Peru. Eur J Plant Pathol. 157:89–102. https://doi.org/10.1007/s10658-020-01983-1

Milholland RD (1972). Histopathology and pathogenicity of Botryosphaeria dothidea on blueberry stems. Phytophathology. 62:654-660

Scarlett KA, Shuttleworth LA, Colllins D, Rothwell CT, Guest DI, Daniel R (2018) Botryosphaeriales associated with stem blight and dieback of blueberry (*Vaccinium* spp.) in New South Wales and Western Australia. Australas Plant Pathol. 48:45-57

Smith BJ (2004). Susceptibility of southern highbush blueberry cultivars to Botryosphaeria stem blight. Small Fruits Rev. 3(1-2):193-201

Smith BJ (2009). Botryosphaeria stem blight of southern blueberries: Cultivar susceptibility and effect of chemical treatments. Acta Hortic. 810

USDA National Agricultural Statistics Service (NASS). https://www.nass.usda.gov/Statistics_by_State/New_Jersey/Publications/Blueberry_Statistics. Accessed 2021-04-23.

Wright AF and Harmon PF (2010) Identification of species in the *Botryosphaeriaceae* Family causing stem blight on southern highbush blueberry in Florida. Plant Dis. 94(8):966-971

Xu C, Zhang H, Zhou Z, Hu T, Wang S, Wang Y, Cao K (2015) Identification and distribution of Botryosphaeriaceae species associated with blueberry stem blight in China. Eur J Plant Pathol. 143(4):737-752