**Title: Assessing the impact of *Xylella fastidiosa* in southern highbush blueberry plants in North Carolina and Georgia**

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**Public Abstract**


Blueberries are an economically important fruit crop in the southeastern U.S., and Georgia (#3), North Carolina (#7), and Florida (#8) rank among the top 8 states nationally in terms of blueberry production. Bacterial leaf scorch (BLS) of blueberry, caused by the bacterial pathogen *Xylella fastidiosa* (Xf) results in leaf scorch, defoliation, decline, and death of affected blueberry plants. BLS was first reported in Georgia blueberries nearly 15 years ago on highly susceptible southern highbush cultivars (*Vaccinium corymbosum* interspecific hybrids). Since then, BLS has become widespread in the blueberry production areas of Georgia and Florida, with isolated reports in other states in the southeastern U.S. Historically, the North Carolina blueberry industry has relied on highbush blueberry and 'rabbiteye' cultivars with higher chill hour requirements; however, in recent years, growers have begun planting increasing numbers of the types of low-chill SHB blueberries that have proven to be highly susceptible to BLS in Georgia. Over the last two years, possible BLS symptoms have been observed in blueberry plantings in eastern North Carolina. Since the presence of Xf in North Carolina blueberries has the potential to have a significant impact on the southeastern U.S. blueberry industry, researchers and extension personnel initiated research with the following objectives: (1) Confirm the identity of Xf detected in SHB blueberries in North Carolina, and compare them to known bacterial leaf scorch causing isolates from Georgia blueberries, (2) Assess how widespread Xf is within southern highbush blueberry plantings in North Carolina, and determine if it is present in alternate host plants nearby or within the same planting, and (3) Evaluate the susceptibility of southern highbush blueberry cultivars grown in North Carolina and Georgia to Xf. While these research efforts are still in progress as of the time of this report, preliminary testing via PCR and AmplifyRP® XRT+ has confirmed the presence of Xf in at least one North Carolina blueberry planting. Subsequent sequencing efforts suggest that the Xf strain infecting blueberries in North Carolina belongs to Xf subspecies *multiplex*, but it does not appear to be closely related to Xf isolates found infecting blueberries in other southeastern U.S. states. Genetic characterization, testing, and assessments will continue in late 2021-2022 and are expected to provide further information regarding the prevalence and potential impacts of Xf on North Carolina blueberries.

**Introduction**

Blueberries are an economically important fruit crop in the southeastern U.S., and Georgia (#3), North Carolina (#7), and Florida (#8) rank among the top 8 states nationally in terms of blueberry production (NASS 2020). Blueberry production is impacted by numerous disease issues, including bacterial leaf scorch, caused by the bacterial pathogen *Xylella fastidiosa* (Xf). This destructive disease causes a marginal leaf scorch on leaves, defoliation, plant decline, and eventual death of affected plants (**Figure 1**) (Brannen et al. 2016). First reported more than a decade ago on southern highbush (SHB) blueberry (*Vaccinium corymbosum* interspecific hybrids) in Georgia, this disease is now widespread in blueberries in Florida as well, and has previously been found in Alabama, Mississippi, Louisiana, Texas, and Kentucky. In Georgia, this disease is one of the leading causes of SHB mortality and is known to limit the productive lifespan of blueberry plantings. Formerly, it was thought that only SHB cultivars could be infected by *Xylella*; however, Xf has also been reported to infect rabbiteye blueberries (*Vaccinium virgatum*) as well, where it can cause typical leaf scorching symptoms under certain conditions (Ferguson 2017).

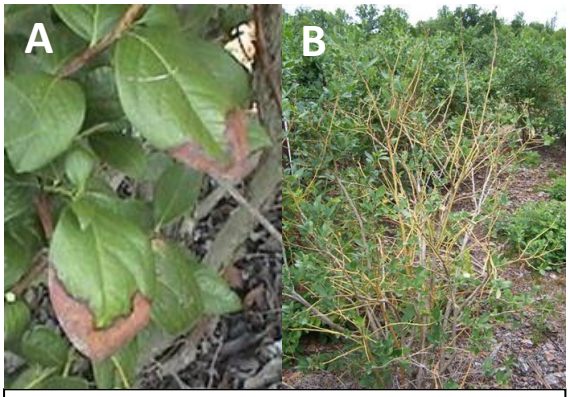


**Figure 1.** Symptoms caused by *Xylella fastidiosa* on blueberry: (A) Marginal leaf scorch. (B) Defoliation. Photos by P. Brannen.

Xf is transmitted by xylem-feeding insects, and it is believed that sharpshooters are responsible for plant-to-plant transmission of the pathogen in blueberry plantings. In addition, Xf has also been shown to be transmitted during plant propagation, when cuttings are taken from infected plants. Blueberry propagation operations which occur outdoors or in screen houses have the potential to be a source of Xf-infected plants if cuttings are taken from infected plants or if insect vectors are allowed to move Xf freely from adjacent infested fields into nursery plants.

Once established in an area, management of bacterial leaf scorch is a challenge, and growers are forced to rely largely upon the identification and removal of infected plants (i.e. 'roguing') to limit the spread of this disease. Molecular methods, which are capable of detecting pre-symptomatic infection, are the preferred means of detection for Xf, but growers often rely on visual identification of bacterial leaf scorch symptoms to make management decisions. Host resistance would be desirable for Xf management in blueberry, but information on relative cultivar susceptibility to Xf is scarce. While most rabbiteye cultivars appear to be tolerant of infection with Xf, field observations suggests that SHB cultivars demonstrate a range of susceptibility to the pathogen. For example, cultivars 'Rebel', 'O'Neal', and 'FL86-19 (V1)' appear to be highly susceptible while cultivar 'Emerald' seems to have some tolerance to bacterial leaf scorch (Brannen et al. 2016).

The species *X. fastidiosa* is composed of at least four genetically-distinct subspecies (subspecies '*fastidiosa*', '*multiplex*', '*pauca*', and '*sandyii*') which differ in terms of their host range, geographic distribution, and primary insect vectors (Parker et al. 2016). Subspecies '*fastidiosa*' isolates are known to infect grapes and muscadines throughout the southeastern U.S., while '*multiplex*' isolates are known to affect peaches, pecan, and several shade trees in the same region. Until recently, all known isolates of Xf from blueberry belonged to the same group of genetically-related individuals within Xf subspecies *multiplex*, but isolates of Xf subsp. *fastidiosa* have been recently identified in naturally infected blueberry fields in Georgia (Di Genova et al. 2020). Evidence suggests that Xf subsp. *fastidiosa* isolates from blueberry in Georgia are closely related to isolates from muscadines in Georgia and Florida, and may have originated from infected wild muscadines (*Vitis rotundifolia*) adjacent to commercial blueberry fields (**Figure 2**).

While bacterial leaf scorch had not been previously reported on blueberries in North Carolina, Xf subsp. *fastidiosa* is endemic within the state on grapes and muscadines and sharpshooter vectors are present. Historically, the North Carolina blueberry industry has relied on highbush blueberry and 'rabbiteye' cultivars with higher chill hour requirements; however, in recent years, as a result of climatic changes and economic pressures for earlier harvests, growers have begun planting increasing numbers of the types of low-chill SHB blueberries (including 'O'Neal' and 'Rebel') that have proven to be highly susceptible to bacterial leaf scorch in Georgia. This has led to heightened concerns regarding the potential for bacterial leaf scorch to become established in North Carolina.

**Figure 2.** Muscadines alongside blueberry field in Georgia infected with Xf subsp. *fastidiosa*. Photo by Dario Di Genova.

During the summer of 2019, marginal leaf scorch and defoliation typical of bacterial leaf scorch was observed in a planting of SHB blueberry cultivar 'Rebel' in southeastern North Carolina. Initial ELISA testing of symptomatic plants indicated that several plants were infected with Xf. A single sample from this location submitted to the Plant Molecular Diagnostic Laboratory at the University of Georgia – Tifton Campus tested positive for Xf using the AmplifyRP® XRT+ test from Agdia® in Fall 2019. These alarming results suggested that bacterial leaf scorch may already be affecting blueberries in North Carolina. To understand the scope of this issue, information is needed regarding the identity of this pathogen, how widespread it is within SHB blueberry plantings in North Carolina, and the relative susceptibility of popular SHB cultivars currently grown in North Carolina. Accordingly, we initiated research with the following objectives: (1) Confirm the identity of Xf detected in southern highbush blueberries in North Carolina, and compare them to known bacterial leaf scorch causing isolates from Georgia blueberries, (2) Assess how widespread Xf is within southern highbush blueberry plantings in North Carolina, and determine if it is present in alternate host plants nearby or within the same planting, and (3) Evaluate the susceptibility of southern highbush blueberry cultivars grown in North Carolina and Georgia to Xf.

## Materials and Methods

**Confirmation of Xf in southern highbush blueberries in North Carolina and genetic comparison between blueberry strains of Xf in North Carolina and Georgia.** In Summer 2021, symptomatic plant material was collected from the blueberry planting in North Carolina were Xf had been previously suspected. Plant tissue was shipped to the Plant Molecular Diagnostic Laboratory in Tifton, Georgia for confirmation of Xf via established methods (Waliullah et al. 2019) including the AmplifyRP® XRT+ test from Agdia® (Elkhart, Indiana) and PCR with the RST31/33 primer pair (Minsavage et al. 1994). Isolation from infected blueberry tissue on Periwinkle Wilt media (Davis et al. 1981) was also attempted. DNA extractions and PCR using Xf-specific primers (**Table 1**) was used to further confirm pathogen identity and allow for genetic comparisons with known strains of Xf present in crop plants in the southeastern U.S. Plant DNA was extracted using a modified CTAB protocol (Doyle and Doyle 1987). PCR products were amplified from the 10 genes listed in **Table 1**. Amplicons were purified using the E.Z.N.A.® Cycle Pure Kit (Omega Bio-Tek, Norcross, GA) and sequenced in both directions via Sanger sequencing by Eurofins Genomics (Louisville, KY). Sequences for each amplicon were reviewed for signal quality on FinchTV 1.5.0 software (Geospiza, Inc.), imported into SeaView 4.6.4 software (Galtier et al. 1996; Gouy et al. 2010) for manual editing and alignment, and analysed via BLAST versus other Xf sequences present in the GenBank database (www.ncbi.nlm.nih.gov/genbank).

**Table 1.** Primers used for PCR amplification and sequencing of Xf genes.

| Gene | Role in Study | Primers (F/R) | Amplicon Size (bp) | Reference |
|---|---|---|---|---|
| *rpoD* | Pathogen detection & genetic characterization | RST-31/RST-33 | 733 | Minsavage et al. 1994 |
| *acvB* | Genetic characterization (MLSA-E) | acvB-F/acvB-R | 743 | Parker et al. 2012 |
| *copB* | Genetic characterization (MLSA-E) | copB-F/copB-R | 607-862 | Parker et al. 2012 |

| | | | | |
|---|---|---|---|---|
| *cvaC* | Genetic characterization (MLSA-E) | cvaC-F/cvaC-R | 330 | Parker et al. 2012 |
| *fimA* | Genetic characterization (MLSA-E) | fimA-F/fimA-R | 557 | Parker et al. 2012 |
| *gaa* | Genetic characterization (MLSA-E) | gaa-F/gaa-R | 1129 | Parker et al. 2012 |
| *pglA* | Genetic characterization (MLSA-E) | pglA-F/pglA-R | 828/829 | Parker et al. 2012 |
| *pilA* | Genetic characterization (MLSA-E) | pilA-F/pilA-R | 405 | Parker et al. 2012 |
| *rpfF* | Genetic characterization (MLSA-E) | rpfF-F/rpfF-R | 825 | Parker et al. 2012 |
| *xadA* | Genetic characterization (MLSA-E) | xadA-F/xadA-R | 1087/1108 | Parker et al. 2012 |

**Assessment of how widespread Xf is within SHB blueberry plantings in North Carolina, and in nearby alternate host plants.** To determine how widespread Xf might be within the region, SHB blueberry plantings in the southeastern North Carolina region were scouted for signs of Xf infection during 2021. When bacterial leaf scorch symptoms were suspected, leaf samples (2-5 samples per site) were collected and shipped to the Plant Molecular Diagnostic Laboratory in Tifton, Georgia for testing as described above. Within sites where Xf was confirmed during 2021, more extensive surveys (up to 100 samples per site) are anticipated to be conducted during 2022 to gauge the proportion of the planting that is currently infected with Xf. In addition to testing the blueberry plants within infected sites, the surrounding vegetation will be tested also, since this may serve as a pathogen reservoir for some strains of Xf.

**Evaluation of the susceptibility of southern highbush blueberry cultivars grown in North Carolina and Georgia to Xf.** As an initial assessment of susceptibility, SHB blueberry plants will be inoculated in the greenhouse during 2022 with Xf isolates obtained from North Carolina blueberries. Experiments will conducted in the UGA Plant Pathology greenhouses in Tifton, Georgia according to the methods utilized by Oliver et al. (2015). SHB cultivars popular in North Carolina, including 'Rebel', 'Farthing', 'Star' and 'Suziblue' will be assessed. Blueberries will be grown in three parts pine bark and one part sand and maintained in the greenhouse for 5-6 months after inoculation. Successful infection of inoculated plants will be confirmed by PCR following DNA extraction from leaves. Inoculated plants will be assessed weekly for the development of typical leaf scorch symptoms or other signs of infection with Xf. The relative susceptibility of each cultivar will be compared following inoculation with North Carolina and Georgia Xf isolates.

## Results

**Confirmation of Xf in southern highbush blueberries in North Carolina and genetic comparison between blueberry strains of Xf in North Carolina and Georgia.** Testing of symptomatic plant material via PCR and AmplifyRP XRT+ revealed a single plant that was infected with Xf. To confirm the identity of the Xf present in this infected blueberry plant, PCR products were amplified, sequenced, and analyzed. Preliminary results suggest that the Xf present in this infected blueberry plant is generally more similar to Xf subsp. *multiplex* isolates from olive trees in California and a red bud tree in Texas than Xf subsp. *multiplex* isolates from blueberry in Georgia and Florida (**Table 2**).

**Table 2.** Genetic similarities between Xf present in NC blueberry and previously sequenced Xf isolates.

| **Gene** | **Most Closely Related Sequences in Genbank** | | | |
|---|---|---|---|---|
| | **Blueberry Xf Isolates** | | **Other Xf Isolates** | |
| | **Isolate (Location)** | **Accession # (% Identity)** | **Host (Location)** | **Accession # (% Identity)** |
| *rpoD* | AlmaEm3 (Georgia) | CP072933.1 (99.03%) | Pecan (Georgia) | MH681048.1 (99.86%) |
| *acvB* | AlmaEm3 (Georgia) | CP072933.1 (99.57%) | Olive (California) | CP052855.1 (99.86%) |
| *copB* | AlmaEm3 (Georgia) | CP072933.1 (99.27%) | Redbud (Texas) | JQ361964.1 (100%) |
| *cvaC* | AlmaEm3 (Georgia) | CP072933.1 (95.44%) | Redbud (Texas) | JQ362013.1 (100%) |
| *fimA* | AlmaEm3 (Georgia) | CP072933.1 (97.23%) | Olive (California) | CP052855.1 (99.80%) |
| *gaa* | AlmaEm3 (Georgia) | CP072933.1 (99.81%) | Olive (California) | CP052855.1 (99.81%) |
| *pglA* | AlmaEm3 (Georgia) | CP072933.1 (97.79%) | Olive (California) | CP052855.1 (100%) |
| *pilA* | AlmaEm3 (Georgia) | CP072933.1 (95.75%) | Redbud (Texas) | JQ362209.1 (99.72%) |
| *rpfF*[a] | AlmaEm3 (Georgia) | CP072933.1 (99.87%) | Olive (California) | CP052855.1 (99.87%) |

| | | | | |
|---|---|---|---|---|
| *xadA* | n.d.[b] | n.d.[b] | n.d.[b] | n.d.[b] |

[a]at the time of this report, only a partial sequence was available for *rpfF;* results above are based on this partial sequence.
[b]not determined (n.d.).; sequencing of this gene is still in progress as of the time of this report.

**Assessment of how widespread Xf is within SHB blueberry plantings in North Carolina, and in nearby alternate host plants.** From April through October 2021, SHB, highbush, and blueberry fields in North Carolina were scouted for bacterial leaf scorch symptoms during routine farm visits. A total of 33 site visits were conducted in 12 counties, with some sites visited repeatedly. Twenty of these site visits took place within six counties (Bladen, Craven, Duplin, New Hanover, Pender and Sampson) in the main blueberry production area of southeastern North Carolina, while the other 13 site visits took place in six counties in other areas of the state where blueberries are grown on a smaller scale for direct-market and pick-your-own sales. Samples were collected only from plants showing potential bacterial leaf scorch symptoms. In total, 12 samples were collected and tested from three widely separated sites in Pender County, NC (**Table 3**). Based on both PCR and AmplifyRP XRT+ testing results, a single plant (sampled twice) of SHB blueberry cultivar 'Rebel' was confirmed as being Xf positive during 2021. (*NOTE: At the time of this report, this objective is currently in progress. As described above, within sites where Xf was confirmed during 2021, more extensive surveys (up to 100 samples per site) are anticipated to be conducted during 2022 to gauge the proportion of the planting that is currently infected with Xf. In addition to testing the blueberry plants within infected sites, the surrounding vegetation that may serve as a pathogen reservoir for some strains of Xf will be tested also.*)

**Table 3.** North Carolina blueberry samples collected and tested for *X. fastidiosa*.

| | | | | | Xf Testing Results | |
|---|---|---|---|---|---|---|
| **Site** | **Location** | **Cultivar** | **Sample** | **Collection Date** | **PCR** | **AmplifyRP®** |
| Site A | eastern Pender County | SHB 'Rebel' | Plant 1 | August 27th, 2021 | - | - |
| | | | Plant 2 | August 27th, 2021 | - | - |
| | | | Plant 3 | August 27th, 2021 | - | - |
| | | | Plant 4 | August 27th, 2021 | - | - |
| | | | Plant 5 | August 27th, 2021 | - | - |
| Site B | eastern Pender County | SHB 'Rebel' | Plant 1 | August 27th, 2021 | - | - |
| | | | Plant 2 | August 27th, 2021 | - | - |
| | | | Plant 3 | August 27th, 2021 | - | - |
| | | | Plant 4 | August 27th, 2021 | - | - |
| | | | Plant 5 | August 27th, 2021 | + | + |
| | | | Plant 5 | September 28th, 2021 | + | + |
| Site C | western Pender County | SHB 'Rebel' | Plant 1 | October 4th, 2021 | - | - |

**Evaluation of the susceptibility of southern highbush blueberry cultivars grown in North Carolina and Georgia to Xf.** As described above, two attempts to isolate Xf from infected North Carolina blueberries were unsuccessful during 2021; therefore, this objective has not yet been completed as of the time of this report. (*NOTE: Additional isolation attempts are anticipated during 2022. Once an isolate of Xf has been obtained from North Carolina blueberries, greenhouse experiments will be conducted to evaluate the susceptibility of SHB blueberry cultivars grown in North Carolina and Georgia to North Carolina Xf isolates.*)

**Discussion**

The presence of Xf in North Carolina blueberries has the potential to have a significant impact on the North Carolina and Southeast U.S. blueberry industries. As such, it is important to confirm whether the observed BLS symptoms in North Carolina are being caused by Xf and subsequently characterize the isolates causing bacterial leaf scorch on blueberries in North Carolina. Based upon the initial testing carried out as a part of these research efforts, the presence of Xf has been confirmed (for the first time) in at least

one SHB blueberry planting in North Carolina. Unexpectedly, the preliminary genetic characterization of the Xf isolate present in North Carolina blueberries suggests that this isolate is not closely related to Xf isolates found previously infecting blueberries in other southeastern U.S. states. The implications of this finding are not fully understood at this time. Genetic differences between Xf isolates can significantly impact important traits including host range and host susceptibility. Since the Xf isolate identified in North Carolina blueberries appears to be more closely related to other Xf subsp. *multiplex* isolates known to infect fruit, nut, ornamental, and shade trees (including olives, pecan, redbud, and red oak trees), this suggests the potential for this isolate to have originated from or to move into adjacent vegetation which could significantly impact potential management strategies for this pathogen. Additional testing for Xf both within and around the affected blueberry planting is anticipated during late 2021-2022. This is expected to provide additional information regarding the origins and spread of the Xf strain present in North Carolina blueberries. Furthermore, isolation of the strain from infected blueberry plants will enable subsequent greenhouse screening of blueberry cultivars grown in North Carolina and Georgia to determine their relative susceptibility to this newly identified strain.

**References**


Brannen, P. M., Chang, C.-J., Boland, R. T., Horton, D. L., and Krewer, G. W. 2016. Bacterial Leaf Scorch of Blueberry. University of Georgia Extension Circular 922. https://extension.uga.edu/publications/detail.html?number=C922&title=Bacterial%20Leaf%20Scorch%20of%20Blueberry.

Davis, M. J., French, W. J., and Schaad, N. W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 6:309-314.

Di Genova, D., Lewis, K.J., Oliver, J.E. 2020. Natural infection of southern highbush blueberry (*Vaccinium corymbosum* interspecific hybrids) by *Xylella fastidiosa* subsp. *fastidiosa*. Plant Disease. 104:2598-2605.

Doyle, J., and Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:11-15.

Ferguson, M. H., Clark, C. A., and Smith, B. J. 2017. Association of *Xylella fastidiosa* with yield loss and altered fruit quality in a naturally infected rabbiteye blueberry orchard. HortScience 52:1073-1079.

Galtier, N., Gouy, M., and Gautier, C. 1996. SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. Comput. Appl. Biosci. 12:543-548.

Gouy, M., Guindon, S., and Gascuel, O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27:221-224.

National Agricultural Statistics Service 2020. 2019 Blueberry Statistics. https://www.nass.usda.gov/Statistics_by_State/New_Jersey/Publications/Blueberry_Statistics/NJ%202019%20Blueberry%20Summary.pdf

Minsavage, G. V., Thompson, C. M., Hopkins, D. L., Leite, R. M. V. B. C., and Stall, R. E. 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84:456-461.

Oliver, J. E., Cobine, P. A., and De La Fuente, L. 2015. *Xylella fastidiosa* isolates from both subsp. multiplex and fastidiosa cause disease on southern highbush blueberry (Vaccinium sp.) under greenhouse conditions. Phytopathology 105:855-862.

Parker, J. K., Havird, J. C., and De La Fuente, L. 2012. Differentiation of *Xylella fastidiosa* strains via multilocus sequence analysis of environmentally mediated genes (MLSA-E). Appl. Environ. Microbiol. 78:1385-1396.

Waliullah, S., Hudson, O., Oliver, J.E., Brannen, P.M., Ji, P., & Ali, M.E. 2019. Comparative analysis of different molecular and serological methods for detection of *Xylella fastidiosa* in blueberry. PLoS ONE. 14(9):e0221903.