Southern Region Small Fruit Consortium Proposal Final Report

Title: Screening blueberry cultivars for stem blight resistance

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Title: Screening blueberry cultivars for stem blight resistance

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Objectives:
The overall goal of this study is to identify blueberry culitvars with ideal resistance or tolerance to Bortryosphaeria stem blight. Specifically, we aim to:
Objective I. Screen southern highbush and rabbiteye blueberry cultivars with ideal resistance or tolerance to Botryosphaeriaceae species commonly found in the Southeast U.S., and
Objective II. Improve the attached-stem assay for more accurate and consistent stem blight resistance screening.

Justification and Description:
Botryosphaeria stem blight: a top limiting factor for blueberry production
Botryosphaeria stem blight is a top limiting disease for the Southestern blueberry industry (East 2019; Flor et al. 2019). Caused by fungal species of the Botryosphaeriaceae family, blueberry stem blight can significantly reduce the productivity of blueberries and even cause plant death, especially in young plantings (Milholland 1995). Since the first reported incidence in North Carolina in 1959 (Milholland 1972), Botryosphaeria stem blight is now found in Alabama, Florida, Georgia, Mississippi, North Carolina, and other states in the U.S. (Creswell & Milholland 1987; East 2019; Smith 2009; Wright & Harmon 2010), and other countries such as Australia (Scarlett et al. 2019), China (Xu et al. 2015), Italy (Guarnaccia et al. 2020), and Peru (Rodríguez-Gálvez et al. 2020) Occurrence of the disease continues to rise under changing climate conditions. In New Zealand, approximately 18% of all blueberries are affected by stem blight, which costs around $500,000 NZ annually due to yield and plant loss (Sammonds et al. 2009). In the U.S., 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).

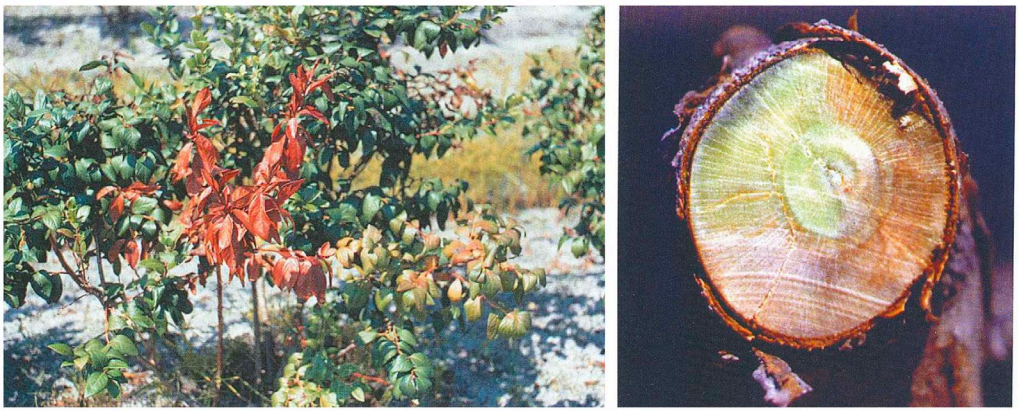

Fig. 1. Symptoms of Botryosphaeria stem blight: twig dieback (left) and internal wood discoloration (right), pictures from Milholland (1995)

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**) (Milholland 1995; Flor et al. 2019). Currently, no chemical is effective for stem blight in the field (Milholland 1995; Smith 2009). As a result, disease management is limited to cultural practices such as proper irrigation, fertilization, and pruning (Milholland 1995) to passively reduce the chance of severe outbreaks. However, **adoption of resistant or tolerant cultivars can provide the most economic, effective, and environmental-friendly solution to minimize losses to blueberry stem blight**.

Lack of a reliable protocol for cultivar screening

Discrepancies in lesion lengths were commonly found among cultivar screening studies, which presents a major challenge for the ienditification of resistant cultivars. A wide range of cultivated and wild blueberry species (*V. corymbosum, V. corymbosum* interspecific hybrid, *V. virgatum* Reade, *V. angustifolium*, *V. corymbosum* × *V. angustifolium*, *V. elliottii, V. darrowii, V. arboretum, V. stamineum*) have been screened for stem blight resistance using detached- (Babiker et al. 2019; Smith 2004, 2009) or attached-stem assays (Polashock and Kramer, 2006). Some studies reported a higher level of resistance in low bush and half-high blueberries compared to cultivated blueberries (highbush and rabbiteye) (Polashock and Kramer 2006), others reported better resistance in rabbiteye than highbush cultivars (Milholland 1995; Smith 2004). However, in a more recent study, no significant effect of species on lesion length was found when 39 accessions of 7 species (*V. corymbosum, V. virgatum* Reade, *V. elliottii, V. darrowii, V. arboretum, V. stamineum*) were inoculated with Botryosphaeriaceae using a detached-stem assay (Babiker et al. 2019). Furthermore, results from artificial inoculation did not correlate well with field observations partially due to harsher conditions during artificial inocularion (Cline et al., 1993; Smith 2004). Among many reasons behind inconsistent results on cultivar resistance to stem blight, a lack of standard and reliable screening methods play a major role. **Therefore, it is critical to improve current screening protocols for more accurate and reliable identification of resistant cultivars.**

The overall goal of this study is to identify blueberry culitvars with ideal resistance or tolerance to Bortryosphaeria stem blight. Specifically, we aim to:
Objective I. Screen southern highbush and rabbiteye blueberry cultivars with ideal resistance or tolerance to Botryosphaeriaceae species commonly found in the Southeast U.S., and
Objective II. Improve the attached-stem assay for more accurate and consistent stem blight resistance screening.

Materials and methods

Pathogenicity test

A pathogenicity test was conducted at the Plant Science Research Center of Auburn University, Auburn, AL, between March 6 and March 27, 2023, using twenty-eight isolates selected from the survey and analysis conducted in Chapter 2 of this work. The test was conducted to assess which fungal families could produce stem blight symptoms on inoculated healthy blueberry plants. The selected isolates included fifteen isolates of Botryosphaeriaceae, seven isolates of Diaporthaceae, and six isolates of Sporocadaceae. Three partially lignified stems chosen from each of twenty-eight blueberry plants (cv. 'Vernon') were inoculated with each of the isolates to assess their ability to cause necrotic lesion on blueberry. Pathogenic isolates were re-isolated from the necrotic lesions produced, and re-cultured in pure form. Nineteen pathogenic isolates were selected for virulence testing.

Experimental design

An attached-stem assay modified from Polashock and Kramer (2006) was used for the pathogenicity test, virulence test, and cultivar screening. Plant containers were placed in plastic trays filled with water to keep them moist. Inoculation was performed in a walk-in growth chamber set at 12-hour day light, 60% relative humidity, and temperature of 25°C. A sterile clip was used to make fresh horizontal cuts on the selected stems, and the cut surface sprayed with 70% ethanol for surface disinfection. A sterile scalpel was used to transfer agar plug of mycelia with the mycelia-side down, from the fungal plate which was previously punched with a sterile 7.8mm diameter cork borer, to the surface of the cut stem. After inoculation, the cut end and agar plug were covered with parafilm (Bemis Inc. WI, USA) to prevent desiccation (**Figure 1a – d**). The parafilm was removed after seven days. Control plants were similarly inoculated with uncolonized PDA, acidified with 85% lactic acid at 0.5 ml/L.

All inoculated plants and controls were arranged in a completely randomized layout and plants were watered as needed. The length of necrotic lesions produced on each stem were measured and imaged every seven days for three weeks. The inoculated stems were harvested, and pathogens were re-isolated from the necrotic lesions produced on the stems to confirm the identity of the pathogen for Koch's postulates. The DNA of each fungal pathogen was extracted using ZymoBiomics™ kit as previously described in Chapter 2, and the ITS region of their DNA was sequenced and compared to the sequences of the ITS region of the original isolates used for inoculation, to fulfil Koch's postulates.

Short and long-term fungal storage

Botryosphaeria stem blight pathogens are known to rapidly lose virulence in culture (Cline et al. 1993). Pathogens isolated in this study were preserved for short term use, by following methods described by Ellis (1979) with slight modifications. Briefly, pure cultures of each isolate grown on APDA were bored with a sterile 7.8 mm diameter cork borer. A sterile scalpel was used to transfer the bored mycelium and APDA medium into 1.5 ml sterile water in screw cap tubes, at 5 cubes per tube. The caps were firmly secured to prevent desiccation and the tubes were stored at 4°C (for short-term preservation) or -80°C (for long-term preservation).

Virulence testing

Fifty-seven blueberry plants (cv. 'Vernon') were inoculated as described above, with eleven Botryosphaeriaceae, four Diaporthaceae, and four Sporocadaceae isolates. The virulence test was conducted to assess the aggressiveness of the selected isolates upon inoculation into living asymptomatic blueberry plants. The isolates were selected based on the results of the pathogenicity test previously conducted as described above. Three plants per isolate and three stems per plant were inoculated, resulting in a total of nine stems inoculated with each isolate. Three plants and a total of nine stems were similarly inoculated with uncolonized APDA as controls. The length of necrotic lesions produced on each stem was measured and imaged every seven days for four weeks. The infected stems were harvested, and the images of necrotic lesions produced on each stem were captured.

Cultivar screening

Two isolates previously identified as *N. parvum* and *Neofusicoccum* sp. were selected based on the results of the virulence testing above and used to screen four blueberry cultivars. Before inoculation, the isolates were cultured on APDA and kept at 4°C under sterile conditions using the short-term storage methods previously described. Three-year-old potted southern highbush blueberry cultivars ('Legacy', 'Miss Alice' and 'Star') and one rabbiteye cultivar ('Vernon') used for the screening were obtained from Fall Creek Nurseries, Oregon, US. The rabbiteye cultivar (Vernon) was included as control cultivar for comparison with the performance of the three highbush cultivars. Prior to inoculation, plants were hedged in June 2023 to promote new shoot growth. The cultivars were inoculated using growth chamber conditions and the inoculation method described above. Inoculation on each cultivar was replicated three times, resulting in a total of nine stems inoculated with each isolate for each cultivar. Three stems on each cultivar were also inoculated with uncolonized APDA and replicated three times, resulting in nine stems per cultivar, as controls. The plants were maintained at Paterson Greenhouse, Department of Horticulture, Auburn University with routine irrigation, fertilization, and weeding. Imaging and measurement of necrotic lesions was carried out every seven days for four weeks.

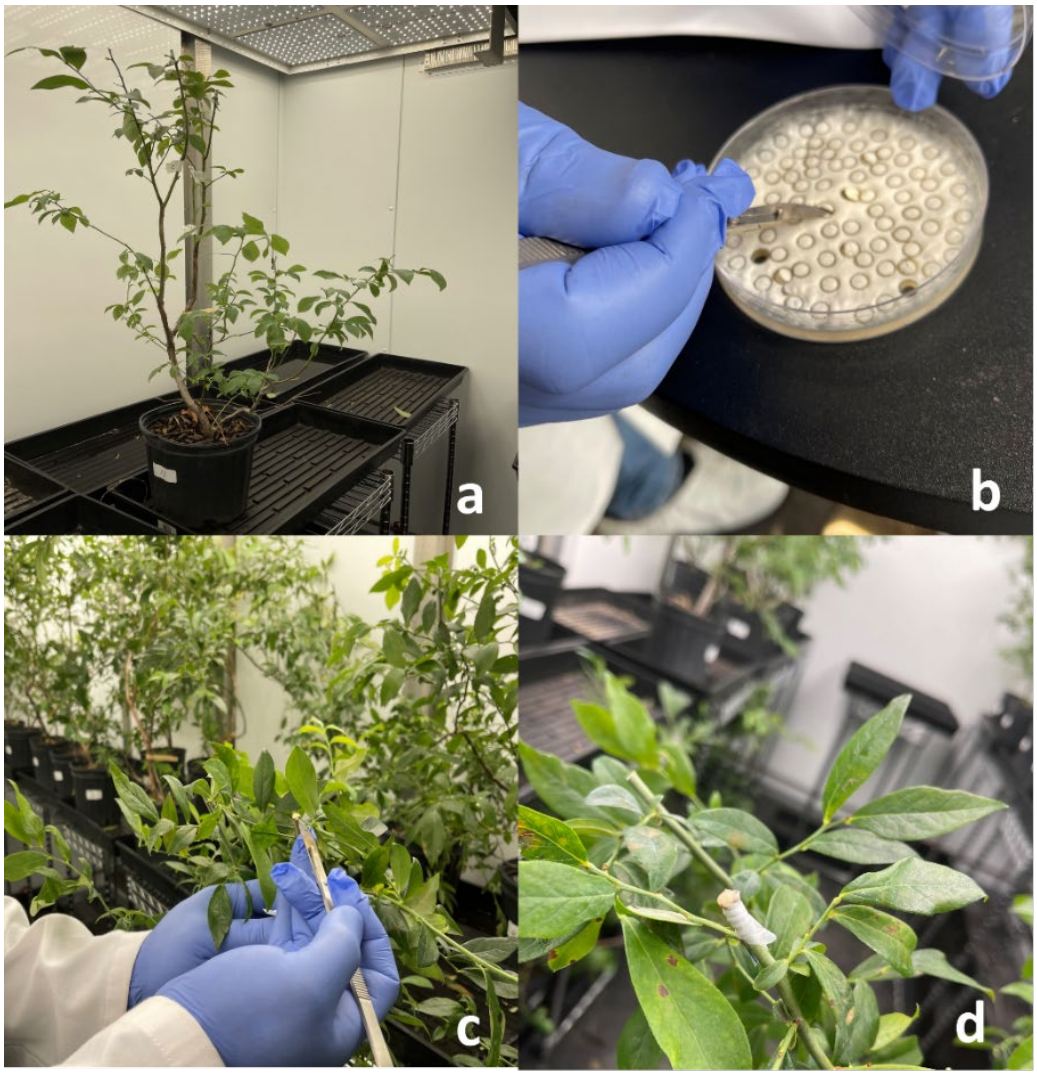

Figure 1. Artificial inoculation of partially lignified, three-year-old potted blueberry plants of cultivar 'Vernon'. The experiment was conducted in a walk-in growth chamber with 12-hour daylight, 60% relative humidity, and temperature of 25°C. Potted blueberry plant (cv. 'Vernon') in the growth chamber (a). Fungal mycelium cultured on APDA, punctured with sterile 7.8mm diameter cork borer (b). Fungal inoculum placed on freshly- made horizontal cut on blueberry stem (c). Inoculum on wounded stem secured with parafilm to prevent rapid desiccation and cross contamination (d).

Statistical analysis

The virulence of the isolates was determined based on lengths of necrotic lesions produced by each of the nineteen isolates on the inoculated 'Vernon' plants after four weeks, using one-way analysis of variance (ANOVA). Isolates that produced lesion length significantly different from the control were identified using Tukey's Honest Significant Difference test (HSD, α=0.05). Multiple comparison of mean lesion length produced by inoculation with each isolate versus the control (inoculation with uncolonized APDA) was conducted using Dunnett's test. Two-way analysis of variance was conducted to determine the resistance level of blueberry cultivars and potential isolate by cultivar interactions based on lesion length at four weeks. Mean comparisons

were conducted using Tukey's Honest Significant Difference test (HSD, α=0.05). All tests were conducted in R (version 4.3.1, R Inc. Boston, USA).

Results

Pathogenicity testing

Out of the twenty-eight isolates tested for pathogenicity, thirteen Botryosphaeriaceae, six Diaporthaceae, and six Sporocadaceae produced necrotic lesions on the inoculated plants (Table 1) and were confirmed as capable of causing dieback symptoms on cut stems. The images of necrotic lesions produced by isolates AU29 (*N. parvum*), AU37 (*N. ribis*), and AU25 (*Neofusicoccum* sp.) seven days after inoculation, are shown in **Figure 2**. The comparison of DNA sequences from the re-isolated pathogens obtained from the pathogenicity test with those of the original isolates used for inoculation confirmed adherence to Koch's postulates.

Infection was readily established in plants inoculated with species from the Botryosphaeriaceae. Necrotic lesion began to grow downwards from the point of inoculation, four days after inoculation for isolates AU13 (*Neofusicoccum ribis*), AU25 (*Neofusicoccum* sp.), and AU27 (*N. ribis*). Isolates AU25 (*Neofusicoccum sp.*) and AU13 (*N. ribis*) produced the longest lesion length in the Botryosphaeriaceae while isolates AU6 (*Lasiodiplodia* sp.), AU2 (*L. brasiliensis*) and AU23 (*Neofusicoccum.* sp.) had the shortest average necrotic lesion among tested isolates (Table 1). Initiation of infection was slower on stems inoculated with Diaporthaceae. In the Diaporthaceae group, isolates AU28 and AU46 (*Diaporthe* sp.) produced the longest lesion length. Isolate AU4 (*Diaporthe* sp.) produced the shortest necrotic lesion in the group. Isolates AU41 (*Neopestalotiopsis* sp.) and AU9 (*Pestalotiopsis* sp.) produced the longest and shortest necrotic lesion on the inoculated stems, respectively. The length of necrotic lesion produced by one isolate from each of the Botryosphaeriaceae and Diaporthaceae, respectively, was not sufficient to confirm their pathogenicity (i.e., was not significantly greater than the uninoculated control)

Table 1 Lesion length produced by isolates of Botryosphaeriaceae, Diaporthaceae, and Sporocadaceae during pathogenicity testing

Family	Isolate	Mean Lesion length	SD
Botryosphaeriaceae	AU25	67.6	10.7
	AU13	54.5	4.7
	AU27	43	9.2
	AU33	36.3	26.7
	AU29	36	6.4
	AU37	32.2	7.9
	AU13	31.9	18.2
	AU8	12.1	8.6
	AU3	4.6	2.2
	AU26	4.3	2.3
	AU6	3.4	2.9
	AU2	2.9	1
	AU23	2.6	1.2
Diaporthaceae	AU28	52.3	4.6
	AU46	49.2	5.7

	AU17	41.2	3
	AU32	36.6	4.5
	AU30	20.7	15.6
	AU46	6	4.9
Sporocadaceae	AU41	13.2	6.7
	AU22	7.7	5.8
	AU38	4.8	0.2
	AU42	10.3	0.4
	AU16	9.5	1.1
	AU9	1.8	8.3
Control	APDA	0	0
	APDA	0.1	0.2

Virulence testing

The Botryosphaeriaceae produced consistently longer necrotic lesion on inoculated stems compared to the Diaporthaceae and Sporocadaceae, with *Neofusicoccum* sp. inducing the overall longest necrotic lesion (isolate AU26). Figure 3 shows the lesion length produced by each isolate, across the three fungal families. The necrotic lesion length produced across the Botryosphaeriaceae family was 54.47 ± 42.98mm. The Diaporthaceae family produced an average lesion length of 10.05 ± 2.89mm. Average lesion length for the Sporocadaceae is 9.98 ± 7.88mm

Analysis of variance revealed a significant difference in mean lesion length produced by at least one of the isolates ($p<0.05$). To determine which specific groups produced necrotic lesion lengths significantly greater than the control, Dunnett's multiple comparison was done to compare lesion length produced by each isolate to that of the lesion length recorded for stems inoculated with uncolonized APDA (**Table 1)**. A total of eight isolates produced lesion length significantly greater than the control: isolates AU26, AU3, AU25 (*Neofusicoccum* sp.), AU8, AU29 (*N. parvum*), AU37 (*N. ribis*), AU6 (*Lasiodiplodia* sp.), and AU1 (*L. citricola*) ($P<0.05$). Lesion length induced by isolates AU13 (*N. ribis*), AU33 (*Botryosphaeria dothidea*), AU5 (*Pseudofusicoccum kimberleyense*), AU32, AU28, AU4, AU30 (*Diaporthe* sp.), AU42 (*Neopestalotiopsis clavispora*), AU38, AU41 (*Neopestalotiopsis* sp.), and AU56 (*Alternaria* sp.) are not significantly greater than the control. The extremely low p-values ($p<0.05$) strongly indicate that isolates AU1, AU3, AU8, and AU26 are highly virulent.

Table 2. Mean lesion length produced by Botryosphaeriaceae, Diaporthaceae, and Sporocadaceae during virulence testing

Species	IsolateNo	Mean lesion length (mm)	SD	Family
Neofusicoccum sp.	AU26	110.2	51.8	Botryosphaeriaceae
Neofusicoccum sp.	AU3	81.3	38.4	Botryosphaeriaceae
Lasiodiplodia citricola	AU1	71.4	68.1	Botryosphaeriaceae
Neofusicoccum parvum	AU8	69.5	39.5	Botryosphaeriaceae
Neofusicoccum ribis	AU37	56.7	22.3	Botryosphaeriaceae
Neofusicoccum sp.	AU25	52.6	25	Botryosphaeriaceae

Lasiodiplodia sp.	AU6	52.3	37.6	Botryosphaeriaceae
Neofusicoccum parvum	AU29	49.7	18.4	Botryosphaeriaceae
Neofusicoccum ribis	AU13	27.2	18.7	Botryosphaeriaceae
Botryosphaeria dothidea	AU33	20.4	6.9	Botryosphaeriaceae
Neopestalotiopsis clavispora	AU42	14.8	13.9	Sporocadaceae
Alternaria sp.	AU56	11.3	5.42	Diaporthaceae
Diaporthe sp.	AU30	11.2	2.63	Diaporthaceae
Diaporthe sp.	AU28	10.4	2.07	Diaporthaceae
Pseudofusicoccum kimberleyense	AU5	9.67	7.65	Botryosphaeriaceae
Neopestalotiopsis sp.	AU38	7.94	2.84	Sporocadaceae
Neopestalotiopsis sp.	AU41	6.43	3.76	Sporocadaceae
Diaporthe sp..	AU4	7.45	2.85	Diaporthaceae
Control	APDA	5.57	4.6	Control

significant difference at P<0.5

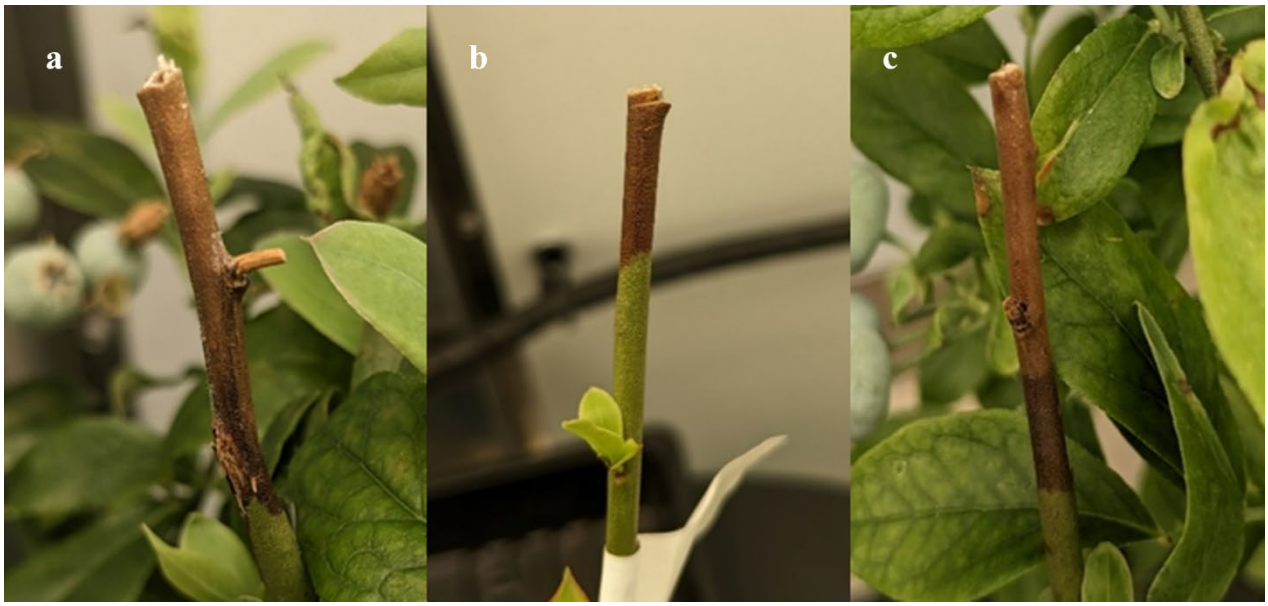

Figure 2. Images of necrotic lesion produced by three *Neofusicoccum spp*. Isolates, 7 days after inoculation**.** Stem inoculated with isolate AU29 (*N. parvum*) (a), AU37 (*N. ribis*) (b), and AU25 (*Neofusicoccum* sp.) (c).

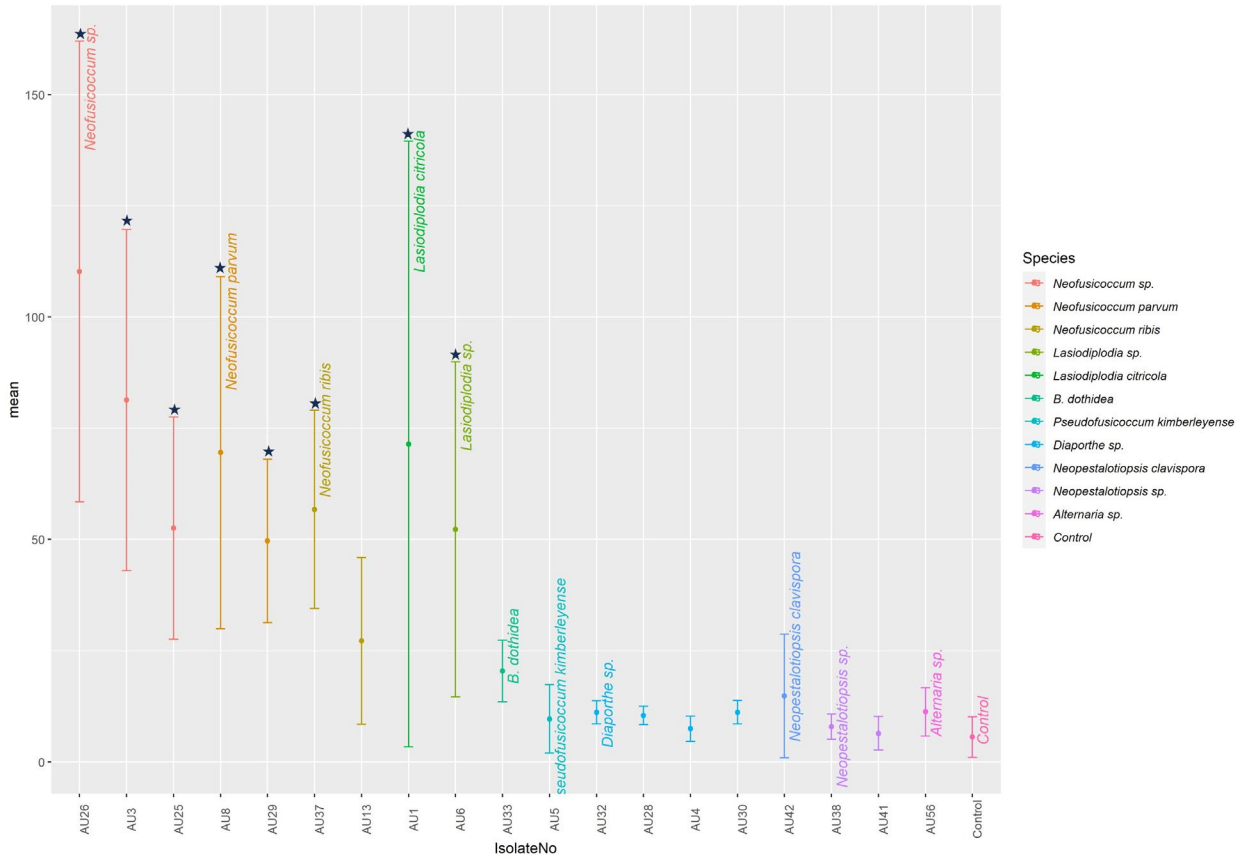

Figure 3. Lesion length produced by isolates from Botryosphaeriaceae, Diaporthaceae, and Sporocadaceae at week four. The error bars represent the standard deviation from the mean lesion length produced by each isolate

Tukey Honest Significance Difference test was conducted to rank the mean lesion length produced by each isolate (* indicates significantly different mean lesion length at $p<0.05$). The result (**Table 2**) confirms that the lesion length produced by isolates AU13 (*N. ribis*), AU33 (*B. dothidea*), AU42 (*Neopestalotiopsis clavispora*), AU56 (*Alternaria* sp.*),* AU30 (*Diaporthe* sp.*),* AU28 (*Diaporthe* sp.*),* AU5 (*Pseudofusicoccum* sp.*),* AU38 (*Neopestalotiopsis clavispora*), AU41 (*Neopestalotiopsis* sp.*),* and AU4 (*Diaporthe* sp.) are not significantly different from the control. Isolates in the Botryosphaeriaceae are similar in virulence as they produce lesion length with apparently overlapping means and range (**Figure 3**), except for isolate AU5 (*Pseudofusicoccum kimberleyense*) which produced mean lesion length of 9.67mm similar to isolates in the Diaporthaceae and Sporocadaceae. Isolate AU26 produced the longest mean lesion length (110.24mm). Isolate AU41 (*Neopestalotiopsis* sp.) produced the least mean lesion length and is not significantly different from other isolates in the Diaporthe and Sporocadaceae group, or the control.

Overall, tested isolates from the Botryosphaeriaceae produced longer lesion lengths compared to isolates from the Diaporthaceae and Sporocadaceae (Table 2). Lesion length by species in the Botryosphaeriaceae continued to increase up to week four when the experiment was terminated (Figure 4). The lesion produced by members of the Diaporthaceae and Sporocadaceae did not increase significantly after the first week. Two distinct isolates AU26 (*Neofusicoccum* sp.) and AU8 (*N. parvum*) were selected for cultivar screening based on the mean lesion length produced, and the deviation from the mean as shown in Table 1. AU26 (*Neofusicoccum* sp.) was selected because it produced the longest lesion length (mean =110.2, SD = 51.8). Isolate AU8 (*N. parvum*)

was selected for cultivar screening instead of isolate AU1 (*Lasiodiplodia citricola*) because it had less deviation from the mean lesion length, even though the two species had similar mean lesion length values (AU1: mean = 71.4, SD = 68.1; AU8: mean = 69.5, SD = 39.5). Images of harvested stems four weeks post inoculation with isolates AU26 (*Neofusicoccum. sp.*), AU8 (*N. parvum*), and isolate AU5 (*Pseudofusicoccum kimberleyense*) are shown in Figure 5.

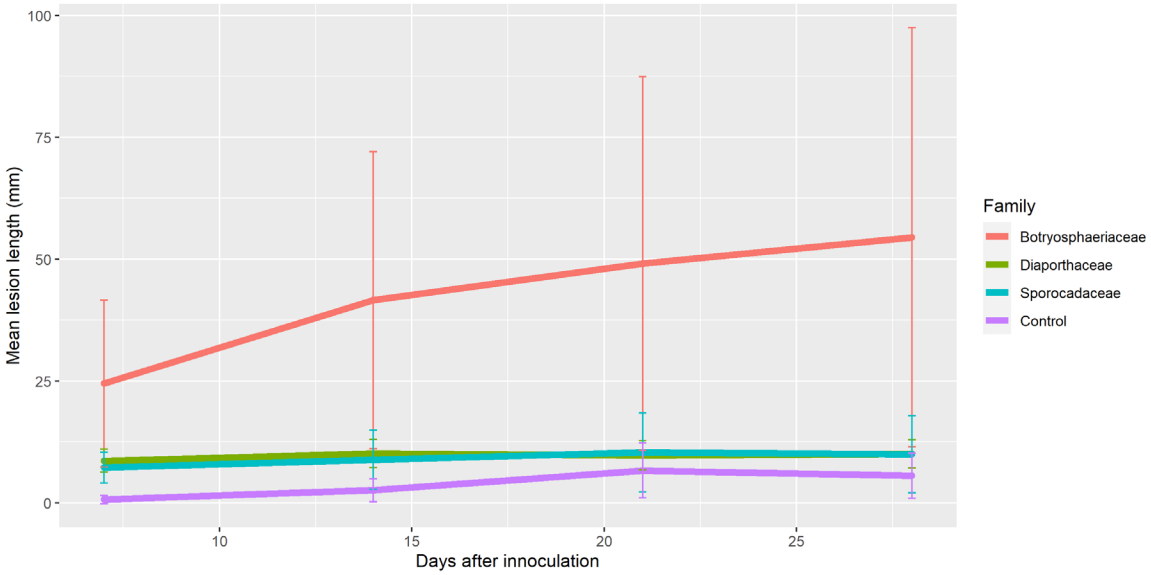

Figure 4. Progression of lesion length produced by Botryosphaeriaceae, Diaporthaceae, and Sporocadaceae isolates from this study four weeks post inoculation.

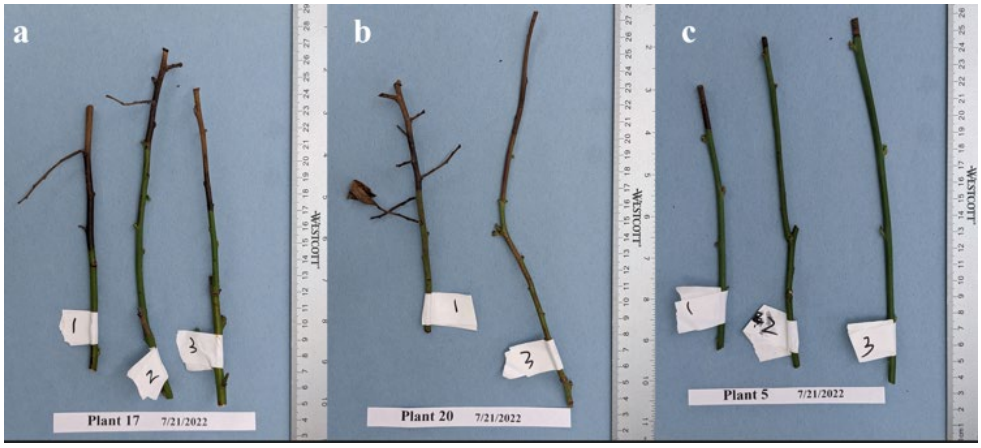

Figure 5. Harvested stems of selected isolates, four weeks post-inoculation. Stems inoculated with isolate AU26 (*Neofusicoccum. sp.*) (a), isolate AU8 (*N. parvum*) (b), isolate AU5 (*Pseudofusicoccum* sp.) (c).

Cultivar screening

The mean lesion length recorded four weeks after the two isolates were used to inoculate 'Legacy', 'Miss Alice', 'Star', and 'Vernon' are shown in Table 2.

Table 2. Susceptibility of four blueberry cultivars inoculated with species of *Neofusicoccum*

Cultivar	Treatment	Mean lesion length (mm)	SD
Legacy	*Neofusicoccum parvum* AU8	56.30	11.91
Miss Alice	*Neofusicoccum parvum* AU8	39.77	20.80
Star	*Neofusicoccum parvum* AU8	42.96	15.03
Vernon	*Neofusicoccum parvum* AU8	54.03	22.95
Legacy	*Neofusicoccum* sp. AU26	41.93	15.22
Miss Alice	*Neofusicoccum* sp. AU26	35.86	13.51
Star	*Neofusicoccum* sp. AU26	43.96	15.28
Vernon	*Neofusicoccum* sp. AU26	56.03	25.73
Legacy	Control (uncolonized APDA)	1.52	0.81
Miss Alice	Control (uncolonized APDA)	1.77	1.10
Star	Control (uncolonized APDA)	1.40	0.73
Vernon	Control (uncolonized APDA)	0.33	0.71

The mean lesion length and the variance for each of the cultivars inoculated with either AU8 (*Neofusicoccum parvum*), AU26 (*Neofusicoccum* sp.), or Control (uncolonized APDA) are presented in Table 2. The two-way ANOVA conducted revealed that the treatment factor ($p = 3.2e^{-13}$) ((Isolates AU26, AU8, and Control) and the cultivars ($p = 0.0272$) had significant effects on the average lesion length produced. However, no treatment x cultivar interaction was detected ($p = 0.2896$). The Tukey Honest Significance Difference Test conducted to compare the fungal pathogen groups indicated that the difference between the means of the AU8 (*N. parvum*) and AU26 (*Neofusicoccum* sp.) groups is not statistically significant ($p = 0.9882$, $\alpha=0.05$). The confidence interval includes zero, and we therefore could not conclude that there was a significant difference between these two groups. However, The Control-AU26 group has a statistically significant difference at the $\alpha=0.05$ level. The confidence interval does not include zero, indicating a significant difference between these two groups. The Control group has a significantly lower mean compared to the AU26 group. Similarly, the P-value for the Control-AU8 group is very close to zero, indicating that the difference in means between this group is statistically significant at the $\alpha=0.05$ level. The confidence interval also does not border zero, implying a significant difference between the two groups. The control group has a significantly lower mean compared to the AU8 group.

Based on the result of this Tukey HSD Test, we infer that there are significant differences in means between the control group and both the AU8 and AU26 groups. However, there is no significant difference in means between the AU26 and the AU8 group. In the AU8 (*Neofusicoccum parvum*) group, the longest average lesion length was recorded on 'Legacy' (56.30) and is thus considered the most susceptible. The shortest lesion length was recorded on 'Miss Alice' (39.77) and is thus considered the most resistant to AU8 (*Neofusicoccum parvum*). In the AU26 (*Neofusicoccum* sp.) group, the longest mean lesion length was recorded on 'Vernon' (56.03), while the shortest mean lesion length was also recorded on 'Miss Alice'(35.86). Similar lesion lengths were recorded on 'Star' and 'Legacy' in this treatment group. Interestingly, for both the AU8 (*Neofusicoccum parvum*) and AU26 (*Neofusicoccum* sp.) groups, 'Miss Alice' had the shortest mean lesion length, suggesting an interaction between the cultivar and isolates AU8 (*Neofusicoccum parvum*) and AU26 (*Neofusicoccum* sp.).

References:

Babiker EM, Stringer SJ, Sakhanokho HF, Smith BJ (2019). Characterization and pathogenicity of stem blight complex isolates associated with stem blight disease on *Vaccinium* species. HortScience, 54(7), 1199-1203. https://doi.org/10.21273/HORTSCI14033-19

Cline WO, Milholland RD, Rooks SD, Ballington JR (1993) Techniques in breeding for resistance to blueberry stem blight caused by *Botryosphaeria dothidea*. Acta Hortic, 346, 107-110. 10.17660/ActaHortic.1993.346.14

Creswell TC, Milholland RD (1987). Responses of blueberry genotypes to infection by *Botryosphaeria dothidea*. Plant Dis. 71 (8), 710–713 http://dx.doi.org/10.1094/PD-71-0710.

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

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

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

Milholland RD (1995) Botryosphaeria stem blight. Pp. 10-11. In: Caruso FL and Ramsdell DC (eds). Compendium of blueberry and cranberry diseases. The American Phytopathological Society Press. St. Paul, MN.

Polashock JJ, Kramer M (2006). Resistance of blueberry cultivars to botryosphaeria stem blight and phomopsis twig blight, HortSci, 41(6), 1457-1461. https://journals.ashs.org/hortsci/view/journals/hortsci/41/6/article-p1457.xml

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

Sammonds J, Billones R, Ridgway HJ, Walter M, Jaspers MV (2009) Survey of blueberry farms for Botryosphaeria dieback and crown rot pathogens. New Zealand Plant Protection 62:238 – 242.

Scarlett KA, Shuttleworth LA, Colllins D, Rothwell CT, Guest DI, Daniel R (2019) 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

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. https://doi.org/10.1094/PDIS-94-8-0966

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. https://doi.org/10.1007/s10658-015-0724-6