LOCATION, TRANSMISSION, AND IMPACT OF *XYLELLA FASTIDIOSA* IN SOUTHERN HIGHBUSH BLUEBERRIES

by

RENEE MARIE HOLLAND

(Under the Direction of Harald Scherm)

ABSTRACT

Bacterial leaf scorch is an emerging disease of southern highbush blueberry caused by the xylem-limited bacterium *Xylella fastidiosa*. This thesis investigated 1) the distribution of the pathogen in stem and root sections of naturally infected blueberry plants; 2) the level of disease transmission through vegetative propagation; and 3) the association between disease resistance and xylem hydraulic conductance in three blueberry cultivars. The pathogen was detected readily in xylem sap from lower stem sections of affected plants, and as disease severity increased, bacterial titer increased in sections of all age classes, particularly root sections. Disease transmission through softwood cuttings from symptomatic mother plants was low but non-negligible (5.1% average). Emerald, a field-resistant cultivar, had higher levels of xylem hydraulic conductance than the susceptible Star and FL 89-16. In diseased plants of FL 89-16, petiole conductance was reduced significantly, potentially explaining the high propensity for leaf scorch-related plant death in this cultivar.

INDEX WORDS: *Xylella fastidiosa*, bacterial leaf scorch, blueberry, *Vaccinium corymbosum*

LOCATION, TRANSMISSION, AND IMPACT OF *XYLELLA FASTIDIOSA* IN SOUTHERN HIGHBUSH BLUEBERRIES

by

RENEE MARIE HOLLAND

B.S., Clemson University, 2008

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

LOCATION, TRANSMISSION, AND IMPACT OF *XYLELLA FASTIDIOSA* IN SOUTHERN HIGHBUSH BLUEBERRIES

by

RENEE MARIE HOLLAND

Major Professor:	Harald Scherm
Committee:	Phillip M. Brannen Robert Teskey

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

DEDICATION

I would like to dedicate this work to my wonderful, supportive family. Thank you to my mother, father, and brother for always being there for me. Your teachings and love carry me through as I strive to reach my dreams.

ACKNOWLEDGEMENTS

For his scientific insight, patience, and encouragement, I would like to thank Dr. Harald Scherm. He has challenged me to acquire new technical skills and to share our meaningful research with the scientific community. He is making me into a quality scientist and for that, I am forever grateful. Thank you to my committee members Drs. Phil Brannen and Robert Teskey for such an open environment in which to discuss research and for your expertise and valuable feedback.

This work would not have been possible without the assistance, guidance, and support of many people. I would like to thank Dr. Rock Christiano for taking the time to train me on molecular techniques and teach me about plants. His mentorship contributed to the success of these projects, and for that, I am very appreciative. I would also like to thank the late Dr. Efrat Gamliel-Atinsky for her contributions to the project on *Xylella fastidiosa* distribution in stems and roots and for the pleasant memories and excellent example she left for the other lab-mates. Thanks are due to Drs. Sydney Everhart and Sara Thomas for being wonderful to work with and so supportive. Also, I would like to thank Dr. Jingjing Yin in Dr. Teskey's lab sincerely for teaching me how to use the high-pressure flow meter, as well as the wonderful people who have helped me with numerous assessments: Andy Shirley, Marie Vernaiz, Ryanna Zoellner, Patrick Savelle, and Justin Ballew. Also, big thanks are due to Charles Laster and Matt Seader for greenhouse support and horticulture farm manager Ryan McNeil for space and assistance at the farm.

Finally, I would like to thank my parents, friends, and fellow graduate students for their positive relationships and wonderful moral support. Thank you for the opportunity to pursue my dreams here at the University of Georgia.

TABLE OF CONTENTS

Page

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Importance and cultivation of blueberries. Blueberries are the economically most important fruit crop in Georgia, having an annual farm gate value of over $200 million (Wolfe and Shepherd 2012). A significant portion of this value (~30%) is generated by the early-maturing, high-value southern highbush blueberries (P. M. Brannen, *personal communication*). Interspecific hybrids between northern highbush (*Vaccinium corymbosum*) and native southern blueberry species (such as *V. darrowii*) (Lang 1993), southern highbush blueberries are grown most successfully in areas with sandy soils that are naturally high in organic matter, sandy soils with the addition of pine bark, or pine bark beds in high-density culture (Fonsah et al. 2006). The cultivation of southern highbush blueberries in the southeastern United States allows growers to take advantage of an early market window (late April to mid-May) that occurs after late-winter imports from South America and before northern highbush blueberries are harvested in North Carolina and New Jersey (Fonsah et al. 2006; Scherm and Krewer 2003). Along with a high demand for greater soil quality and agrichemical inputs, southern highbush blueberries in general are more susceptible to diseases than the native rabbiteye (*V. virgatum*) cultivars traditionally grown in the Southeast (Scherm and Krewer 2003).

Bacterial leaf scorch of blueberry. Bacterial leaf scorch, caused by the fastidious bacterium *Xylella fastidiosa*, is a lethal disease that affects several commonly grown cultivars of southern highbush blueberries such as FL 86-19 (V1), Star, and Rebel (Brannen et al. 2008; Chang et al. 2009). The disease is prevalent in the major blueberry-producing states in the

Southeast, including Georgia, Florida, and North Carolina. It is thought to be transmitted by xylem-feeding sharpshooter leafhoppers, primarily the glassy-winged sharpshooter, *Homalodisca vitripennis* (Tertuliano et al. 2010).

Bacterial leaf scorch was observed first in 2004 in the southern Georgia blueberry production region as a new disorder referred to as "yellow twig" or "yellow stem" affecting primarily cultivar FL 86-19. Initial symptoms noted were chlorosis and subsequent necrosis of older leaves. The necrosis begins at the margin of the leaf and then progresses throughout the entire leaf lamina. These leaves appear scorched, not unlike drought or fertilizer burn symptoms. Other symptoms include abnormally thin new shoots, fewer flower buds on these shoots, and the development of a yellow "skeleton-like" appearance when affected leaves drop. Death of the plant follows this leaf drop and typically occurs within 2 years after onset of symptoms. These symptoms on blueberry were similar to those seen on grape with Pierce's disease and on plum with leaf scald disease, both caused by *X. fastidiosa*. The similarity of these diseases prompted enzyme-linked immunosorbent assay (ELISA) tests and isolations targeting the bacterium to be conducted. In 2006, ELISA testing of two leaf and two root samples from symptomatic blueberry plants proved to be positive for *X. fastidiosa* (Chang et al. 2009). Isolations were unsuccessful from the leaf samples, but the root samples provided positive isolations. Koch's postulates were fulfilled when re-isolations were successful from inoculated symptomatic plants (4 out of 8 southern highbush Southern Belle, 2 out of 6 rabbiteye Powderblue, and 8 out of 8 southern highbush FL86-19) (Chang et al. 2009). Rabbiteye cultivar Premier remained asymptomatic (0 out of 8 inoculated plants).

Isolation and detection of *X. fastidiosa*. When Koch's postulates were completed for bacterial leaf scorch on blueberry as described above (Chang et al. 2009), successful isolation

was accomplished only from root tissues; however, ELISA tests were positive from both aboveground and root tissues. *Xylella fastidiosa* has also been detected via microscope visualization in the roots of peach trees asymptomatic for phony peach disease with 14.3 to 85.7% of roots testing positive; typically 100% of roots positive in symptomatic trees (Aldrich et al. 1992). In muscadine grape (*Vitis rotundifolia*), American bunch grape (*V. labrusca*), and French grape hybrids (*V. vinifera* × *V. labrusca*), *X. fastidiosa* has been isolated from the petioles of Pierce's disease-symptomatic branches (Chang et al. 1990). Using ELISA, the bacterium was detected in leaf and stem samples in June from asymptomatic American elm (*Ulmus americana*) and sycamore (*Platanus occidentalis*) that had leaf scorch symptoms in the canopy during the previous September (Sherald and Lei 1991). The pathogen has also been detected in symptomatic, but not in asymptomatic, extracts from red maple (*Acer rubrum*), red oak (*Quercus rubra*), and red mulberry (*Morus rubra*) (Sherald and Lei 1991). In summary, movement and concentrations of *X. fastidiosa* in the xylem of different host tissues (e.g., petioles compared with stems or roots) appears to be host species-specific.

Spread and systemic movement of *X. fastidiosa*. *Xylella fastidiosa*, being xylem-limited, is vectored mainly by xylem-feeding leafhoppers (Mizell et al. 2003; Costa et al. 2006), of which the glassy-winged sharpshooter (GWSS) is most prevalent in southern highbush blueberry plantings (Tertuliano et al. 2010). The GWSS is native to the southeastern United States and is the largest leafhopper in Georgia, measuring 15 to 20 mm in length at adult maturity. Piercing, sucking insects, they feed on the xylem of more than 100 plant species (Turner and Pollard 1959; Blua et al. 1999; Purcell and Saunders 1999; Conklin and Mizell 2002; Almeida and Purcell 2003; Hoddle et al. 2003; Costa et al. 2006). The bacterium colonizes the foregut of the insect. When acquired by an adult, it is persistent through the life of the insect.

Nymphs are not efficient in transmitting *X. fastidiosa* because the bacterium is lost during molting. In blueberry, southern highbush cultivars Emerald, Star, and FL 86-19, which are field-resistant, moderately susceptible, and highly susceptible to bacterial leaf scorch, respectively, were tested for their attractiveness to the GWSS. In a 5-day choice test, where field-collected GWSS adults were caged with potted plants of all three cultivars, the insects settled significantly more frequently on field-resistant cultivar Emerald compared with moderately susceptible Star and highly susceptible FL 86-19 (Tertuliano et al. 2012). Cumulatively, over the 5-day test, 52.7, 25.5, and 17.7% of adults settled on Emerald, Star, and FL 86-19, respectively. In a no-choice test, where field-collected GWSS adults were caged with potted plants of each cultivar individually, overall host plant acceptance was similar for all three cultivars with 89.3 to 92.1% of adults settling on the plants (Tertuliano et al. 2012). In the no-choice test, GWSS adults settled significantly more frequently on the stems (79.3%) vs. leaves (11.2%). They also preferred the middle portion of the shoot (56.9%) compared with the upper and lower shoot portions, 7.8% and 14.7%, respectively (Tertuliano et al. 2012). Overall, the study showed that 1) host field resistance is not related to plant attractiveness to the GWSS, and 2) GWSS settle and feed most frequently on the middle portions (current and previous season's growth) of blueberry plants.

There is currently no information on the location (different tissue types and ages) and concentrations of *X. fastidiosa* in blueberry plants affected by bacterial leaf scorch, indicating an important knowledge gap. In hosts such as alfalfa, citrus, grape, and peach, *X. fastidiosa* can spread through manual grafting and natural root grafts in the field (He et al. 2000). This documents the systemic nature of the bacterium. As *X. fastidiosa* replicates, it moves up and down the xylem. In citrus, for example, downward movement of *X. fastidiosa* holds true for the case of top grafting with budsticks and subsequent translocation of the bacterium to the root

system of citrus rootstocks (Almeida et al. 2001). There is conflicting data on whether or not bacterial multiplication needs to be at high concentrations for systemic movement to occur (Hill and Purcell 1995; Almeida et al. 2001). Through bacterial aggregate formation, *X. fastidiosa* restricts water and nutrient movement in the plant, resulting in the onset of symptoms (Hopkins 1989; Mizell et al. 2003; Li et al. 2007). For example, bacterial aggregates and tyloses have been found in tracheary elements of diseased vs. healthy petioles of grape (Tyson et al. 1985). Physical blockage of xylem vessels is thought to be a major (but not the sole) contributing factor to leaf scorch development and plant death (Hopkins 1989; Mizell et al. 2003).

The bacterium is capable of moving through xylem conduits without crossing pit membranes from stem or petiole into leaf veins (Thorne et al. 2006). Chatelet et al. (2006) concluded that open vessels of primary xylem in grape provided long-distance pathways without impediment from stem to leaves, allowing rapid spread of *X. fastidiosa in planta*.

Management of bacterial leaf scorch. Rates of new infections in southern Georgia have decreased during the past 2 years (H. Scherm and P. M. Brannen, *personal communication*), possibly because of unusually cool winters that could have delayed emergence of vector populations, altered the phenology of alternative hosts of the vector or bacterium, and/or cured young bacterial infections that occurred late in the season (Ledbetter et al. 2009; Meyer and Kirkpatrick 2011; Feil and Purcell 2001; Lieth et al. 2011). With a return to more normal winter weather, it is anticipated that active spread of bacterial leaf scorch will resume. Little is known about the epidemiology of bacterial leaf scorch in blueberry, and there are currently no effective management options for the disease in existing blueberry plantings. For new plantings, choosing cultivars with lower disease susceptibility, such as Emerald (Brannen et al. 2008), is the best

option. The mechanism behind cultivar resistance or tolerance in southern highbush blueberry is currently unknown.

In relation to the systemic nature of the bacterium, a question that is frequently asked by blueberry producers is whether early-stage infections in affected plantings can be pruned out by removing shoots that are just starting to show symptoms. A related question is whether more seriously affected plantings can be cured by flail-mowing plants to ground level at the end of the season when symptoms are most apparent. Whether or not these strategies will be successful depends on where the bacterium is located in plants of different severity classes.

Pruning has been used previously for management of diseases caused by *X. fastidiosa*. For Pierce's disease of grape, movement and bacterial location within the plant have been investigated, whereby the date of inoculation affected within-plant movement of the bacterium in the field (Feil et al. 2003). Vines that were inoculated earlier in the season (April to May) developed more extensive and severe Pierce's disease symptoms than those inoculated later in the season (June to August) (Feil et al. 2003). This affected the percentage of vine recovery after the winter, such that only 54% of vines from the earlier inoculated batch recovered over the winter (i.e., became disease-free) compared with 88% of vines from the later inoculated batch (Feil et al. 2003). Of the recovered vines in this study, half had noticeably shorter new canes on the spur from winter pruning. This suggests that pruning in late winter may have removed all infected portions of the canes from some vines, leaving the vine uninfected (Feil et al. 2003).

In coffee, where *X. fastidiosa* causes a bacterial leaf scorch disease, pruning procedures of varying intensity were evaluated for control of disease incidence in two commercial coffee cultivars (Queiroz-Voltan et al. 2006). The three pruning methods assessed were traditional, skeleton cut, and trunking (Queiroz-Voltan et al. 2006). In traditional pruning, plants were cut

0.5 m down from the apex of the plant. In skeleton cut, 0.5 m of the branches from the main trunk of the plant were kept, whereas in trunking, the plants were cut 0.5 m from the ground. When plants of a severely affected cultivar underwent dramatic pruning such as skeleton cut or trunking, the proportion of stems with xylem vessel obstruction was lowered (Queiroz-Voltan et al. 2006). This indicates that drastic pruning could be advantageous for the control of *X. fastidiosa* when disease incidence is high.

In other *X. fastidiosa* pathosystems, such as those involving shade trees, it is unknown whether pruning is effective in stopping the spread of disease within tree populations (Gould and Lashomb 2007). In blueberries, selective pruning, hedging, or more aggressive flail-mowing of plants could be implemented in an attempt to cure infected plants from *X. fastidiosa*. Whether such strategies will be effective depends on where in the plant the bacterium is located as symptoms become first apparent. If *X. fastidiosa* has already moved into the roots at an early stage of infection, even aggressive mowing would be ineffective in removing infections. Thus, there is a need to determine the presence or absence in tissue types of different age of the bacterium in plants at varying levels of disease severity.

Vegetative propagation and disease transmission. Given the systemic nature of *X. fastidiosa* infections, it is likely that disease transmission could occur through vegetative propagation. In grape, hardwood cuttings from plants infected by *X. fastidiosa* proved to be less successful in rooting than cuttings from asymptomatic plants (Krawitzky et al. 2008). In peach and pecan, grafting infected (hardwood) scions onto healthy rootstocks resulted in infected trees, with variable transmission rates, usually between 20 and 50% (Hutchins et al. 1953; Sanderlin and Melanson 2008). Hot water treatment of pecan scions lowered transmission significantly (Sanderlin and Melanson 2008). It is not clear if and how these results with hardwood cuttings

can be extrapolated to blueberry, where propagation occurs via softwood cuttings (Haralson 2009), pointing toward another important knowledge gap.

Host resistance to *X. fastidiosa* and its relation to xylem water flow. Populations of *X. fastidiosa* increased rapidly in petioles of susceptible *V. vinifera* but only gradually in tolerant and resistant *V. rotundifolia* (Fry and Milholland 1990). Differences in susceptibility to diseases caused by *X. fastidiosa* exist not only among plant species, but also at the cultivar level. For example, an evaluation of almond cultivar susceptibility to almond leaf scorch, caused by *X. fastidiosa*, using disease ratings and an assessment of movement and symptom development within inoculated trees showed that cultivars Sonora and Solano were the most susceptible (Cao et al. 2011). Mission, Price, Peerless, and Ne Plus Ultra were intermediate in susceptibility, and Carmel, Padre, Butte, and Thompson were the least susceptible for maintaining populations of the pathogen over the winter (Cao et al. 2011).

There is limited information and conflicting data with regard to disease resistance and xylem structure and function. In grape, Fritschi et al. (2008) concluded that occlusion of xylem conduits did not appear to be the mechanism of resistance to Pierce's disease. Furthermore, there was a low number of infested vessels in the cross-sections of grape leaf petioles affected by the disease (Mollenhauer and Hopkins 1976). Mollenhauer and Hopkins (1976) indicated that the morphology of tracheary cells differed significantly in Pierce's disease-infected susceptible and resistant grape species, but according to Chatelet et al. (2011), grape varieties examined showed no differences in stem or petiole vascular anatomy.

Less conflicting results may be obtained when xylem function (i.e., the effect on water flow), as opposed to xylem anatomy and structure, is investigated in relation to disease development and host resistance. Water flow studies have been conducted in grapes affected by

Pierce's disease. Leaf and petiole hydraulic conductance (a measure of xylem water flow) was measured in cv. Chardonnay, showing that hydraulic conductance in these tissues decreased with increasing disease severity (Choat et al. 2009). Furthermore, water stress was shown to increase susceptibility to Pierce's disease based on a positive correlation between the concentration of *X. fastidiosa* and symptom severity in plants that were water-deficient (Choat et al. 2009). In *Quercus palustris* and *Q. rubra*, hydraulic conductivity of petioles infected with *X. fastidiosa* decreased significantly when compared with petioles of healthy trees; as the season progressed and symptoms appeared, hydraulic conductivity decreased rapidly to zero (McElrone et al. 2008). Hopkins (1989) demonstrated that stem hydraulic conductivity was significantly lower in elm leaf scorch and phony peach-affected trees. Similarly, node and petiole xylem flow resistance was 60 to 200 times greater in grapevines affected by Pierce's disease (Hopkins 1989). No such information on xylem function in relation to disease severity or cultivar resistance is currently available for bacterial leaf scorch on blueberry.

Hydraulic conductance as a measure of xylem water flow. Hydraulic conductance (K_h) measures the efficiency of bulk flow through a material and is reported as the flow rate per unit pressure driving force (in units of kg s^{-1} MPa^{-1}). A whole shoot or root system can be measured, as well as stem sections or fine roots. Hydraulic conductance can be standardized to cross-sectional sapwood area for sapwood-specific conductance (K_{sa}) or to the total leaf area that the measured segment supports for leaf-specific conductance (K_{lsp}). Hydraulic conductance or conductivity is often referenced in the literature, and the difference between the two measurements is that conductivity takes into account the length of the measured segment.

There are several methods for measuring hydraulic conductance, including the high-pressure method, the evaporative flux method, the vacuum pump method, and the centrifugal

method. The high-pressure method measures the flow rate under high pressure (up to 600 psi = 4.14 MPa). These measurements can be taken under transient or quasi-steady state pressure conditions. The hydraulic conductance is the slope of the flow rate vs. change in pressure. In the evaporative flux method, a leaf is placed in an environment favorable for transpiration, water uptake can be measured using a balance, and leaf water potential, which represents the driving force, is determined by using a pressure bomb (Sack et al. 2002). Leaf hydraulic conductance can be calculated as flow rate/balancing pressure. The vacuum pump method involves pulling water through the sample (i.e., stem, leaf, or root) using a vacuum pump while measuring flow rates (Sack et al. 2002). The principle behind the centrifugal method is to spin a xylem segment by its center and generate negative pressures in the sample and a positive hydrostatic pressure difference across the sample by using centrifugal force (Cochard 2002). The ends of the sample are to be immersed in water and under a pressure difference, the water will flow through the sample, allowing for the calculation of hydraulic conductance as the flow rate divided by the change in pressure (Cochard 2002). Among these various approaches, the high-pressure method is the most flexible for collecting hydraulic conductance data on stems with varying diameters, petioles, and whole shoots.

Several precautions must be taken to obtain meaningful hydraulic conductivity data. In order to minimize air bubbles, it is recommended to use deionized water that has been degassed (Espino and Schenk 2011). When taking a measurement, hydraulic conductance can decrease with time, and this can begin within the first few minutes (Sperry et al. 1988). Possible explanations include air coming out of the fluid going through the sample and causing blockage, swelling of tissue around the vessels causing a narrowing of the vessel diameter, or swelling of intervascular pit membranes, and particulate clogging (Sperry et al. 1988). Blockage can occur

naturally as well as through emboli from water stress or winter freezing of xylem sap (Sperry et al. 1988).

It is also critical to make sure the initial segment cut from the plant is long enough so that the vessels that are cut on the ends do not extend into the central portion of the segment which will be used for measurement (Sperry et al. 1988). This cuts down on air bubbles in the tested segment. When collecting samples in the field, samples must be wrapped in plastic bags to prevent drying. In the laboratory, the samples can be soaked for 15 min (Sperry et al. 1988).

In angiosperms, wood anatomy and density determine hydraulic conductivity. When assessing sapwood-specific stem conductivity (K_{sa}) for 3005 woody species, variation in this measurement was due to the size to number ratio of the mean vessel lumen area to the number per unit area (Zanne et al. 2010). It was concluded that the higher the size to number ratio, the faster K_{sa} would be.

In blueberries, hydraulic conductivity has been tested, using the pressure chamber method, in the roots of flooded and unflooded northern highbush cultivar Bluecrop subjected to increased CO_2 levels and reduced O_2 levels (Davies and Flore 1986). Results showed that root conductivity was lower and the effect of increasing CO_2 levels on carbon assimilation less for flooded than unflooded plants after short (1-2 days), intermediate (10-14 days) and long-term (35-40 days) flooding (Davies and Flore 1986). Little is known about hydraulic conductance or conductivity in relation to disease in blueberries.

PROJECT OBJECTIVES AND HYPOTHESES

The overall goal of this thesis is to better understand disease transmission and host resistance in the *X. fastidiosa* – blueberry pathosystem. Aspects being investigated include

within-plant pathogen distribution in naturally infected plants, disease transmission through vegetative propagation, and the relationship between disease resistance and xylem hydraulic conductance. The study has the following specific objectives:

1. Evaluate the distribution of *X. fastidiosa* in stem and root tissues of different age classes in relation to natural disease severity.
2. Determine transmission rates of *X. fastidiosa* via softwood cuttings from infected mother plants, and assess growth and development of daughter plants derived from infected mother plants.
3. Examine the relationship between xylem hydraulic conductance and field resistance to bacterial leaf scorch in southern highbush blueberry cultivars.

Hypotheses:

1. Plant-level disease severity affects how far the bacterium has advanced toward the base of naturally infected plants.
2. There is no transmission of *X. fastidiosa* from symptomatic mother plants via asymptomatic cuttings.
3. Field-resistant southern highbush cultivars have greater xylem hydraulic conductance in stem and petioles, and – when infected – the decrease in hydraulic conductance with increasing disease severity is lower than in susceptible cultivars.

LITERATURE CITED

Aldrich, J. H., Gould, A. B., and Martin, F. G. 1992. Distribution of *Xylella fastidiosa* within roots of peach. Plant Dis. 76: 885-888.

Almeida, R. P. P., Pereira, E. F., Purcell, A. H., and Lopes, J. R. S. 2001. Multiplication and movement of a citrus strain of *Xylella fastidiosa* within sweet orange. Plant Dis. 85: 382-386.

Almeida, R. P. P., and Purcell, A. H. 2003. Transmission of *Xylella fastidiosa* to grapevines by *Homalodisca coagulata* (Hemiptera: Cicadellidae). J. Econ. Entomol. 96: 264-271.

Blua, M. J., Phillips, P. A., and Redak, R. A. 1999. A new sharpshooter threatens both crops and ornamentals. California Agriculture 53(2): 22-25.

Brannen, P. M., Scherm, H., and Chang, C.J. 2008. Survey of cultivar differences in bacterial leaf scorch incidence among southern highbush blueberries. Dixie Blueberry News 8: 6-7.

Cao, T., Connell, J. H., Wilhelm, M., and Kirkpatrick, B. C. 2011. Influence of inoculation date on the colonization of *Xylella fastidiosa* and the persistence of almond leaf scorch disease among almond cultivars. Plant Dis. 95: 158-165.

Chang, C. J., Donaldson, R., Brannen, P., Krewer, G., and Boland, R. 2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. HortScience 44: 413-417.

Chang, C. J., Robacker, C. D., and Lane, R. P. 1990. Further evidence for the isolation of *Xylella fastidiosa* on nutrient agar from grapevines showing Pierce's disease symptoms. Can. J. Plant Pathol. 12: 405-408.

Chatelet, D. S., Matthews, M. A., and Rost, T. L. 2006. Xylem structure and connectivity in grapevine (*Vitis vinifera*) shoots provides a passive mechanism for the spread of bacteria in grape plants. Ann. Bot. 98: 483-494.

Chatelet, D. S., Wistrom, C. M., Purcell, A. H., Rost, T. L., and Matthews, M. A. 2011. Xylem structure of four grape varieties and 12 alternative hosts to the xylem-limited bacterium *Xylella fastidiosa*. Ann. Bot. 108: 73-85.

Choat, B., Gambetta, G. A., Wada, H., Shackel, K. A., and Matthews, M. A. 2009. The effects of Pierce's disease on leaf and petiole hydraulic conductance in *Vitis vinifera* cv. Chardonnay. Physiol. Plantarum 136: 384-394.

Cochard, H. 2002. A technique for measuring xylem hydraulic conductance under high negative pressures. Plant Cell Environ. 25: 815-819.

Conklin, T. and Mizell, R. F. 2002. Glassy-winged sharpshooter, *Homalodisca vitripennis* (=*coagulata*) (Germar) (Insecta: Hemiptera: Cicadellidae: Cicadellinae). EENY-274. University of Florida IFAS Extension, Gainesville, FL.

Costa, H. S., Guzman, A., Hernandez-Martinez, R., Gispert, C., and Cooksey, D. A. 2006. Detection and differentiation of *Xylella fastidiosa* strains acquired and retained by glassy-winged sharpshooters (Hemiptera: Cicadellidae) using a mixture of strain-specific primer sets. J. Econ. Entomol. 99(4): 1058-1064.

Davies, F. S., and Flore, J. A. 1986. Flooding, gas exchange and hydraulic root conductivity of highbush blueberry. Physiol. Plantarum 67(4): 545-551.

Espino, S., and Schenk, H. J. 2011. Mind the bubbles: achieving stable measurements of maximum hydraulic conductivity through woody plant samples. J. Exp. Bot. 62: 1119-1132.

Feil, H., Feil, W. S., and Purcell, A. H. 2003. Effects of date of inoculation on the within-plant movement of *Xylella fastidiosa* and persistence of Pierce's disease within field grapevines. Phytopathology 93: 244-251.

Feil, H., and Purcell, A. H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Dis. 85: 1230-1234.

Fonsah, E. G., Krewer, G., Harrison, K., and Bruorton, M. 2006. Economic analysis of producing southern highbush blueberries in Georgia. Bulletin 1303, College of Agricultural and Environmental Sciences, Univ. of Georgia, Athens.

Fritschi, F. B., Lin, H., and Walker, M. A. 2008. Scanning electron microscopy reveals different response pattern of four *Vitis* genotypes to *Xylella fastidiosa* infection. Plant Dis. 92: 276-286.

Fry, S. M., and Milholland, R. D. 1990. Response of resistant, tolerant, and susceptible grapevine tissues to invasion by the Pierce's disease bacterium, *Xylella fastidiosa*. Phytopathology 80: 66-69.

Gould, A. B., and Lashomb, J. H. 2007. Bacterial leaf scorch (BLS) of shade trees. Plant Health Instructor. DOI: 10.1094/PHI-I-2007-0403-07.

Haralson, J. C. 2009. Pathogens associated with blueberry cutting failure in south Georgia nurseries and their control. M. S. thesis. University of Georgia, Athens.

He, C. X., Li, W. B., Ayres, A. J., Hartung, J. S., Miranda, V. S., and Teixeira, D. C. 2000. Distribution of *Xylella fastidiosa* in citrus rootstocks and transmission of citrus variegated chlorosis between sweet orange plants through natural root grafts. Plant Dis. 84: 622-626.

Hill, B. L., and Purcell, A. H. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85: 1368-1372.

Hoddle, M. S., Triapitsyn, S. V., and Morgan, D. J. W. 2003. Distribution and plant association records for *Homalodisca coagulate* (Hemiptera: Cicadellidae) in Florida. Florida Entomologist 86: 89-91.

Hopkins, D. L. 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27: 271-290.

Hutchins, L. M., Cochran, L. C., Turner, W. F., and Weinberger, J. H. 1953. Transmission of phony disease virus from tops of certain affected peach and plum trees. Phytopathology 43: 691–696.

Krawitzky, M., Montague, T., and Hellman, E. 2008. Propagation success of grapevines infected with *Xylella fastidiosa*. Page 41 in: Proceedings of the 2nd Annual National Viticulture Research Conference. University of California, Davis.

Lang, G. A. 1993. Southern highbush blueberries: Physiological and cultural factors important for optimal cropping of these complex hybrids. Acta Hortic. 346: 72-80.

Ledbetter, C. A., Chen, J., Livingston, S., and Groves, R. L. 2009. Winter curing of *Prunus dulcis* cv 'Butte,' *P. webbii* and their interspecifc hybrid in response to *Xylella fastidiosa* infections. Euphytica 169: 113-122.

Li, Y., Hao, G., Galvani, C. D., Meng, Y., De La Fuente, L., Hoch, H. C., and Burr, T. J. 2007. Type I and type IV pili of *Xylella fastidiosa* affect twitching motility, biofilm formation and cell-cell aggregation. Microbiology 153: 719-726.

Lieth, J. H., Meyer, M. M., Yeo, K.-H., and Kirkpatrick, B. C. 2011. Modeling cold curing of Pierce's disease in *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' grapevines in California. Phytopathology 101: 1492-1500.

McElrone, A., Jackson, S., and Habdas, P. 2008. Hydraulic disruption and passive migration by a bacterial pathogen in oak tree xylem. J. Exp. Bot. 59: 2649-2657.

Meyer, M. M., and Kirkpatrick, B. C. 2011. Exogenous applications of abscisic acid increase curing of Pierce's disease-affected grapevines growing in pots. Plant Dis. 95: 173-177.

Mizell, R. F., Anderson, P. C., Tipping, C., and Brodbeck, B. 2003. *Xylella fastidiosa* diseases and their leafhopper vectors. ENY-683 (IN174). University of Florida IFAS Extension, Gainesville, FL.

Mollenhauer, H. H., and Hopkins, D. L. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. Physiol. Plant Pathol. 9: 95-100.

Purcell, A. H., and Saunders, S. R. 1999. Glassy-winged sharpshooters expected to increase plant disease. California Agriculture 53(2): 26-27.

Queiroz-Voltan, R. B., Cabral, L. P., Paradela Filho, O., and Fazuoli, L. C. 2006. Prune efficiency in the control of *Xylella fastidiosa* in coffee trees. Bragantia 65(3): 433-440.

Sack, L., Melcher, P. J., Zwieniecki, M. A., and Holbrook, N. M. 2002. The hydraulic conductance of the angiosperm leaf lamina: a comparison of three measurement methods. J. Exp. Bot. 53: 2177-2184.

Sanderlin, R. S. and Melanson, R. A. 2008. Reduction of *Xylella fastidiosa* transmission through pecan scion wood by hot-water treatment. Plant Dis. 92: 1124-1126.

Scherm, H., and Krewer, G. 2003. Blueberry production in Georgia: Historical overview and recent trends. Small Fruits Rev. 2: 83-91.

Sherald, J. L., and Lei, J. D. 1991. Evaluation of a rapid ELISA test kit for detection of *Xylella fastidiosa* in landscape trees. Plant Dis. 75: 200-203.

Sperry, J., Donnelly, J., and Tyree, M. 1988. A method for measuring hydraulic conductivity and embolism in xylem. Plant Cell Environ. 11: 35-40.

Tertuliano, M., Scherm, H., and Horton, D. 2010. Population dynamics and feeding preference of the glassy-winged sharpshooter [*Homalodisca vitripennis* (Germar)] (Hemiptera:

Cicadellidae), vector of *Xylella fastidiosa*, on southern highbush blueberry. (Abstr.) Entomological Society of America 58th Annual Meeting, San Diego, CA.

Tertuliano, M., Srinivasan, R., and Scherm, H. 2012. Settling behavior of the glassy-winged sharpshooter, *Homalodisca vitripennis*, vector of *Xylella fastidiosa*, on southern highbush blueberry cultivars. Entomol. Exp. Appl. 143: 67-73.

Thorne, E. T., Young, B. M., Young, G. M., Stevenson, J. F. Labavitch, J. M., Matthews, M. A., and Rost, T. L. 2006. The structure of xylem vessels in grapevine (*Vitaceae*) and a possible passive mechanism for the systemic spread of bacterial disease. Am. J. Bot. 93: 497-504.

Turner, W. F., and Pollard, H. N. 1959. Insect transmission of phony peach disease. USDA Technical Bulletin 1183.

Tyson, G. E., Stojanovic, B. J., Kuklinski, R. F., DiVittorio, T. J., and Sullivan, M. L. 1985. Scanning electron microscopy of Pierce's disease bacterium in petiolar xylem of grape leaves. Phytopathology 75: 264-269.

Wolfe, K., and Shepherd, T. 2012. 2011 Georgia Farm Gate Value Report. AR-12-09, Center for Agribusiness and Economic Development, University of Georgia, Athens.

Zanne, A. E., Westoby, M., Falster, D. S., Ackerly, D. D., Loarie, S. R., Arnold, S. E. J., and Coomes, D. A. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. Am. J. Bot. 97: 207-215.

CHAPTER 2

DISTRIBUTION OF *XYLELLA FASTIDIOSA* IN BLUEBERRY STEM AND ROOT SECTIONS IN RELATION TO DISEASE SEVERITY IN THE FIELD

Holland, R. M., Christiano, R. S. C., Gamliel-Atinsky, E., and Scherm, H. 2013. To be submitted to *Plant Disease*.

Distribution of *Xylella fastidiosa* in blueberry stem and root sections in relation to disease severity in the field

R. M. Holland, R. S. C. Christiano, E. Gamliel-Atinsky, and H. Scherm

Department of Plant Pathology, University of Georgia, Athens 30602

ABSTRACT

Xylella fastidiosa causes bacterial leaf scorch, a new disease of southern highbush blueberry in the southeastern United States. Infections occlude the xylem of affected plants, causing drought-like symptoms and eventually plant death. To assess the likelihood of mitigation of bacterial leaf scorch through cultural practices such as pruning or hedging of affected plants, we determined the localization and population density of *X. fastidiosa* in naturally infected blueberry plants with varying levels of bacterial leaf scorch severity. Stem segments from the current season's growth to the base of the plant, as well as root segments, were sampled from plants that were either asymptomatic or had light, moderate, or severe symptoms in three plantings affected by the disease. Xylem sap was extracted from each segment and population densities of *X. fastidiosa* were determined using real-time quantitative PCR with species-specific primers. Detection frequencies were lowest (but non-zero) in xylem sap from asymptomatic plants and highest in plants with severe symptoms. In asymptomatic plants, detection was generally least frequent (0 to 20.0%) in top and root sections, and highest (4.6 to 55.6%) in middle and base stem sections. As disease severity increased, detection frequencies in roots increased to >80% in two plantings and to 60% in the third planting. Overall, detection frequencies were highest (>80%) in middle and base stem sections of plants from the moderate

and severe disease classes. The lowest bacterial titers (averaging 0 to 2.1×10^1 colony-forming units/50 µl of sap) were observed in top and root sections of asymptomatic plants, whereas the highest titers (generally between 10^4 and 10^5 colony-forming units/50 µl sap) were obtained from middle, base, and root sections of plants from the moderate and severe classes. The presence of the pathogen in middle and base stem sections at low disease severity indicates rapid distribution of *X. fastidiosa* throughout the plant. Since the pathogen accumulates in the roots at moderate and high disease severity levels, management strategies such as pruning and mowing are unlikely to be effective in curing affected plants from bacterial leaf scorch.

INTRODUCTION

Bacterial leaf scorch, caused by the xylem-limited bacterium *Xylella fastidiosa*, is a lethal disease of susceptible southern highbush blueberry cultivars such as FL 86-19, Star, and Rebel (Brannen et al. 2008; Chang et al. 2009). Symptoms include marginal necrosis of the leaves, yellowing of stems, dieback, and eventually plant death. The bacterium is vectored by xylem-feeding leafhoppers, of which the glassy-winged sharpshooter, *Homalodisca vitripennis*, is most prevalent in blueberry plantings in southern Georgia (Tertuliano et al. 2010).

Field surveys conducted in 2007 indicated that bacterial leaf scorch was widespread and damaging on susceptible cultivars in the Georgia blueberry belt (Brannen et al. 2008). Currently, management options are limited to the use of resistant cultivars (such as Emerald) in new plantings and application of insecticides for control of the vector in existing plantings. The latter approach is hampered by concerns about application timing, limited efficacy, and potential for overuse of insecticides. This prompted the investigation of cultural practices for managing the

disease. One such practice may be the application of pruning to remove infected tissues and to slow or prevent disease increase and plant death.

Pruning has been used previously for managing diseases caused by *X. fastidiosa*. For Pierce's disease of grape, the date of inoculation affects persistence of the bacterium in the field (Feil et al. 2003). In California, grapevines inoculated earlier in the season (April to May) developed more extensive and severe Pierce's disease symptoms than those inoculated later in the season (June to August) (Feil et al. 2003). This affected the percentage of vine recovery after the winter, such that only 54% of vines recovered from the earlier inoculated batch compared with 88% of vines from the later inoculated batch (Feil et al. 2003). Of the recovered vines, half had noticeably shorter new canes from winter pruning. The authors concluded that mitigation of the disease in these vines was likely associated with late-winter pruning, which may have removed all infected portions of affected canes thereby leaving the vine uninfected (Feil et al. 2003).

In coffee, where *X. fastidiosa* causes a bacterial leaf scorch disease, pruning procedures of varying intensity were evaluated for control of disease incidence in two commercial cultivars (Queiroz-Voltan et al. 2006). The three pruning methods assessed were traditional pruning, skeleton cut, and trunking (Queiroz-Voltan et al. 2006). In traditional pruning, plants were cut 0.5 m down from the apex of the plant. In skeleton cut, 0.5 m of the branches from the main trunk of the plant were kept, whereas in trunking, the plants were cut 0.5 m from the ground. When plants of a severely affected cultivar underwent dramatic pruning such as skeleton cut or trunking, the proportion of stems with xylem vessel obstruction was lowered (Queiroz-Voltan et al. 2006). This indicates that drastic pruning could be advantageous for the control of *X. fastidiosa* when disease incidence is high.

In blueberry, selective pruning, mechanical hedging of tops (Williamson et al. 2004; Krewer et al. 2004), or more aggressive flail-mowing of plants close to ground level could be implemented in an attempt to cure infected plants from *X. fastidiosa*. Whether such strategies will be effective depends on where in the plant the bacterium is located as symptoms start to develop. The pathogen is thought to be inoculated into current-season growth, given that the glassy-winged sharpshooter, the most prevalent potential vector of *X. fastidiosa* in Georgia blueberry plantings, settles and feeds primarily on new growth (Tertuliano et al. 2012). In advanced stages of infection, the pathogen can be isolated from roots of affected southern highbush blueberry plants (Chang et al. 2009). If *X. fastidiosa* moves rapidly into the roots at early stages of infection, even aggressive pruning would be ineffective in curing affected plants. Thus, it is important to determine the presence or absence of the bacterium in tissue sections of different ages and in plants with varying levels of disease severity. Based on these considerations, the objective of this study was to evaluate the distribution of *X. fastidiosa* in stem and root segments of different age classes in relation to natural disease severity in southern highbush blueberry.

MATERIALS AND METHODS

Sample collection. Three southern highbush blueberry plantings affected by bacterial leaf scorch were sampled for stem and root sections. Cultivars FL 86-19 and Bluecrisp (both highly susceptible) were utilized in 2009, whereas Star (moderately susceptible) was sampled in 2010. In each field, 10 asymptomatic plants as well as 10 plants each with light, moderate, or severe visual symptoms of bacterial leaf scorch (Fig. 2.1) were selected in September or early October when symptoms are generally most pronounced (total of 40 plants per field, except for

cultivar Star where the light disease severity class was not included). Within each plant, a representative main stem was selected and dissected into sections (20 to 35 cm long) corresponding to different age classes, i.e., current year's fall growth, current year's spring growth, last year's growth, and lower trunk sections of different diameter classes for a total of up to six aboveground stem sections per plant (Fig. 2.2). In addition, root segments from the same sector within the plant where the stem originated were sampled. Cuts were made on asymptomatic plants before sampling from symptomatic plants. In addition, the pruning shears used to make these cuts were surface-disinfested with disinfecting wipes (active ingredients: 0.184% n-alkyl dimethyl benzyl ammonium chloride and 0.184% n-alkyl dimethyl ethylbenzyl ammonium chloride) (Clorox, Oakland, CA) between cuts. Samples were placed into plastic sample bags and returned to the laboratory on ice.

Xylem sap extraction and pathogen detection. Segments were trimmed to ~15 to 20 cm length with an additional fresh cut made to each side of the stem section, and xylem sap was extracted from each stem or root section using mechanical pressure applied with a vise. A micropipette was used to retrieve the extruding xylem sap, which was then collected into sterile microcentrifuge tubes and stored at −80^{o}C. To determine bacterial titer in the sap, total DNA was extracted from typically 50 µl of thawed sap using the PowerPlant Pro DNA isolation kit (MO BIO, Carlsbad, CA) and subjected to real-time quantitative PCR with species-specific primers EFTu_3 – Fwd 5- TGA GGT GGA AAT TGT TGG CAT T -3 and EFTu_3 – Rev 5- AGC CTG ACC TTG ATC CAA TAA -3. PCR reaction volume was a total of 12 µl which included 3 µl template DNA, 6 µl Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA), 0.5 µl 10 µM forward primer EFTU_3 FW, 0.5 µl 10 µM reverse primer EFTU_3 Rev, and 2 µl sterile distilled deionized water. Reaction cycling conditions were as follows: 95°C for 10 min,

40 cycles of 95°C for 10 s, 60°C for 1 min, 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, and 60°C for 15 s. The resulting Ct values were converted into colony-forming units (CFU) per 50 µl of sap based on a standard curve starting with 10^8 CFU/mL of *X. fastidiosa* DNA diluted 1:10 through 10^2 CFU/mL in 50 µl of xylem sap taken from disease-free plants.

To facilitate data presentation and analysis, PCR detection data from aboveground sections were pooled into upper (current year), mid (previous year), and lower (older than 1 year) strata, and averages calculated for these strata (as well as for the root segments) for each cultivar and disease severity class. One-way analysis of variance (SAS v. 9.3; SAS Institute, Cary, NC) was applied to compare detection data across these four different strata within each severity class, with plant (replicate) serving as a blocking variable. The analysis was done separately for detection incidence (proportion of positive samples) and bacterial titer (CFU/50 µl of sap).

RESULTS

Detection frequency. As expected, frequency of detection of *X. fastidiosa* in xylem sap by real-time quantitative PCR was lowest (but non-zero) in asymptomatic plants and highest in plants with severe symptoms (Figs. 2.3 through 2.5). Detection frequencies on a per plant level were 11.4, 38.7, and 3.2% in asymptomatic plant sections and 76.8, 92.1, and 85.2% in severe plant sections of Bluecrisp, FL 86-19, and Star, respectively (Table 2.1). In asymptomatic plants, detection was generally least frequent (zero in the case of Bluecrisp) in top and root sections, and highest (up to 55.6% in the case of FL 86-19) in middle and base stem sections. As disease severity increased, detection frequencies remained lowest in top sections, but increased to >80% in root sections. A deviation from this pattern was observed in moderately susceptible Star, where detection frequency in the root remained <60% (Fig. 2.5). Overall, detection frequencies

were highest (>80%) in middle and base sections of plants from the moderate and severe classes (Figs. 2.3 through 2.5).

Bacterial titers. Bacterial population densities in xylem sap (Figs. 2.6 through 2.8) closely mirrored the detection frequency data discussed above. The lowest titers (averaging 0 to 2.1×10^1 CFU/50 µl of sap) were observed in top and root sections of asymptomatic plants, whereas the highest titers (generally between 10^4 and 10^5 CFU/50 µl of sap) were obtained from middle, base, and root sections of plants from the moderate and severe classes. Moderately susceptible Star had higher bacterial titers in the top, middle, and base stem sections (typically between 10^3 and 10^4 CFU/50 µl of sap) and lower titers in roots (~10^2 CFU/50 µl of sap), but these population densities were not significantly different among stem sections and roots ($P > 0.05$). Bacterial populations were higher in FL 86-19 than in the other two cultivars, most likely because of the higher overall disease severity observed in this field (data not shown).

DISCUSSION

This study shows that pruning, hedging, or flail-mowing are unlikely to be effective management options for bacterial leaf scorch in southern highbush blueberry. This was deduced by observing high detection frequencies and bacterial titers of *X. fastidiosa* in the lower stem sections of symptomatic plants. In addition, the pathogen was readily detected in roots of symptomatic plants, indicating that removal of aboveground plant parts will be ineffective in depleting bacterial reservoirs within the plant.

In a diagnostic context, our findings suggest that pathogen detection from xylem sap is most likely to be successful when testing middle and base stem sections in asymptomatic plants and base stem sections and roots in symptomatic plants. This is based on the evidence that the

target pathogen was more frequently detected in these strata and that even in asymptomatic plants or plants with low disease severity levels, the highest bacterial titer was generally found in the base stem sections and roots. Initially bacterial titer is low in the roots, but as symptoms progress, the bacteria appear to accumulate in the roots. This suggests rapid systemic spread of the pathogen, possibly by twitching motility, which allows *X. fastidiosa* to move basipetally from the inoculation site against the xylem flow (De la Fuente et al. 2007; Cursino et al. 2011; Meng et al. 2005). Our results showing high bacterial titer of *X. fastidiosa* in roots is consistent with the study completing Koch's postulates for bacterial leaf scorch of blueberry, in which the pathogen was readily isolated from roots but not from aboveground plant parts (Chang et al. 2009). Other pathosystems in which *X. fastidiosa* appears to be accumulating in the roots include citrus (Almeida et al. 2001; He et al. 2000) and peach (Aldrich et al. 1992; Hutchins et al. 1953), in both of which the pathogen causes a stunting disease. Our findings on blueberry seem to contradict the paradigm that the pathogen does not accumulate in the roots of hosts affected by *X. fastidiosa*-induced leaf scorching disease (Henneberger 2003).

It is not certain where *X. fastidiosa* is inoculated into blueberry plants. However, since the glassy-winged sharpshooter, the most prevalent potential pathogen vector observed in blueberry plantings in Georgia (Tertuliano et al. 2010), settles primarily on current and previous season's growth (Tertuliano et al. 2012), it is likely that the bacterium is inoculated into these sections. In blueberry, it is important to note that previous and current year's shoots may branch off directly from stem base sections; as such the pathogen may bypass the middle stem sections, reaching the base and roots more quickly than if traversing the full hierarchy of sections from top to bottom as shown in Fig. 2.2. Another possible explanation for the high bacterial titer in the bottom portions of these plants could be inverse hydraulic redistribution, i.e., the downward or

lateral movement of water depending on where dry and moist soil layers are in the soil profile (Prieto et al. 2012). In particular, overhead irrigation is used commonly in southern highbush blueberries, and this could be responsible for creating the conditions for hydraulic redistribution to occur such that leaves are kept wet while bark mulch or surface soil remains dry. Water would then be taken up by the leaves and redistributed to water-deficient roots by downward movement of xylem fluid. Even if *X. fastidiosa* is inoculated into upper, younger portions of the plant, it could be redistributed readily into base sections and roots through this process.

The fact that *X. fastidiosa* was detected in asymptomatic plants in this study was not surprising. All three fields sampled had a high incidence of the disease, and as such it is likely that asymptomatic plants had been exposed to the bacterium. Indeed, detection frequency of the pathogen from asymptomatic plants was highest in FL 86-19 (38.7%), the field with the highest disease incidence and severity. Yet, average bacterial titers were low even in these plants (<20 cells/50 µl). The high prevalence of *X. fastidiosa*-infected plants in our test fields, along with the high sensitivity of the PCR assay used in our study (~10^2 CFU/50 µl sap), may explain the fact that pathogen detection from asymptomatic plants was not uncommon.

It has been suggested that bacterial multiplication needs to be at high concentrations for systemic movement of *X. fastidiosa* to occur (Hill and Purcell 1995), but the results shown in this study may suggest otherwise, given that the bacterial titer was low in the top stem sections, yet higher titers could be detected in the lower stem sections of even asymptomatic plants. Compared with a study in grape where infections were readily pruned out in late winter (Feil et al. 2003), suggesting that the bacterium did not move quickly from distal inoculation sites, the results of our study on blueberry showed that the bacterium was already present in the middle and base stem sections of asymptomatic plants and even further in the roots of symptomatic

plants. Thus, even the use of drastic pruning techniques, such as applied successfully to mitigate bacterial leaf scorch in coffee (Queiroz-Voltan et al. 2006), would likely be ineffective, again due to the rapid establishment of the pathogen in the lower base sections and roots of the blueberry plant.

In conclusion, this study determined the distribution of *X. fastidiosa* in stem and root sections of naturally infected southern highbush blueberry plants, showing that the bacterium reaches lower stem sections and even the roots at relatively low levels of disease severity. Results therefore indicate that pruning, hedging, or flail-mowing are unlikely to be effective management options. For routine disease diagnosis, this study also demonstrates that xylem sap from lower stem sections and roots can be tested using quantitative real-time PCR to confirm the disease *in planta*.

LITERATURE CITED

Aldrich, J. H., Gould, A. B., and Martin, F. G. 1992. Distribution of *Xylella fastidiosa* within roots of peach. Plant Dis. 76: 885-888.

Almeida, R. P. P., Pereira, E. F., Purcell, A. H., and Lopes, J. R. S. 2001. Multiplication and movement of a citrus strain of *Xylella fastidiosa* within sweet orange. Plant Dis. 85: 382-386.

Brannen, P. M., Scherm, H., and Chang, C.J. 2008. Survey of cultivar differences in bacterial leaf scorch incidence among southern highbush blueberries. Dixie Blueberry News 8: 6-7.

Chang, C. J., Donaldson, R., Brannen, P., Krewer, G., and Boland, R. 2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. HortScience 44: 413-417.

Cursino, L., Galvani, C. D., Athinuwat, D., Zaini, P. A., Li, Y., De La Fuente, L., Hoch, H. C., Burr, T. J., and Mowery, P. 2011. Identification of an operon, Pil-Chp, that controls twitching motility and virulence in *Xylella fastidiosa*. MPMI 24: 1198-1206.

De La Fuente, L., Montanes, E., Meng, Y., Li, Y., Burr, T. J., Hoch, H. C., and Wu, M. 2007. Assessing adhesion forces of type I and type IV pili of *Xylella fastidiosa* bacteria by use of microfluidic flow chamber. Appl. Environ. Microb. 73: 2690-2696.

Feil, H., Feil, W. S., and Purcell, A. H. 2003. Effects of date of inoculation on the within-plant movement of *Xylella fastidiosa* and persistence of Pierce's disease within field grapevines. Phytopathology 93: 244-251.

He, C. X., Li, W. B., Ayres, A. J., Hartung, J. S., Miranda, V. S., and Teixeira, D. C. 2000. Distribution of *Xylella fastidiosa* in citrus rootstocks and transmission of citrus variegated chlorosis between sweet orange plants through natural root grafts. Plant Dis. 84: 622-626.

Henneberger, T. S. 2003. Effects of low temperature on populations of *Xylella fastidiosa* in sycamore. M. S. thesis. University of Georgia, Athens.

Hill, B. L., and Purcell, A. H. 1995. Multiplication and movement of *Xylella fastidiosa* within grapevine and four other plants. Phytopathology 85: 1368-1372.

Hutchins, L. M., Cochran, L. C., Turner, W. F., and Weinberger, J. H. 1953. Transmission of phony disease virus from tops of certain affected peach and plum trees. Phytopathology 43: 691–696.

Krewer, G., Stanaland, D., Nesmith, S., and Mullinix, B. 2004. Post-harvest hedging and pruning of three year pruning trial on 'Climax' and 'Tifblue' rabbiteye blueberry. Small Fruits Review 3: 203-212.

Meng, Y. Z., Li, Y. X., Galvani, C. D., Hao, G. X., Turner, J. N., Burr, T. J., and Hoch, H. C. 2005. Upstream migration of *Xylella fastidiosa* via pilus-driven twitching motility. J. Bacteriol. 187: 5560-5567.

Prieto, I., Armas, C., and Pugnaire, F. I. 2012. Water release through plant roots: new insights into its consequences at the plant and ecosystem level. New Phytol. 193: 830-841.

Queiroz-Voltan, R. B., Cabral, L. P., Paradela Filho, O., and Fazuoli, L. C. 2006. Prune efficiency in the control of *Xylella fastidiosa* in coffee trees. Bragantia 65(3): 433-440.

Tertuliano, M., Scherm, H., and Horton, D. 2010. Population dynamics and feeding preference of the glassy-winged sharpshooter [*Homalodisca vitripennis* (Germar)] (Hemiptera: Cicadellidae), vector of *Xylella fastidiosa*, on southern highbush blueberry. (Abstr.) Entomological Society of America 58th Annual Meeting, San Diego, CA.

Tertuliano, M., Srinivasan, R., and Scherm, H. 2012. Settling behavior of the glassy-winged sharpshooter, *Homalodisca vitripennis*, vector of *Xylella fastidiosa*, on southern highbush blueberry cultivars. Entomol. Exp. Appl. 143: 67-73.

Williamson, J. G., Davies, F. S., and Lyrene, P. M. 2004. Pruning blueberry plants in Florida. HS985, IFAS Extension, University of Florida, Gainesville.

Table 2.1. Overall detection frequency (across all stem and root segments[a]) of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from naturally infected southern highbush blueberry plants in three fields sampled in 2009 and 2010.

Disease severity	Detection frequency (%)		
class	Bluecrisp (2009)	FL 89-16 (2009)	Star (2010)
Asymptomatic	11.4	38.7	3.2
Low	47.7	48.6	…[b]
Moderate	71.4	92.7	75.4
High	76.8	92.1	85.2

[a] Segments sampled shown in Fig. 2.2, with ten replicates for each segment type.
[b] Low severity class not sampled in this field.

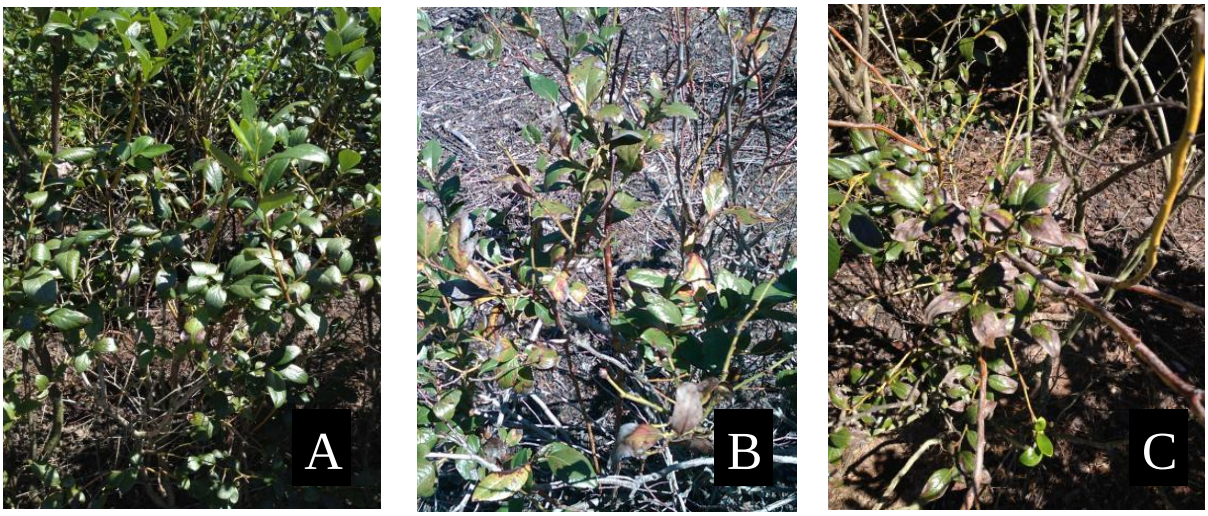

Figure 2.1. Examples of bacterial leaf scorch severity levels used to classify individual plants for xylem sap sampling to detect *Xylella fastidiosa* in naturally infected southern highbush blueberry plants in the field: light (A), moderate (B), and severe (C).

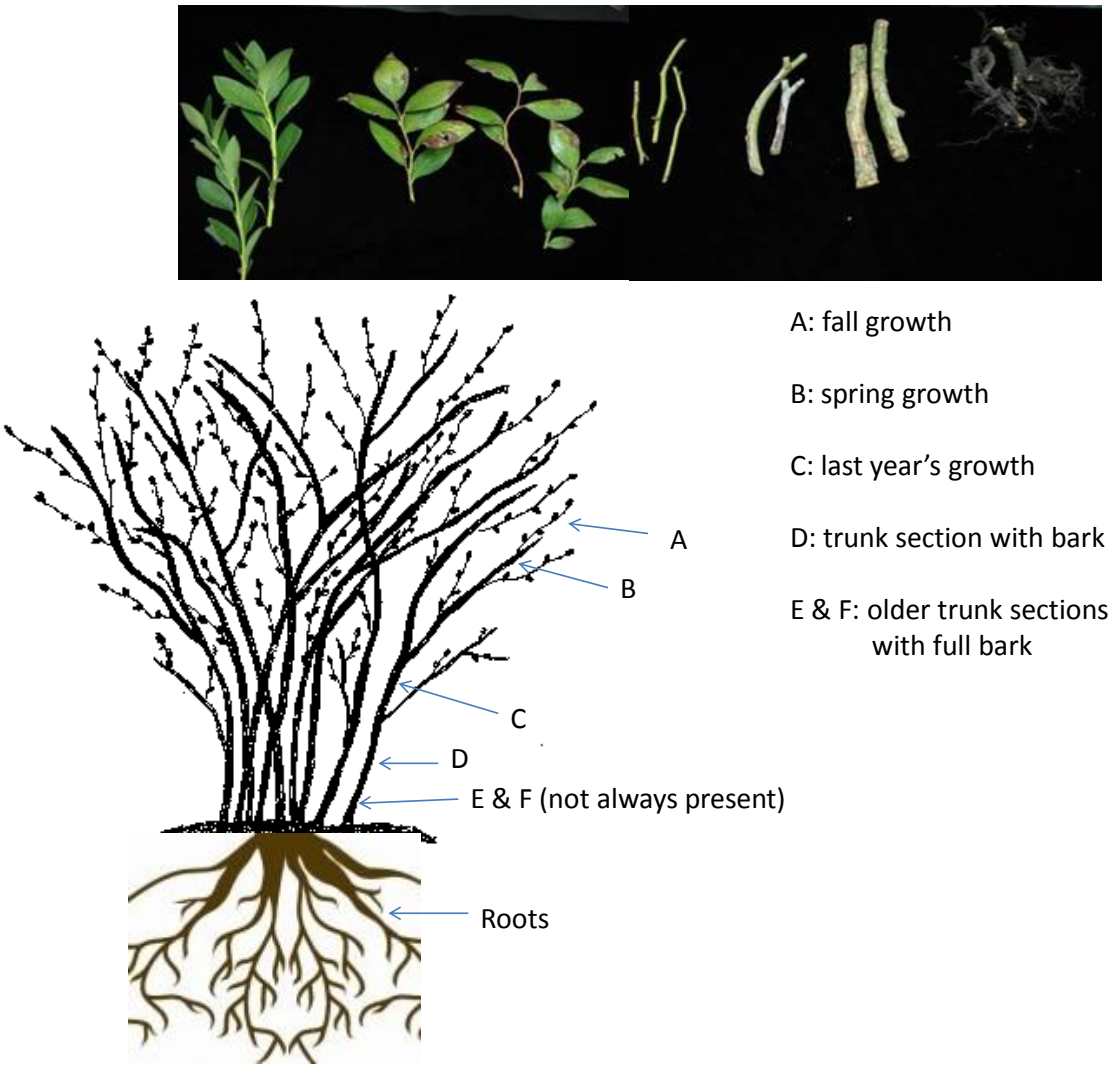

Figure 2.2. Stem and root sections used for xylem sap sampling to detect *Xylella fastidiosa* in naturally infected southern highbush blueberry plants in the field (diagram courtesy of Ohio State University Extension).

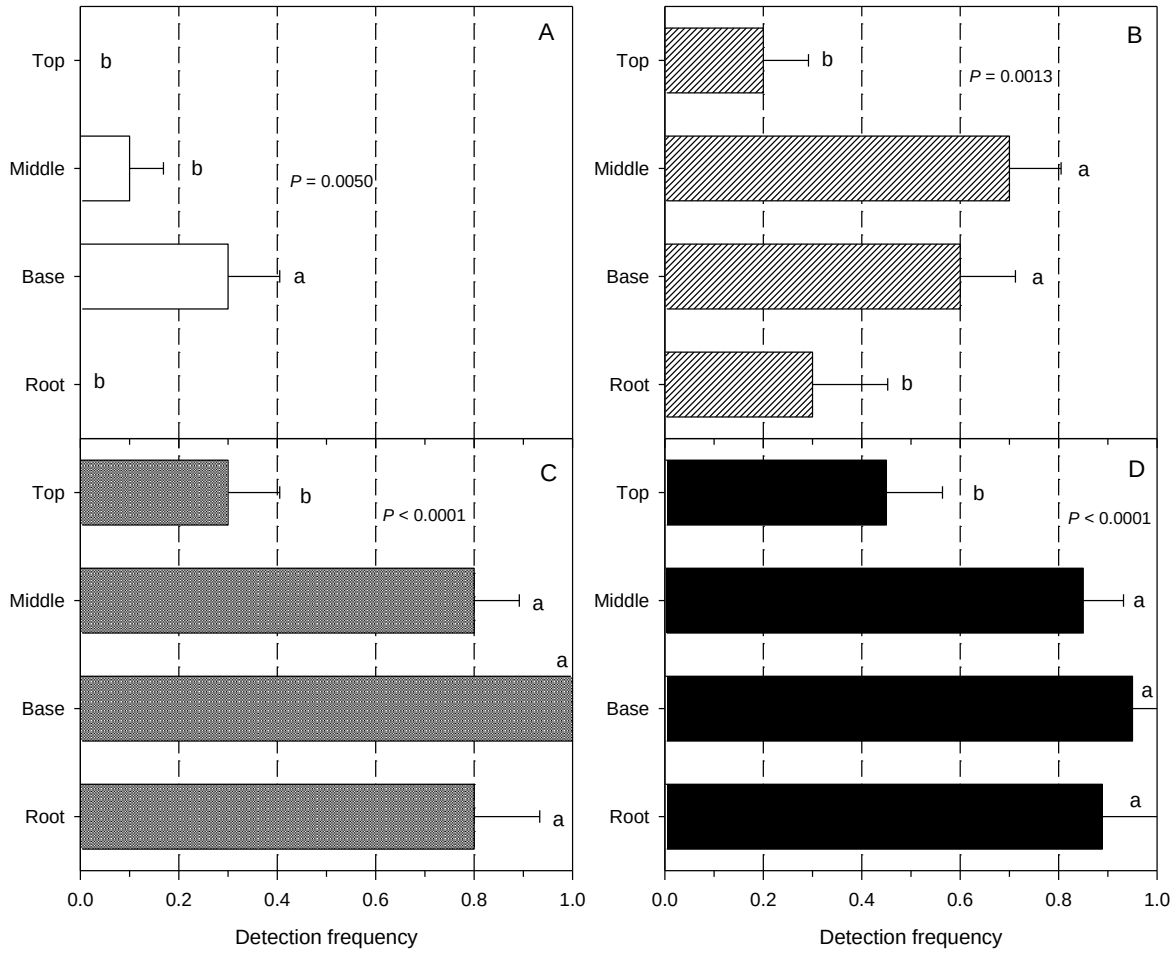

Figure 2.3. Detection frequency of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of Bluecrisp southern highbush blueberry (2009) from asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

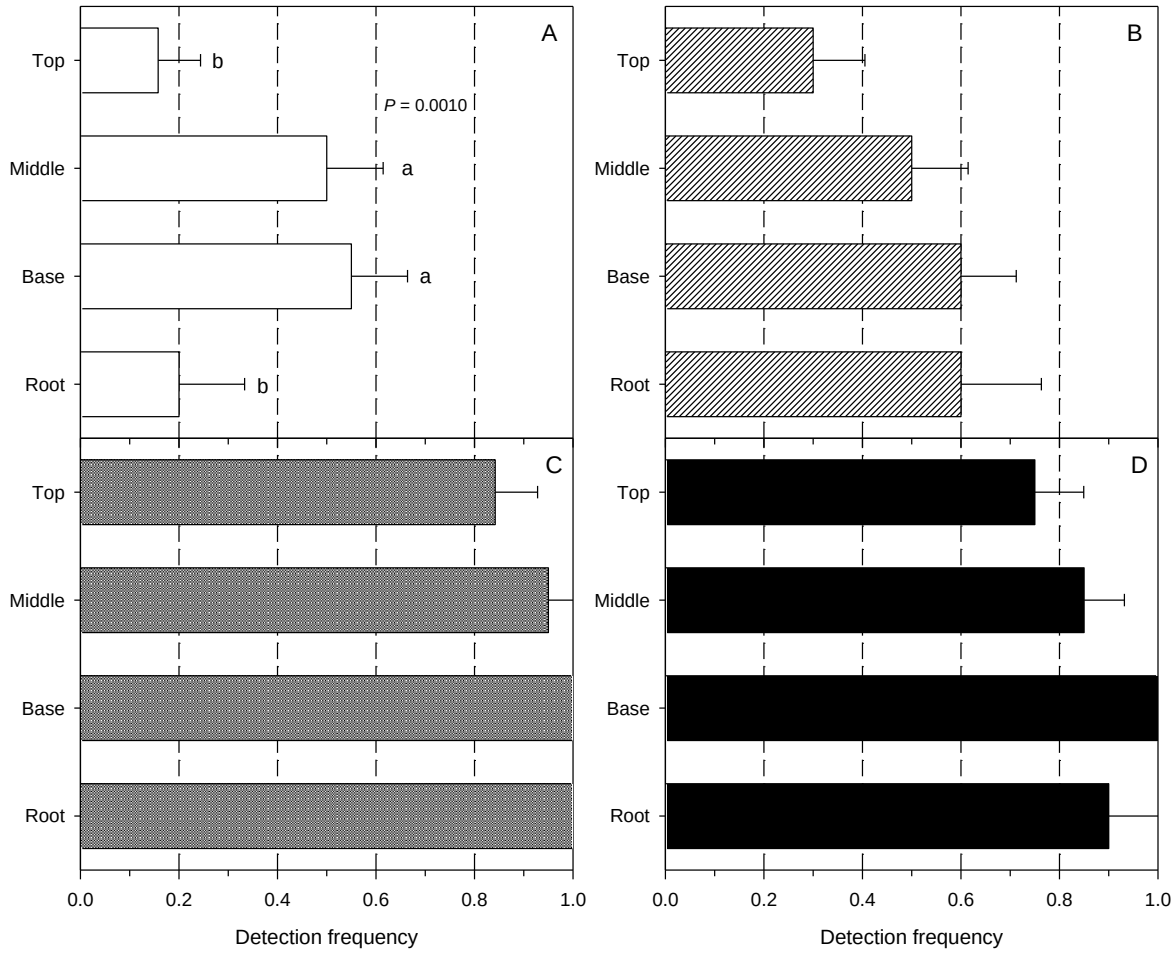

Figure 2.4. Detection frequency of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of FL 86-19 southern highbush blueberry (2009) from asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

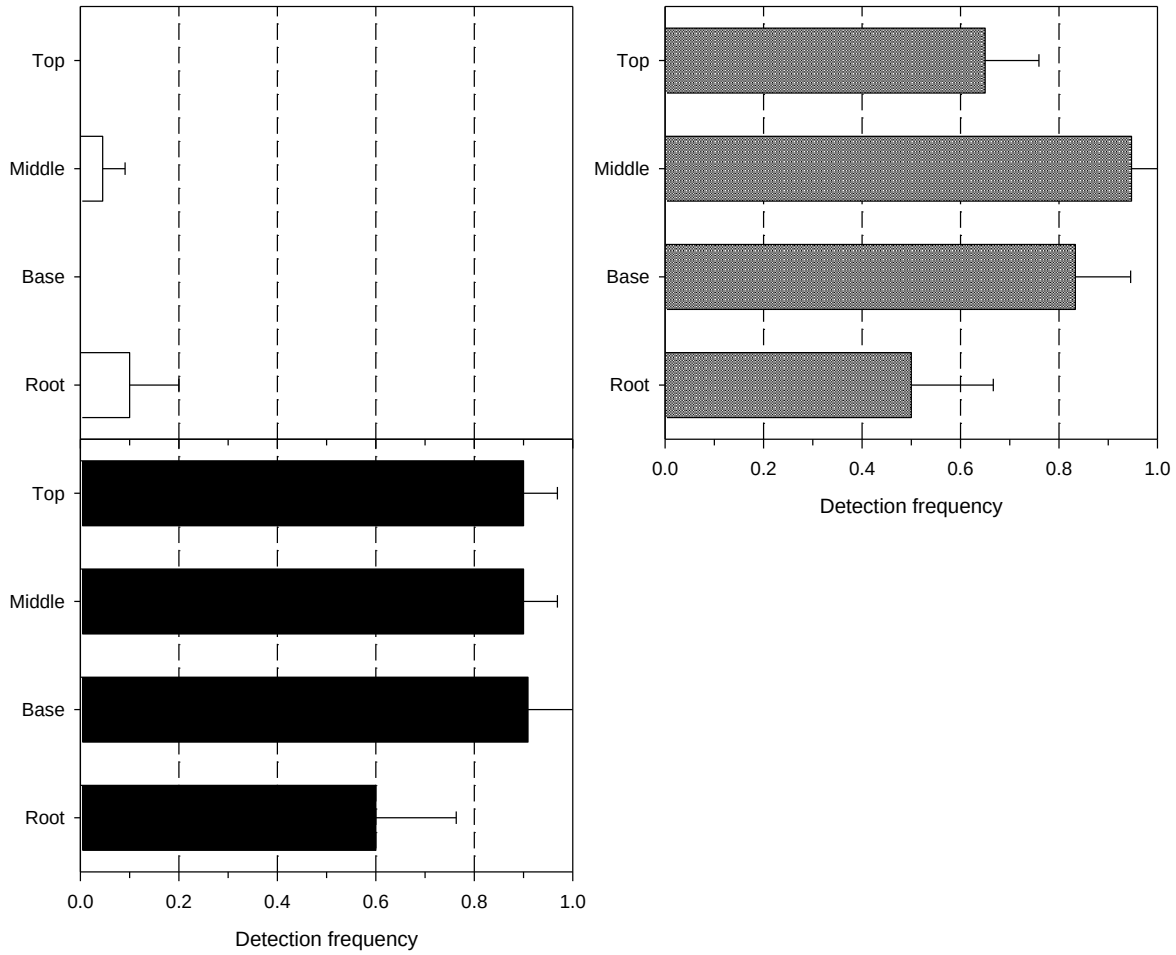

Figure 2.5. Detection frequency of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of Star southern highbush blueberry (2010) from asymptomatic plants (**A**) and those having moderate (**B**) and severe (**C**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, there were no statistically significant differences among sections according to Fisher's protected LSD test ($P > 0.05$).

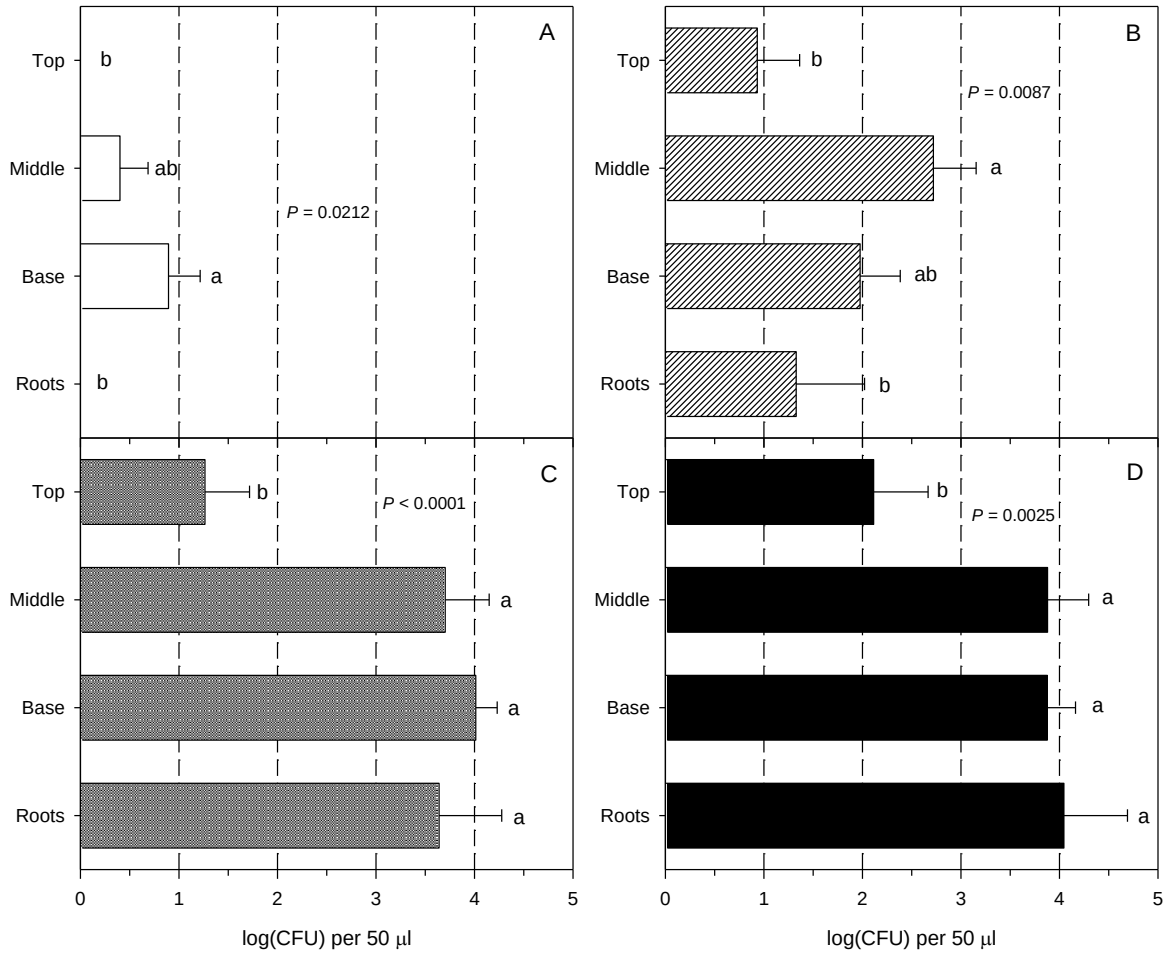

Figure 2.6. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of Bluecrisp southern highbush blueberry (2009) from asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

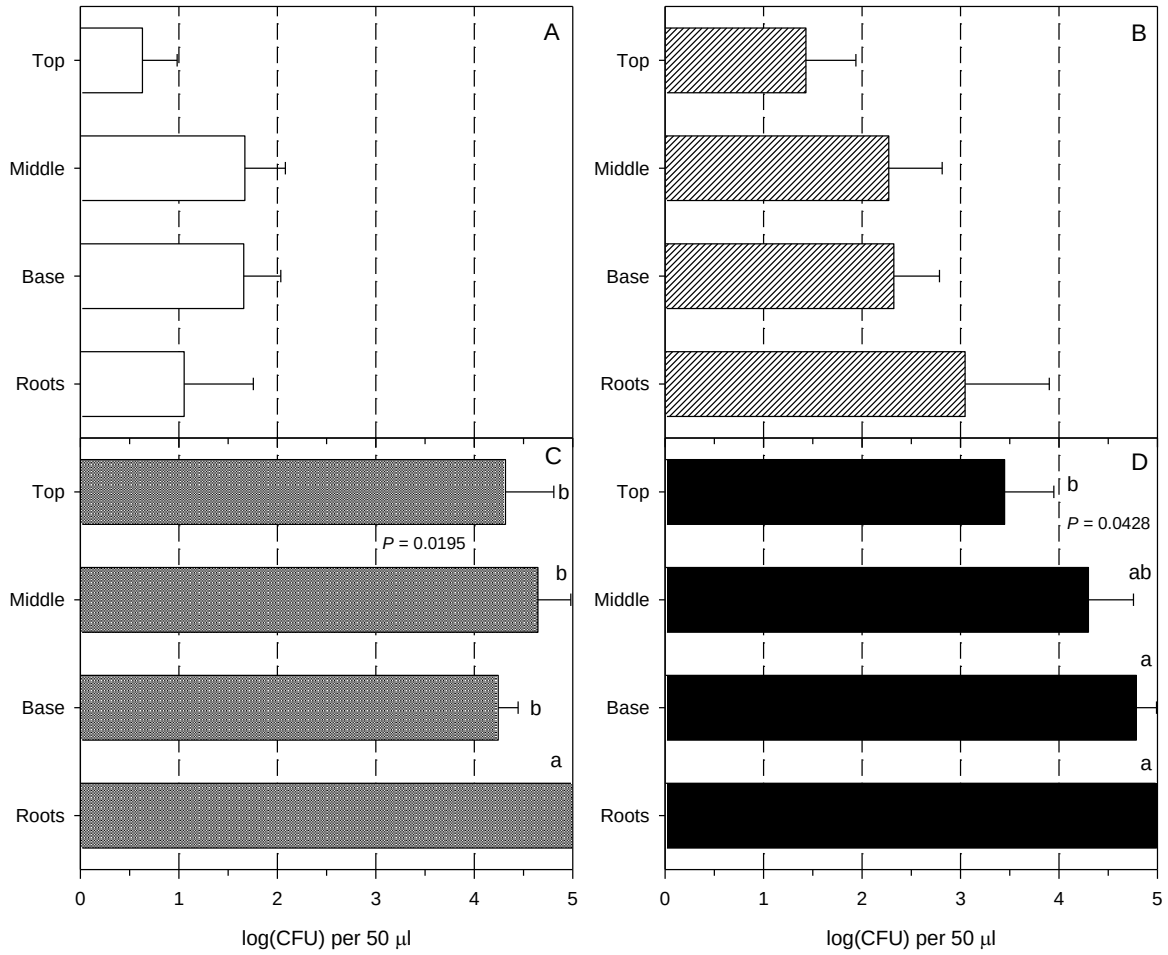

Figure 2.7. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of FL 86-19 southern highbush blueberry (2009) from asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, means followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

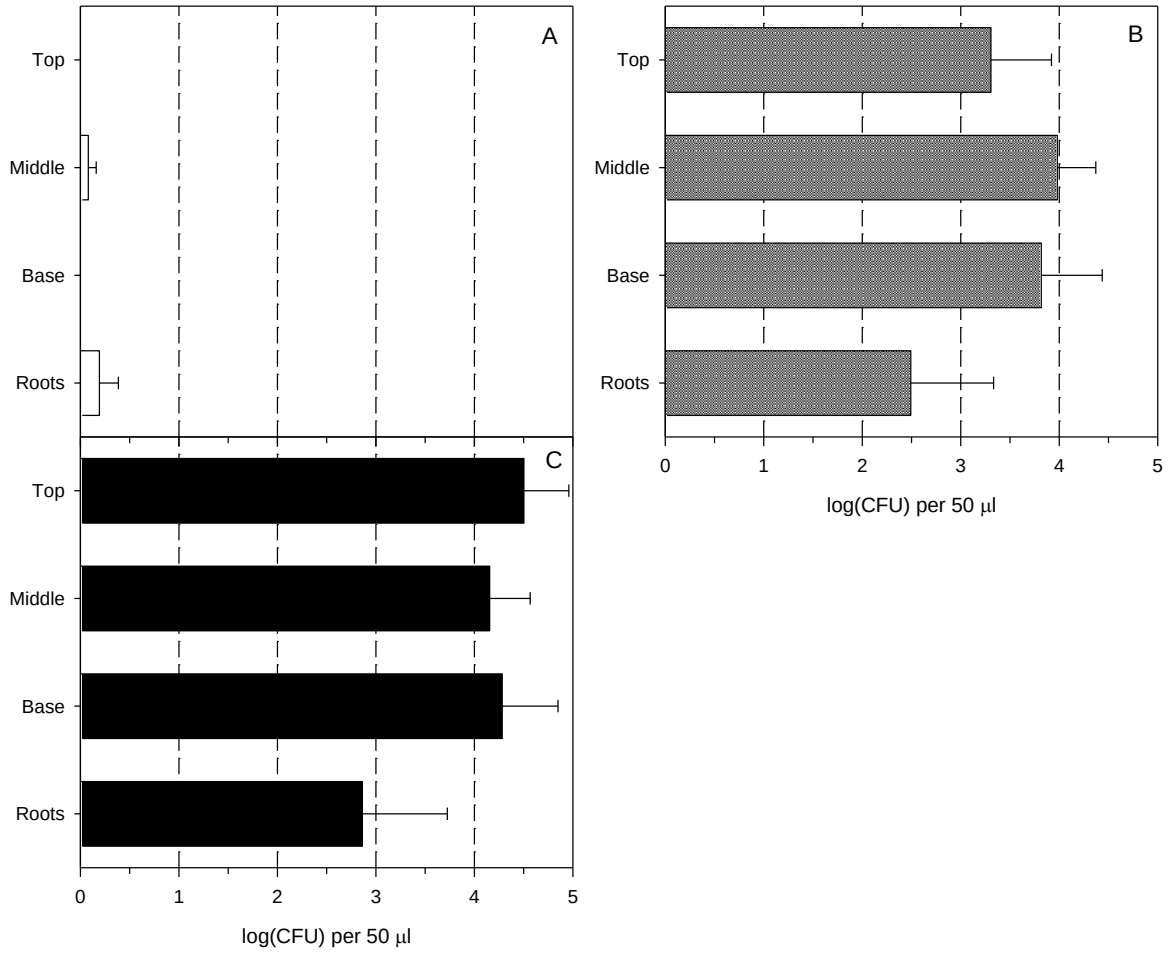

Figure 2.8. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in different stem sections (top to bottom of plant) and roots of Star southern highbush blueberry (2010) from asymptomatic plants (**A**) and those having moderate (**B**) and severe (**C**) bacterial leaf scorch symptoms (cf. Fig. 2.1). Values are means and standard errors. Within each disease severity class, there were no statistically significant differences among sections according to Fisher's protected LSD test ($P > 0.05$).

CHAPTER 3

TRANSMISSION OF BACTERIAL LEAF SCORCH, BLUEBERRY RED RINGSPOT VIRUS, AND BLUEBERRY NECROTIC RINGBLOTCH-ASSOCIATED VIRUS THROUGH SOFTWOOD CUTTINGS

Holland, R. M., Christiano, R.S.C., and Scherm, H. 2013. To be submitted to *Plant Disease*.

Transmission of bacterial leaf scorch, *Blueberry red ringspot virus*, and *Blueberry necrotic ringblotch-associated virus* through softwood cuttings

R. M. Holland, R. S. C. Christiano, and H. Scherm

Department of Plant Pathology, University of Georgia, Athens, 30602

ABSTRACT

With the rapid expansion of blueberry acreage in the southeastern United States, especially that of early-maturing, high-value southern highbush blueberries, the incidence and severity of systemic or locally systemic diseases has increased. These include bacterial leaf scorch (BLS) caused by the xylem-limited bacterium *Xylella fastidiosa*, red rinspot caused by *Blueberry red ringspot virus* (BRRV), and necrotic ringblotch caused by *Blueberry necrotic ringblotch-associated virus* (BNRBaV). It is unknown to what degree these diseases are spread via vegetative propagation. Asymptomatic softwood cuttings were collected from mother plants symptomatic for BLS, BRRV, or BNRBaV in commercial plantings of southern highbush blueberry cultivar Star in June and September of 2010 and 2011. Cuttings were allowed to root for 8 to 10 weeks on a mist bench, followed by transplanting and overwintering in a greenhouse. The following spring, plants were moved into an insect-proof screenhouse and monitored for symptom development for a period of 12 to 27 months after initial collection of cuttings. Disease status of the mother plant did not consistently affect mortality, rooting success, or defoliation of cuttings on the mist bench, although cuttings derived from BRRV and BLS plants had numerically higher mortality levels than the control in all four trials. At the time of transplanting of rooted cuttings from the mist bench to the greenhouse, cuttings from BRRV mother plants

showed a high incidence of visual symptoms (32.5 to 97.5%), whereas the BLS cuttings remained asymptomatic. However, in three of four trials cuttings derived from BLS mother plants tested positive (1.9 to 25.0% incidence) for *X. fastidiosa* based on real-time PCR with species-specific primers. During the first 1.5 months after transplanting in the greenhouse, vegetative growth of BLS plants was reduced significantly in three trials compared with the untreated control, although BLS plants were asymptomatic at that time. After 3 months in the greenhouse, BRRV plants were symptomatic in all four trials (37.5 to 97.5% incidence), whereas symptoms resembling BLS and BNRBaV were observed in one trial each (17.5 and 1.4% incidence, respectively). The percentage of BLS and BNRBaV plants that tested positive by PCR in the latter trial was 9.5 and 0%, respectively. After plants had been maintained in the screenhouse for one or two growing seasons, between 54.3 and 96.8% of BRRV plants were symptomatic, whereas all BLS and BNRBaV plants were asymptomatic. However, comprehensive testing of all BLS plants by real-time PCR revealed positives in three of four trials, with an overall incidence (across all trials) of 5.1%. In conclusion, BBRV is transmitted readily via softwood cuttings; symptoms become apparent early, suggesting that the disease can be eliminated by rogueing symptomatic plants. BNRBaV, the other extreme, does not appear to be transmitted through vegetative propagation. Transmission rates of *X. fastidiosa* through softwood cuttings are low, but the disease can remain latent during the propagation process, thereby eluding visual detection and posing a significant risk.

INTRODUCTION

Blueberry production in Georgia has grown exponentially over the past three decades and has placed Georgia at a rank of third in acreage (Scherm and Krewer 2003) and fifth in

production in the United States (USDA 2012). Whereas rabbiteye blueberries (*Vaccinium virgatum*) became well-established commercially in Georgia from the 1950s to 1980s (Scherm and Krewer 2003), these blueberry types ripen relatively late in early to mid-summer when prices have already dropped due to availability of highbush blueberry fruit from other production regions such as North Carolina (Williamson and Lyrene 2004). Thus, there was a need for developing early-maturing blueberry varieties suitable for southern production regions. As a result, blueberry breeding programs in Florida, North Carolina, and Georgia began to focus on southern highbush blueberry cultivars (*Vaccinium corymbosum* interspecific hybrids) that flower and mature earlier than rabbiteye cultivars (Ballington et al. 1997; Lyrene et al. 2003; Lyrene 2006; NeSmith 2007; Ballington 2008). Growers quickly realized the profitability of southern highbush blueberries, and acreage has increased exponentially starting in the 1990s (Scherm and Krewer 2003).

Due to drastic increase in grower demand for southern highbush blueberry cultivars, demand for vegetatively propagated nursery stock, mostly derived from softwood cuttings (Haralson 2009), increased concomitantly. Key aspects of blueberry propagation in Georgia, including the management of root and stem rot diseases affecting softwood cuttings, have been reviewed previously (Haralson 2009; Haralson et al. 2013). However, there has been no research on transmission of systemic viral and bacterial diseases in southern highbush blueberry propagation systems in the southeastern United States.

At the field scale, the rapid expansion of the southern highbush blueberry acreage in the Southeast was accompanied by an increase in the incidence and severity of systemic or locally systemic diseases (Brannen et al. 2008). These include bacterial leaf scorch (caused by the xylem-limited bacterium *Xylella fastidiosa*), red ringspot (caused by *Blueberry red ringspot*

virus, BRRV), and necrotic ringblotch (caused by *Blueberry necrotic ringblotch-associated virus*, BNRBaV). To what degree these diseases are spread through horticultural practices, including vegetative propagation, is currently unknown.

Bacterial leaf scorch is the most serious of the three aforementioned diseases, capable of killing susceptible blueberry cultivars within a few years of infection (Chang et al. 2009). In a field survey in Georgia (Brannen et al. 2008), most farms (71.1%) were positive for the presence of bacterial leaf scorch in at least one field, although incidence and severity varied, largely based on cultivar and age of the planting. At the field level, the disease was present in 41.9% of 167 fields surveyed, indicating that it was widespread. The causal bacterium *X. fastidiosa* is transmitted through xylem-feeding leafhoppers, of which the glassy-winged sharpshooter (*Homalodisca vitripennis*) is most common in blueberry plantings in Georgia (Tertuliano et al. 2010). Transmission of bacterial leaf scorch through vegetative propagation has not been investigated in blueberry. In wine grape, rooting success of (hardwood) cuttings infected by *X. fastidiosa* was lower than that of asymptomatic cuttings (Krawitzky et al. 2008), indicating that at least some infected cuttings may be an epidemiological dead end because they fail to root. In peach and pecan, grafting infected (hardwood) scions onto healthy rootstocks resulted in infected trees, with variable transmission rates usually between 20 and 50% (Hutchins et al. 1953; Sanderlin and Melanson 2008). Hot water treatment of pecan scions lowered transmission of *X. fastidiosa* significantly (Sanderlin and Melanson 2008). It is not clear if and how these results with hardwood cuttings can be extrapolated to blueberry, where propagation occurs primarily via softwood cuttings. Furthermore, we hypothesize that *X. fastidiosa* transmission rates vary depending on the time of year when cuttings are taken, being lower in the spring, after potential winter curing of bacterial population densities through cool temperatures (Ledbetter et al. 2009;

Meyer and Kirkpatrick 2011; Feil and Purcell 2001; Lieth et al. 2011), than in the fall, after extensive feeding of leafhoppers on new growth during the summer.

BRRV is common in Georgia, having occurred on 19 out of 45 farms (42.2%) and in 25 of 167 southern highbush fields (14.9%) surveyed in 2008 (Scherm et al. 2008). Symptoms are most pronounced in the fall, hence it is easy for infected plants not to be recognized in the spring when cuttings for propagation are commonly taken. The literature indicates that BRRV is readily transmitted through grafting (Hutchinson and Varney 1954), suggesting that transmission via cuttings should be high. No insect vector spreading the disease in the field has been identified to date (Martin et al. 2012), hence vegetative propagation may be the only means of spread.

BNRBaV causes a new viral disease of southern highbush blueberry characterized by irregularly shaped concentric rings or blotches with green centers on the upper surface and undersides of the leaves (Martin et al. 2012). The dark brown to purplish-black rings can eventually coalesce and develop into solid necrotic spots or blotches. These symptoms are distinct from the red, thin-margined rings caused by BRRV. Symptoms were initially observed in 2006 in southern Georgia in a few locations, but by 2008, symptoms of necrotic ringblotch were seen in multiple locations throughout major blueberry production areas. Double-stranded RNA was present, suggesting a virus as the causal agent, and with further characterization, four single-stranded RNA segments have been observed and the size of the nucleotide genome determined to be 14 kb (Quito-Avila and Martin 2012). An eriophyid mite has been associated with BNRBaV-symptomatic plants (Burkle et al. 2012). Morphology of the mite, a wandering foliar feeder, suggests it might be a new species in the genus *Calacarus* (Burkle et al. 2012). So far, symptoms are only found on the leaf, indicating limited systemic movement, and it is not clear whether the pathogen inhabits other areas of the plant. Given the localized nature of symptoms in the leaves

and the deciduous growth habit of blueberry, it appears unlikely that the pathogen would be transmitted readily through vegetative propagation, but there is currently no experimental data to support or refute this hypothesis.

The primary objective of this study was to determine the level of transmission of bacterial leaf scorch, BRRV, and BNRBaV through vegetative propagation via asymptomatic softwood cuttings taken from symptomatic mother plants at different times of the year (in spring or fall). A secondary objective was to determine the performance (rooting success and vegetative growth of young plants) derived from softwood cuttings taken from mother plants affected by the three diseases.

MATERIALS AND METHODS

Collection and rooting of cuttings. Softwood cuttings were taken in commercial plantings of mature Star southern highbush blueberry in June (spring cuttings) and September (fall cuttings) of 2010 and 2011 (Table 3.1). The fields were selected such that they were similar in age but differed in the types of disease present as determined by visual assessment or prior PCR testing: 1) asymptomatic plants; 2) plants with bacterial leaf scorch symptoms; 3) plants with BRRV symptoms; and 4) plants with BNRBaV symptoms. Cuttings were 15 and 20 cm in length, and only asymptomatic cuttings (i.e., sections not showing visual symptoms) were collected from the symptomatic mother plants. Ca. 300 cuttings were collected per field and stored in large plastic bags on ice overnight in a cold room.

The next day a sterile scalpel was used to make a 2-cm vertical incision at the base of each cutting from the fall collections to facilitate rooting; there was no such wounding for the spring cuttings. The bases of the cuttings were subsequently dipped into 2,500 ppm potassium-

indole-acetic acid plant growth hormone before sticking into 36-well trays containing milled pine bark. Four trays (replicates) were prepared for each of the four treatments. The trays were arranged in a randomized complete block design on a mist bench in a greenhouse for rooting. Greenhouse conditions were characterized by a temperature range of 21 to 31°C for spring cuttings with 14 h of daylight and 11 to 26°C for fall cuttings with 12 h of daylight. During the rooting of the fall 2011 cuttings, 3 weeks after sticking, the temperature in the greenhouse rose temporarily to ~37°C due to a heater malfunction, resulting in leaf scorching and temporary defoliation of cuttings.

Rooting assessment and transplanting. Eight to ten weeks after sticking in the four trials, a visual assessment of the cuttings was made, examining each for characteristic visual symptoms of the three diseases. At the same time, it was noted whether each cutting was alive, had rooted, had formed new vegetative growth, and whether defoliation (leaf loss) had occurred. Cuttings were uprooted and the volume of the root system determined by measuring length, width, and depth with a digital caliper. On the same day, 10 live cuttings each for asymptomatic and BRRV and 20 each for bacterial leaf scorch and BNRBaV treatments per replicate were transplanted into 2.8-liter pots containing 3:1 fine pine bark:sand and 14-14-14 NPK fertilizer; these cuttings were subsequently raised in a greenhouse at 21 to 29°C, watering them every other day and fertilizing once a month with a 20-20-20 NPK liquid fertilizer. The remaining cuttings (not transplanted) were frozen and stored for PCR testing. .

Growth and disease assessments. A visual disease and vegetative growth assessment was done 1.5 months after transplanting, and again 3 months after transplanting for each trial. The growth assessment consisted of measuring the cumulative length of all vegetative shoots formed on each plant. After overwintering in the greenhouse, plants were transplanted to larger

5.6 or 8.4-liter pots and moved to an insect-proof screenhouse (51 X 25 mesh) in the spring (April) and monitored monthly for symptom development during the season. Any leaves with symptoms resembling bacterial leaf scorch or BNRBaV were sampled and frozen for PCR analysis. No molecular confirmation was done for BRRV because previous work in our laboratory documented a perfect relationship between presence of BRRV visual symptoms and PCR detection using primer set RRSV3/RRSV4 (Polashock et al. 2009). All plants derived from the *X. fastidiosa* mother plants, regardless whether symptomatic or asymptomatic, were sampled in September 2012 for PCR testing. Samples typically consisted of 12 leaves with attached petioles per plant, collecting symptomatic leaves when present.

PCR testing for bacterial leaf scorch and BNRBaV. For rooted cuttings sampled destructively at the time of transplanting, a CTAB protocol (Porebski et al. 1997) was used for DNA extraction from 100 mg of stem tissue, whereas the MoBio PowerPlant Pro kit (MoBio, Carlsbad, CA) kit was used for DNA extraction from petioles (50 mg) of leaves sampled from plants in the greenhouse or screenhouse. Real-time PCR was conducted in the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) to detect *X. fastidiosa* using species-specific primers EFTu_3 – Fwd 5- TGA GGT GGA AAT TGT TGG CAT T -3 and EFTu_3 – Rev 5- AGC CTG ACC TTG ATC CAA TAA -3. PCR reaction volume was a total of 12 µl which included 3 µl template DNA from petiole extract, 6 µl Power SYBR Green Master Mix (Applied Biosystems), 0.5 µl 10 µM forward primer EFTU_3 FW, 0.5 µl 10 µM reverse primer EFTU_3 Rev (see Chapter 2), and 2 µl sterile distilled deionized water. Reaction cycling conditions were as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s, 60°C for 1 min, and 1 cycle of 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, and 60°C for 15 s. Samples with Ct values <35 and melting temperatures 76.5 to 78°C were considered positive for *X. fastidiosa*.

For samples suspect of BNRBaV, the RNeasy Plant Mini Kit (Qiagen, Valencia, CA) was used to extract total RNA from 100 mg of leaf tissue. Then using the Qiagen OneStep RT-PCR protocol, reverse transcriptase-PCR was used to detect the pathogen with genus-specific primers 31– Fwd 5- CCA GTT TGG AGG AAT TGC AT -3 and 31– Rev 5- GCG TTT CAG CAC CAC TAA C – 3 (R. R. Martin, unpublished). Reaction cycling conditions in the Veriti 96-Well Thermal Cycler (Applied Biosystems) were as follows: 50°C for 30 min, 95°C for 15 min, 40 cycles of 94°C for 30 s, 55°C for 20 s, 72°C for 45s, and one cycle of 72°C for 10 min. The amplicon was 432 bp and was visualized on a 1% agarose gel.

RESULTS

Performance of cuttings. After the 8 to 10-week rooting period, mortality of control cuttings from asymptomatic mother plants was generally low (<10%), except for the fall 2011 trial (16.0% mortality) in which the greenhouse had overheated temporarily (Table 3.2). In all four trials, mortality was numerically highest in cuttings from BRRV and *Xylella*-infected mother plants. However, this effect was statistically significant in only one trial for BRRV and in two trials for *X. fastidiosa*. Cuttings derived from mother plants with BNRBaV had mortality levels statistically similar to the untreated control in all four trials.

The rooting percentage for the control 10 weeks after sticking ranged from 72.9 to 99.3% (Table 3.2). Although BRRV cuttings had lower rooting percentages in two trials, this effect was not statistically significant. Thus, rooting was unaffected by the cutting source, i.e., whether or not the mother plants had been symptomatic. Root volume was similarly unaffected by treatment. Overall, the fall 2011 trial had the lowest root volumes, presumably because of the stress caused when the greenhouse overheated.

New vegetative growth was generally produced by <30% of the cuttings during the rooting period and was affected more by trial than by treatment (Table 3.2). Interestingly, the trial with the weakest root growth (fall 2011) had the highest frequency of cuttings producing new growth. In only one trial (spring 2011) was there a significant effect of cutting source, viz. a significantly higher frequency of new growth in the BNRBaV cuttings compared with all other treatments. Overall, however, there was no compelling evidence for a consistent effect of cutting source on vegetative growth during the rooting phase. A similar conclusion applied to defoliation of cuttings during the rooting period, where BRRV and *Xylella* cuttings had significantly higher defoliation levels (up to 21.2%) than the control in one trial each. Data on defoliation was collected in only three of the four trials (the fall 2011 trial was excluded because of the leaf damage caused by overheating of the greenhouse).

Disease symptoms on rooted cuttings. As stated previously, care was taken to obtain asymptomatic cuttings from symptomatic plants when the cuttings were collected in the field. As such, when the cuttings were stuck, they were asymptomatic. After the 8 to 10-week rooting period only the BRRV cuttings consistently showed symptoms typical of the corresponding disease (chlorotic mottling and spotting), with incidence levels ranging from 32.5 to 97.5% (Table 3.3). In 2010, a high percentage of the *Xylella* cuttings (57.2 and 40.2% for spring and fall, respectively) also showed symptoms of BRRV. This was the case because BRRV was relatively prevalent in the planting where the 2010 *Xylella* cuttings were taken, despite our best efforts to avoid *Xylella*-affected mother plants that also had BRRV symptoms. In 2011, a different *Xylella* planting was used for propagation, and infection with BRRV was no longer a problem. Overall, our data show that BRRV transmits readily from mother plant to cutting, and that BRRV symptoms appear relatively soon during the propagation process.

Symptoms typical of BNRBaV were observed very rarely in the rooting trays, with only cuttings in the spring 2011 trial from the BNRBaV-affected mother plants being symptomatic at an incidence of 4.9% (Table 3.3). For *Xylella*, no characteristic symptoms were observed during the rooting period in any of the trials; however, real-time PCR testing on *Xylella* cuttings that were not transplanted proved to be positive in three of the four trials with percentages of PCR positives being 1.9, 25.0, and 18.5% for fall 2010, spring 2011, and fall 2011, respectively (Table 3.3).

Performance of plants 1.5 months after transplanting. In general, vegetative plant growth was more vigorous for the plants derived from spring cuttings than those from fall cuttings (Table 3.4). This was likely due to more favorable conditions for growth of the transplanted cuttings during summer than in the fall and winter. In three of the four trials, vegetative growth (cumulative length of all shoots produced per plant) was significantly lower in plants derived from *Xylella* cuttings than in those from healthy or BNRBaV cuttings, even though the *Xylella* plants were still asymptomatic. This was not unexpected since the *Xylella*-affected mother plants from which these cuttings were taken were less vigorous (due to the effects of the disease) than the mother plants belonging to the other three treatments. As such, the reduced growth is more likely due to reduced overall vigor of the cuttings than to a direct effect of *X. fastidiosa* on the cuttings after sticking or transplanting. There was a significant reduction in vegetative growth in the BRRV plants in two out of four trials, whereas the BNRBaV plants grew as well as those derived from healthy mother plants (Table 3.4).

At 1.5 months after transplanting between 16.6 and 84.4% of the plants derived from BRRV cuttings showed symptoms of the disease (Table 3.3). There were no symptoms of BNRBaV, except for the spring 2011 trial in which the incidence of symptoms resembling

BNRBaV was 1.3%. No symptoms typical of bacterial leaf scorch were observed 1.5 months after transplanting, confirming the earlier observations made in the rooting trays. No PCR testing was conducted on the symptomatic BNRBaV plants since only few leaves were symptomatic and needed to be kept so that disease transmission could be monitored in later growth stages. Also, no PCR testing was conducted for *Xylella* plants because there were no symptoms expressed at this time.

Performance of plants 3 months after transplanting. When vegetative growth was measured in the greenhouse 3 months after transplanting of rooted cuttings, growth was lower in plants derived from BRRV and *Xylella* cuttings than in those from control cuttings (Table 3.4). This was significant in two of the four trials. Visual disease incidence continued to be highest for BRRV cuttings, ranging from 37.5 to 97.5%. Only one of the four trials showed characteristic visual symptom development for plants from BNRBaV and *Xylella* cuttings, with an incidence of 1.4 and 17.5%, respectively. Leaf samples from these symptomatic plants were tested by PCR, and the percentage that proved to be PCR positive was 9.5 and 0% *X. fastidiosa* and BNRBaV, respectively.

Disease incidence in fall 2012. After plants derived from the rooted cuttings had spent their first winter in the greenhouse, they were transplanted into 5.6 or 8.4-liter pots in April and moved to an insect-proof screenhouse outdoors. These plants were then monitored periodically for symptom development. A comprehensive disease assessment on all plants was conducted in September of 2012, 27 and 25 months after the 2010 cuttings had been taken and 15 and 12 months after the 2011 cuttings had been collected. Visual disease incidence continued to be highest for BRRV cuttings, ranging from 54.3 to 96.8% across the four trials (Table 3.3). No BNRBaV plants showed any visual symptoms, and in only one out of four trials did plants

derived from *Xylella* cuttings show symptoms (2.5% incidence). However, when petioles from all *Xyllela* plants (whether symptomatic or not) were tested by real-time PCR, three of four trials had PCR positives at 1.3, 6.2, and 14.3% incidence, respectively (Table 3.3). Overall transmission percentages, across all plants in the four trials were 78.6% for BRRV (based on visual symptoms), 0% for BNRBaV (based on visual symptoms), and 5.1% for *X. fastidiosa* (based on PCR assessment).

DISCUSSION

This study confirmed that BRRV is transmitted readily through vegetative propagation, and that typical symptoms are observed at early stages in the propagation process (in the rooting trays or soon thereafter). In contrast, BNRBaV transmission through softwood cuttings appears to be minimal, and the few plants observed having symptoms resembling necrotic ringblotch could not be confirmed by PCR. Lastly, although there may be little to no symptom expression in cuttings from *X. fastidiosa*-infected mother plants, the pathogen can be detected by PCR during various plant growth stages. Transmission of *X. fastidiosa* through vegetative propagation was low, but non-negligible (5.1% across all trials).

When interpreting these results it needs to be borne in mind that data were based on asymptomatic cuttings taken from symptomatic plants. It is likely that higher transmission percentages would be obtained if symptomatic cuttings are collected and rooted. However, in a practical context, such cuttings would likely be discarded by nursery operators prior to sticking, and hence would be of limited relevance in the propagation process.

After 8 to 10 weeks on a mist bench, survival and rooting of cuttings derived from symptomatic plants (for any of the three diseases) were not consistently affected compared with

cuttings from asymptomatic control plants. Thus, infected cuttings do not constitute an epidemiological dead end because they perform similar to asymptomatic cuttings in the rooting trays. This contrasts with the findings for hardwood cuttings in grape, where asymptomatic cuttings had better rooting success than cuttings from *Xylella*-infected plants (Krawitzky et al. 2008).

The only cuttings that consistently developed symptoms of the corresponding disease during the rooting phase were those from BRRV-affected mother plants, regardless of whether the cuttings were collected in the spring or fall. Thus, in a practical context, these plants could be rogued readily prior to transplanting them from the mist bench into larger containers. This is not the case for *X. fastidiosa*, where rooted cuttings are symptomless after 8 to 10 weeks on the mist bench but where the pathogen could be detected in up to 25% of these rooted cuttings by real-time PCR testing. Thus, although BRRV is transmitted more readily through the propagation process than *X. fastidiosa*, the latter poses a greater risk because it remains asymptomatic for a much longer period. Even at the end of the experiment, up to 27 months after the earliest cuttings had been collected and stuck, visual symptom development continued to be absent for *Xylella* plants, although the pathogen was detected in 5.1% of plants by PCR.

It has been suggested that BNRBaV transmission through vegetative propagation in blueberry would be minimal (Martin et al. 2012) because of the lack of systemic movement of the pathogen *in planta*, and this was confirmed in the present study. We noted a few plants with symptoms resembling necrotic ringblotch during the propagation process (up to 1.3% incidence in one trial), but these plants tested negative by PCR. Since there is generally an excellent relationship between BNRBaV symptoms and PCR test results (Martin et al. 2012), it is likely that the symptoms observed on the potted plants in the present study were not due to BNRBaV.

As such, there is no conclusive experimental evidence to date that BNRBaV can be transmitted via vegetative propagation.

The three diseases investigated here present interesting case studies on the role of vegetative propagation in facilitating disease spread. BBRV is transmitted readily but symptoms become apparent early and the disease therefore should be relatively easy to eliminate by rogueing symptomatic plants early in the propagation process. BNRBaV, the other extreme, does not appear to be transmitted through vegetative propagation. Transmission rates of *X. fastidiosa* through softwood cuttings are low, but the disease can remain latent during the propagation process, thereby eluding visual detection and posing a significant risk. Hence, further research is needed to investigate improved clean propagation strategies such as nurserystock certification, tissue culture, or thermotherapy to mitigate the risk of disease transmission.

ACKNOWLEDGEMENTS

We thank blueberry growers and county extension agents for access to their fields for sampling, Tanisha Robinson for her technical support with testing for *Blueberry necrotic ringblotch-associated virus*, and Amy Savelle, Charles Laster, Matt Seader, and Ryan McNeil for providing greenhouse and farm support.

LITERATURE CITED

Ballington, J. R., Rooks, S. D., Cline, W. O., Meyer, J. R., and Milholland, R. D. 1997. The North Carolina State University blueberry breeding program – toward *V. covilleanum*? Acta Hort. 446: 243-247.

Ballington, J. R. 2008. The role of interspecific hybridization in blueberry improvement. Acta Hort. 810: 49-60.

Brannen, P. M., Scherm, H., and Chang, C.J. 2008. Survey of cultivar differences in bacterial leaf scorch incidence among southern highbush blueberries. Dixie Blueberry News 8: 6-7.

Burkle, C., Olmstead, J. W., and Harmon, P. F. 2012. A potential vector of *Blueberry necrotic ring blotch virus* and symptoms on various host genotypes. (Abstr.) Phytopathology 102:S4.17

Chang, C. J., Donaldson, R., Brannen, P., Krewer, G., and Boland, R. 2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. HortScience 44: 413-417.

Feil, H., and Purcell, A. H. 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. Plant Dis. 85: 1230-1234.

Haralson, J. C. 2009. Pathogens associated with blueberry cutting failure in south Georgia nurseries and their control. M. S. thesis. University of Georgia, Athens.

Haralson, J. C., Brannen, P. M., NeSmith, D. S., and Scherm, H. 2013. Chemical control of Cylindrocladium and Rhizoctonia root rots in blueberry propagation. Crop Protection 44: 1-5.

Hutchins, L. M., Cochran, L. C., Turner, W. F., and Weinberger, J. H. 1953. Transmission of phony disease virus from tops of certain affected peach and plum trees. Phytopathology 43: 691–696.

Hutchinson, M. T., and Varney, E. H. 1954. Ringspot-A virus disease of cultivated blueberry. Plant Dis. Rep. 38: 260-262.

Krawitzky, M., Montague, T., and Hellman, E. 2008. Propagation success of grapevines infected with *Xylella fastidiosa*. Page 41 in: Proceedings of the 2nd Annual National Viticulture Research Conference. University of California, Davis.

Ledbetter, C. A., Chen, J., Livingston, S., and Groves, R. L. 2009. Winter curing of *Prunus dulcis* cv 'Butte,' *P. webbii* and their interspecifc hybrid in response to *Xylella fastidiosa* infections. Euphytica 169: 113-122.

Lieth, J. H., Meyer, M. M., Yeo, K.-H., and Kirkpatrick, B. C. 2011. Modeling cold curing of Pierce's disease in *Vitis vinifera* 'Pinot Noir' and 'Cabernet Sauvignon' grapevines in California. Phytopathology 101: 1492-1500.

Lyrene, P. M., Vorsa, N., and Ballington, J. R. 2003. Polyploidy and sexual polyploidization in the genus *Vaccinium*. Euphytica 133: 27-36.

Lyrene, P. M. 2006. Breeding southern highbush and rabbiteye blueberries. Acta Hort. 715: 29-36.

Martin, R. R., Polashock, J. J., and Tzanetakis, I. E. 2012. New and emerging viruses of blueberry and cranberry. Viruses 4: 2831-2852.

Meyer, M. M., and Kirkpatrick, B. C. 2011. Exogenous applications of abscisic acid increase curing of Pierce's disease-affected grapevines growing in pots. Plant Dis. 95: 173-177.

NeSmith, D. S. 2007. Blueberry cultivar development at the University of Georgia. A progress report for 2006. Pages 60-73 in: Proc 13th Southeast Blueberry Conf., Savannah, GA.

Polashock, J. J., Ehlenfeldt, M. K., and Crouch, J. A. 2009. Molecular detection and discrimination of *Blueberry red ringspot virus* strains causing disease in cultivated blueberry and cranberry. Plant Dis. 93: 727-733.

Porebski, S., Bailey, L. G., and Baum, B. R. 1997. Modification of a CTAB DNA Extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol. Biol. Rep. 15: 8-15.

Quito-Avila, D. F., and Martin, R. R. 2012. *Blueberry necrotic ring blotch virus* represents a unique genus of plant RNA viruses. (Abstr.) Phytopathology 102:S4.96.

Sanderlin, R. S. and Melanson, R. A. 2008. Reduction of *Xylella fastidiosa* transmission through pecan scion wood by hot-water treatment. Plant Dis. 92: 1124-1126.

Scherm, H., and Krewer, G. 2003. Blueberry production in Georgia: Historical overview and recent trends. Small Fruits Rev. 2: 83-91.

Scherm, H., Brannen, P. M., and Cline, W. O. 2008. Blueberry red ringspot virus: Prevalence in Georgia and North Carolina, and yield losses associated with the disease. Southern Region Small Fruit Consortium. Grant code: 2008-04.

Tertuliano, M., Scherm, H., and Horton, D. 2010. Population dynamics and feeding preference of the glassy-winged sharpshooter [*Homalodisca vitripennis* (Germar)] (Hemiptera: Cicadellidae), vector of *Xylella fastidiosa*, on southern highbush blueberry. (Abstr.) Entomological Society of America 58th Annual Meeting, San Diego, CA.

Williamson, J. G., and Lyrene, P. M. 2004. The Florida blueberry industry: A decade of growth. Proc. Fla. State Hort. Soc. 117: 234-235.

Table 3.1. Sources of asymptomatic softwood cuttings collected from mature, symptomatic southern highbush blueberry plants (cv. Star) to determine transmission by vegetative propagation of bacterial leaf scorch (caused by *Xylella fastidiosa*), *Blueberry red ringspot virus* (BRRV), and *Blueberry necrotic ringblotch-associated virus* (BNRBaV).

Treatment	Spring 2010 (8 June)	Fall 2010 (31 Aug.)	Spring 2011 (14 and 22 June)	Fall 2011 (15 Sept.)
Asymptomatic	New Hanover Co., NC (Farm A)	Bacon Co. (Farm E)	Bacon Co. (Farm E) & Clinch Co. (Farm G)	Clinch Co. (Farm G)
BRRV	Appling Co. (Farm B)	Appling Co. (Farm B)	Appling Co. (Farm B)	Appling Co. (Farm B)
BNRBaV	Bladen Co., NC (Farm C)	Berrien Co. (Farm F)	Clinch Co. (Farm H)	Clinch Co. (Farm H)
Bacterial leaf scorch	Brantley Co. (Farm D)	Bacon Co. (Farm E)	Clinch Co. (Farm I)	Clinch Co. (Farm I)

Table 3.2. Growth of Star southern highbush blueberry softwood cuttings taken from mother plants that were either asymptomatic (healthy) or symptomatic for *Blueberry red ringspot virus* (BRRV), *Blueberry necrotic ringblotch-associated virus* (BNRBaV), or *Xylella fastidiosa* (BLS) during incubation on a mist bench for 8 to 10 weeks in four separate trials[a].

Trial and treatment[b]	Mortality (%)	Rooting (%)	Root volume (cm^3)	New growth (%)	Defoliation (%)
Spring 2010					
Healthy	3.5 b	80.6	127.4	10.6	0.76
BRRV	22.9 a	65.4	109.8	8.3	8.5
BNRBaV	2.8 b	93.7	158.8	8.6	0.71
BLS	7.6 b	76.4	128.0	21.9	8.6
P-value	0.0002	0.2365	0.3204	0.2418	0.0635
Fall 2010					
Healthy	0.69 b	99.3	123.4	5.0	1.4 b
BRRV	3.6 b	100	136.0	2.8	0 b
BNRBaV	0 b	99.3	129.1	0	0.69 b
BLS	9.0 a	97.7	139.1	2.4	9.9 a
P-value	0.0020	0.5920	0.2265	0.6432	0.0291
Spring 2011					
Healthy	9.7 b	76.4	79.4	22.3 b	2.1 b
BRRV	13.2 b	71.5	75.8	18.4 b	21.2 a
BNRBaV	0 b	87.5	91.1	55.6 a	0 b
BLS	32.6 a	63.2	80.8	10.6 b	4.5 b
P-value	0.0196	0.1573	0.0962	<0.0001	0.0027
Fall 2011					
Healthy	16.0	72.9	43.6	24.8	--[c]
BRRV	29.9	58.3	31.6	29.9	--
BNRBaV	5.6	78.5	43.7	24.8	--
BLS	26.4	66.7	29.4	35.9	--
P-value	0.0990	0.4182	0.4729	0.6830	--

[a]All cuttings were asymptomatic when collected in the field. Means followed by the same letter for each column and trial are not significantly different according to Fisher's protected LSD test.
[b]In most cases, different sets of mother plants were used in different trials (Table 3.1).
[c]Missing data (greenhouse overheated temporarily and caused defoliation of cuttings).

Table 3.3. Symptom development and real-time PCR confirmation on Star southern highbush blueberry softwood cuttings taken from mother plants that were symptomatic for *Blueberry red ringspot virus* (BRRV), *Blueberry necrotic ringblotch-associated virus* (BNRBaV), or *Xylella fastidiosa* (BLS)[a].

Trial and treatment[b]	Rooting tray (8 to 10 weeks)		Greenhouse (1.5 months)	Greenhouse (3 months)		Screenhouse (12 to 27 months)	
	Visual symptom incidence (%)	PCR positives for BLS (%)	Visual symptom incidence (%)	Visual symptom incidence (%)	PCR positives (%)	Visual symptom incidence (%)	PCR positives for BLS (%)
Spring 2010							
BRRV	51.3	--	45.5	80.5	--	73.5	--
BNRBaV	0	--	0	0	--	0	--
BLS	0	0	0	0	--	0	0
Fall 2010							
BRRV	97.5	--	84.4	97.5	--	74.4	--
BNRBaV	0	--	0	0	--	0	--
BLS	0	1.9	0	0	--	2.5	1.3
Spring 2011							
BRRV	32.5	--	16.6	37.5	--	54.3	--
BNRBaV	4.9	--	1.3	0	--	0	--
BLS	0	25.0	0	0	--	0	6.2
Fall 2011							
BRRV	78.1	--	18.8	66.7	--	96.8	--
BNRBaV	0	--	0	1.4	0	0	--
BLS	0	18.5	0	17.5	9.5	0	14.3

[a]All cuttings were asymptomatic when collected in the field.
[b]In most cases, different sets of mother plants were used in different trials (Table 3.1).

Table 3.4. Vegetative growth (total shoot length per plant) on Star southern highbush blueberry softwood cuttings taken from mother plants that were either asymptomatic or symptomatic for *Blueberry red ringspot virus* (BRRV), *Blueberry necrotic ringblotch-associated virus* (BNRBaV), or *Xylella fastidiosa* (BLS) 1.5 and 3 months after transplanting in the greenhouse in four separate trials[a]

	Vegetative growth (cm)[c]	
Trial and treatment[b]	1.5 months	3 months
Spring 2010		
Asymptomatic	70.6 a	306.2 a
BRRV	43.2 b	220.1 b
BNRBaV	66.0 a	276.7 a
Xylella (BLS)	46.9 b	214.1 b
P-value	<0.0001	0.0018
Fall 2010		
Asymptomatic	25.4	209.9
BRRV	18.8	165.3
BNRBaV	25.9	218.4
Xylella (BLS)	17.4	179.6
P-value	0.1147	0.1619
Spring 2011		
Asymptomatic	56.4 a	153.4/167.4 bc
BRRV	41.7 ab	185.5 ab
BNRBaV	56.7 a	247.1 a
Xylella (BLS)	19.6 b	122.6 c
P-value	0.0177	0.0107
Fall 2011		
Asymptomatic	16.7 a	70.6 a
BRRV	8.0 c	52.7 b
BNRBaV	13.2 ab	71.6 a
Xylella (BLS)	10.9 bc	45.8 b
P-value	0.0071	0.0011

[a]All cuttings were asymptomatic when collected in the field. Means followed by the same letter for each column and trial are not significantly different according to Fisher's protected LSD test.
[b]In most cases, different sets of mother plants were used in different trials (Table 3.1).
[c]Total cumulative shoot growth per plant.

CHAPTER 4

XYLEM HYDRAULIC CONDUCTANCE IN SOUTHERN HIGHBUSH BLUEBERRY CULTIVARS WITH DIFFERENT LEVELS OF FIELD RESISTANCE TO BACTERIAL LEAF SCORCH

Holland, R. M., Yin, J., Teskey, R., and Scherm, H. 2013. To be submitted to *Plant Disease*.

Xylem hydraulic conductance in southern highbush blueberry cultivars with different levels of field resistance to bacterial leaf scorch

R. M. Holland, J. Yin, R. Teskey, and H. Scherm

First and fourth authors: Department of Plant Pathology, University of Georgia, Athens 30602

Second and third authors: Warnell School of Forestry and Natural Resources, University of Georgia, Athens, 30602

ABSTRACT

Xylella fastidiosa causes bacterial leaf scorch, a new disease of southern highbush blueberry in the southeastern U.S. The bacterium colonizes and occludes the xylem of affected plants, causing drought-like symptoms and eventually plant death. Here, we investigate host xylem functionality in response to natural infection by *X. fastidiosa* in blueberry cultivars differing in field resistance to the disease. Using a high-pressure flow meter, hydraulic conductance per stem cross-sectional area (K_{sa}) was measured on field-collected stem sections from 5 to 6-year-old plants of cultivars FL 86-19 (highly susceptible), Star (intermediate), and Emerald (field-resistant) in fall 2011 and 2012. In asymptomatic plants, K_{sa} values were typically greater for Emerald than for Star or FL 86-19 for all tissue types tested (lignified stems, partially lignified stems, green shoots, and petioles) across both years, with differences being statistically significant for lignified and partially lignified stems. This shows overall greater capacity for water flow in the xylem system of Emerald. When K_{sa} of sections having <20% foliar leaf scorch severity was compared with that of more severely affected sections (≥20% foliar leaf scorch severity) in Star and FL 86-19, K_{sa} was higher in the less severely affected sections for all tissue

types except the current-season green shoots. Consistently across both years, petiole K_{sa} was reduced considerably and significantly in severely affected sections of the highly susceptible FL 89-16. Loss of xylem function in the petiole may help explain the greater propensity for plant death in FL 86-19 compared with Star in response to infection by *X. fastidiosa*.

INTRODUCTION

Bacterial leaf scorch, caused by the xylem-limited bacterium *Xylella fastidiosa*, is a new disease of blueberry that has emerged in the southeastern United States within the past decade (Chang et al. 2009; Harmon and Hopkins 2009). The disease primarily affects southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrids) (Brannen et al. 2008), causing scorching and marginal necrosis of leaves, yellowing of stems, dieback, and eventually plant death. The causal agent is transmitted by xylem-feeding leafhoppers (of which the glassy-winged sharpshooter, *Homalodisca vitripennis*, is most prevalent on blueberry) (Tertuliano et al. 2010) and colonizes and occludes the water-conducting tissues of affected plants, causing drought-like symptoms and eventually plant death. In a 2008 field survey in Georgia, most farms (71.1%) were positive for bacterial leaf scorch in at least one field, and the disease was present in 41.9% of the 167 fields surveyed (Brannen et al. 2008). Most rabbiteye blueberry cultivars (*Vaccinium virgatum*) and some southern highbush cultivars, such as Emerald and Millenia, appear to be resistant to the disease, but the widely planted FL 86-19 has shown high susceptibility. Star, the most widely planted southern highbush cultivar in Georgia and Florida, shows intermediate susceptibility to the disease (Brannen et al. 2008). There are notable differences in bacterial leaf scorch intensity among these blueberry cultivars of similar age planted in the same field, with some highly susceptible cultivars (such as FL 86-19) dying within a few years and others such as

Emerald remaining asymptomatic and unaffected over many years at the same location. Previous work has shown that these differences are not due to differential attractiveness of the cultivars to the glassy-winged sharpshooter (Tertuliano et al. 2012), pointing toward a plant-related mechanism being responsible for the observed field resistance.

The scorching symptoms associated with the disease suggest that the xylem has been compromised and that the plant is undergoing water stress (McElrone et al. 2003). Thus, xylem anatomy and structure in relation to infection by *X. fastidiosa* may be at least partially responsible in determining host resistance vs. susceptibility. In wine grape, Fritschi et al. (2008) concluded that reduction of occlusion of xylem conduits did not appear to be the mechanism of resistance to Pierce's disease (also caused by *X. fastidiosa*). Furthermore, there were low numbers of infested vessels in the cross-sections of grape leaf petioles affected by the disease (Mollenhauer and Hopkins 1976). Mollenhauer and Hopkins (1976) indicated that the morphology of tracheary cells differed significantly in Pierce's disease-infected susceptible and -resistant grape species, but according to Chatelet et al. (2011), grape varieties examined showed no differences in stem or petiolar vascular anatomy.

Less conflicting results may be obtained when xylem function (i.e., the effect on water flow), as opposed to xylem anatomy, is investigated in relation to *X, fastidiosa* disease development and host resistance. Water flow studies have been conducted in wine grapes affected by Pierce's disease (Choat et al. 2009). Leaf and petiole hydraulic conductance (a measure of xylem water flow) were measured in cv. Chardonnay, and it was reported that hydraulic conductance in these tissues decreased with increasing disease severity. Furthermore, there was a positive correlation between the concentration of *X. fastidiosa* and symptom severity in plants that were irrigation-deficient, suggesting that water stress increased susceptibility to

Pierce's disease (Choat et al. 2009). In pin oak and red oak, hydraulic conductivity of petioles affected by oak leaf scorch decreased significantly when compared with petioles of healthy trees, and as the season progressed and symptoms appeared, hydraulic conductivity rapidly decreased to zero (McElrone et al. 2008). Hopkins (1989) demonstrated that stem hydraulic conductivity was significantly lower in elm leaf scorch and phony peach-affected trees. Similarly, node and petiole xylem flow resistance was 60 to 200 times greater in grapevines affected by Pierce's disease (Hopkins 1989). No such information on xylem function in relation to disease severity or cultivar disease resistance is currently available for bacterial leaf scorch on blueberry.

Xylem hydraulic conductance is a measure of the plant's capacity for water movement and may be related to disease resistance in one of two ways. The objective this study was to quantify xylem hydraulic conductance in relation to bacterial leaf scorch in three southern highbush blueberry cultivars differing in their level of field resistance to the disease. We hypothesized that 1) blueberry cultivars will differ in innate hydraulic conductance (in the absence of disease), indicating that some cultivars will be less prone to pathogen-induced disruption of the water flow, and 2) the rate of decrease of hydraulic conductance with increasing disease severity will differ in these cultivars, providing another potential mechanism for differences in disease resistance.

MATERIALS AND METHODS

Sample collection. Plant samples for measuring hydraulic conductance were collected in a commercial blueberry planting affected by bacterial leaf scorch near Bristol, GA, in October 2011 and September 2012. The planting was 5 to 6 years old and included plants of various cultivars, including Emerald (field-resistant), Star (moderately resistant), and FL 86-19 (highly

susceptible). Disease severity varied from asymptomatic to severe symptoms for Star and FL 86-19, whereas no plants with bacterial leaf scorch symptoms were found for Emerald.

Using surface-disinfested pruners, stems from plants that differed in visual disease severity were cut at the base and the cut end was secured with parafilm. A moist towelette was placed around the parafilm to prevent xylem embolism, and the entire stem was placed in a large 144-L plastic bag. The bag was then sealed to create a humidity chamber. For Star and FL 86-19, at least eight asymptomatic stems and eight symptomatic stems were collected in this manner, whereas for Emerald only asymptomatic stems were available. Samples were transported back to the laboratory in an air-conditioned vehicle and stored in a cold room until testing 1 to 4 days later.

Hydraulic conductance measurements and disease assessment. Hydraulic conductance (K_h) of stem sections was measured using a high-pressure flow meter (Dynamax, Houston, TX) in transient mode (Tyree et al. 1995). These measurements were taken on stem segments with different tissue age classes starting from the base of the stem up through the tip with leaves. The sections were subsequently grouped into three classes, namely lignified stems, partially lignified stems, and green shoots with leaves (current-season growth). All stems were re-cut under water to avoid cavitation before being connected to the flow meter (Heath et al. 1997). A series of three measurements was taken from the apical segment (current-season growth). First, the entire segment with leaves attached was measured, followed by removal of the leaves under water with a razor blade. After reconnecting the segment with only petioles attached, a second measurement was taken. Lastly, petioles were removed under water and the shoot section alone was measured. This series of measurements allowed the petiole resistance to be calculated (petioles were too small to be measured directly). Using the hydraulic conductance

(K_h) values, calculations were made for stem area-specific hydraulic conductance, K_{sa} (accounting for section diameters). Leaf area was scanned digitally and leaf disease severity (percent necrotic leaf area) determined using Quant V.1.0.1 software (Vale et al. 2001).

Real-time PCR confirmation of infection. To confirm the presence of *X. fastidiosa* in symptomatic plants, DNA was extracted from 50 mg of petioles from symptomatic Star and FL 86-19 samples in September 2012 using the MoBio PowerPlant Pro DNA Isolation kit (MoBio, Carlsbad, CA). Real-time PCR was conducted in the 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) using species-specific primers EFTu_3 – Fwd 5- TGA GGT GGA AAT TGT TGG CAT T -3 and EFTu_3 – Rev 5- AGC CTG ACC TTG ATC CAA TAA -3. PCR reaction volume was a total of 12 µl which included 3 µl template DNA from petiole extract, 6 µl Power SYBR Green Master Mix (Applied Biosystems), 0.5 µl 10 µM forward primer EFTU_3 FW, 0.5 µl 10 µM reverse primer EFTU_3 Rev, and 2 µl sterile distilled deionized water. Reaction cycling conditions were as follows: 95°C for 10 min, 40 cycles of 95°C for 10 s, 60°C for 1 min, and 1 cycle of 95°C for 15 s, 60°C for 1 min, 95°C for 15 s, and 60°C for 15 s. Samples with Ct values <35 and melting temperatures 76.5 to 78°C were considered positive for *X. fastidiosa*.

Data analysis. In the first step of the analysis, one-way analysis of variance followed by Fisher's protected LSD test was applied to determine differences in K_{sa} among the three disease-free cultivars, using measurements from the asymptomatic segments only. In the second step, two symptom severity classes, asymptomatic and low disease severity (<20% necrotic leaf area as determined by image analysis) vs. moderate and high disease severity (≥20% necrotic leaf area) were compared for each cultivar and stem segment class using the non-parametric median test. All analyses were conducted using SAS 9.2 for Windows (SAS Institute, Cary, NC).

RESULTS

Disease severity and pathogen confirmation. Stem sections were collected from both asymptomatic and symptomatic plants. In 2011, disease severity, as measured by image analysis of foliar leaf scorch symptoms, ranged from 13.1 to 77.3% (mean 25.3%) in Star and 4.5 to 44.8% (mean 22.3%) in FL 89-16. In 2012, the corresponding values were 4.7 to 49.4% (mean 25.2%) in Star and 15.2 to 56.1% (mean 31.5%) in FL 89-16. As stated previously, no bacterial leaf scorch symptoms were observed on Emerald in either year. All symptomatic Star and FL 89-16 plants subjected to real-time PCR analysis tested positive for *X. fastidiosa*.

Hydraulic conductance in asymptomatic tissue. In asymptomatic plants, hydraulic conductance per stem cross-sectional area (K_{sa}) was greatest (up to 20.5 kg s^{-1} m^{-1} MPa^{-1}) in lignified stems and partially lignified stems (i.e., older stem sections toward the base of the plant) and lowest (not exceeding 3.0 kg s^{-1} m^{-1} MPa^{-1}) in current-season green shoots with leaves attached (Figs. 4.1 and 4.2). In both years, Emerald (field-resistant) had significantly higher K_{sa} than the moderately susceptible Star and/or the highly susceptible FL 89-16 in both lignified stems and partially lignified stems. In 2012, Emerald also had significantly higher K_{sa} than Star and/or FL 89-16 in green shoots and petioles (Fig. 4.2), but this was not observed in 2011 (Fig. 4.1).

Hydraulic conductance in symptomatic tissue. In both years, for both susceptible cultivars (Star and FL 89-16), and in all tissue types tested except for the green shoots, K_{sa} was greater in sections having <20% leaf scorch severity than in those with ≥20% disease severity (Figs. 4.3 and 4.4). However, this effect was statistically significant only for the highly susceptible FL 86-19, specifically for partially lignified stems in 2011 and for petioles in 2011

and 2012. In 2012, petiole K_{sa} in FL 89-16 plants having ≥20% disease severity was zero (Fig. 4.4).

In green shoots (with leaves attached), K_{sa} was greater in more severely affected plants, for both cultivars and both years (Figs. 4.3 and 4.4). However, this effect was not statistically significant. This reverse in trend in current-season green shoots may be attributed to a transient physiological change in xylem vessel length or diameter in order to compensate for the stress of disease.

DISCUSSION

This study documents differences in hydraulic conductance among southern highbush blueberry cultivars with varying susceptibility to bacterial leaf scorch disease. The results supported our hypothesis that blueberry cultivars would differ in innate water flow capacity in the absence of disease. In asymptomatic plants, K_{sa} was typically higher in field-resistant Emerald compared with moderately susceptible Star and highly susceptible FL 86-19 for all tissue types, and this was statistically significant for the older stem sections (lignified and partially lignified stems) in both years. This increased xylem efficacy in Emerald may contribute to its reduced susceptibility to xylem occlusion and/or embolism caused by by *X. fastidiosa*.

It has been shown previously in other plant species that hydraulic conductance can differ among cultivars or varieties. For apple, cultivar Galaxy had a higher hydraulic conductance than cultivar Granny Smith due to its 7% higher water use per trunk cross-sectional area and 19% higher water use per leaf area (González-Talice et al. 2012). In grape, cultivars Silvaner and Airen were more sensitive to xylem embolism in the stem than Grenache and Syrah in dry conditions (Chouzouri and Schultz 2005), however, in the internode segments, hydraulic

conductance was greatest for Airen and Silvaner, followed by Grenache and Syrah (Chouzouri and Schultz 2005). Data suggests that hydraulic conductance is correlated with plant architecture and anatomy. For example, at the node position basal to the shoot apex, the hydraulic conductance of Silvaner was four-fold higher than Syrah in the field (Chouzouri and Schultz 2005). Other studies also show differences among cultivars in water flow efficiency. In maize under water stress, cultivar Nongda 60 had a 54.6% increase in root hydraulic conductivity, whereas Zhongdan 2 and Shandan 11 had a decrease in root hydraulic conductivity by 50.9 and 63.0%, respectively (Tao et al. 2005). In sugarcane under drought stress, when hydraulic properties of entire root systems and isolated roots were characterized, there were differences among cultivars in both root- and leaf-specific hydraulic conductance (Saliendra 1991).

The current study also supported our second hypothesis that the rate of decrease of hydraulic conductance with increasing disease severity would differ among cultivars. Specifically, results showed that K_{sa} decreases with an increase in disease severity for both moderately susceptible Star and highly susceptible FL 86-19 for lignified stems, partially lignified stems, and petioles. In particular, the petioles of FL 86-19 were significantly impacted by higher disease severity, providing a possible explanation for why this cultivar has a higher propensity for death when affected by bacterial leaf scorch. Our results are in agreement with studies on Pierce's disease of grape, phony peach and oak bacterial leaf scorch, in which hydraulic conductance or conductivity decreased rapidly with an increase in disease severity in susceptible species or varieties (Choat et al. 2009; McElrone et al. 2008; Hopkins 1989). A phytoplasma disease has also affected hydraulic conductance. Affected *Spartium junceum* stems, showing dieback symptoms under drought stress, had a pronounced loss of hydraulic conductivity (40 and 60%) in fasiciate diseased stems (flat stems) and witches broom diseased

stems (shortened stems), respectively (Lo Gullo et al. 2000). These affected stems also had an unfavorable ratio of weighted conduit radius to total green surface area, hence efficiency in supplying the transpiring area of the plant with water was strongly reduced (Lo Gullo et al. 2000).

Although previous anatomical studies, particularly those relating to grape, show conflicting data as to whether there are significant differences in the xylem structure of Pierce's disease susceptible vs. resistant varieties (Mollenhauer and Hopkins 1976; Chatelet et al. 2011), it is important to note that such differences in the varieties may only appear at certain times of the season. A good example for this is provided in elm, where the presence of larger vessels in field elm than in Siberian elm early in the season explains why the former is more susceptible to Dutch elm disease than the latter (Solla et al. 2005); these larger vessels facilitate the spread of pathogen propagules and are more prone to embolism (Solla et al. 2005).

Vessel anatomy may differ in the highly susceptible FL 86-19 compared with moderately susceptible Star and field-resistant Emerald, contributing to a lower K_{sa}. FL 86-19 and particularly its petioles may also be more vulnerable to embolism, although this remains to be documented experimentally. According to Zimmerman's segmentation hypothesis, upper portions of the plant, including leaves and twigs, are more prone to cavitation due to low water potential in these distal parts (Hacke et al. 1996). If water stress is induced, embolism would occur in the petioles first, then in the conduits of branches because petiole xylem is more vulnerable to cavitation than branch xylem and xylem pressure is lower in the leaves (Hacke et al. 1996).

The pathogen could directly affect hydraulic conductance as well. For example, formation of *X. fastidiosa* aggregates or biofilms on xylem vessel walls could reduce surface

tension sufficiently to cause air bubble formation, rendering the xylem vessel non-functional (Tyree and Zimmermann 2002). It has been hypothesized that *X. fastidiosa* produces cell wall-degrading enzymes that enlarge the pore size of intervessel pit membranes. Using colloidal gold microspheres, the porosity of pit membranes in healthy grapevine was determined to be between 5 and 20 nm, which is too small to permit free vessel-to-vessel passage of the bacterium (Pérez-Donoso et al. 2010). In non-symptomatic stem segments from inoculated shoots or non-inoculated shoots from a two-shooted vine that had been inoculated in the second shoot, 20-nm microspheres moved through the xylem and collected at the distal end (Pérez-Donoso et al. 2010). This indicated that there was an increased pit membrane pore size, possibly due to a decrease in pit membrane polysaccharide integrity; this could have been a consequence of the presence of the pathogen, even though Pierce's disease symptoms were absent (Pérez-Donoso et al. 2010). When two cell wall-degrading enzymes, endo-1,4-beta-glucanase from *X. fastidiosa* and polygalactuoronase from *Aspergillus niger*, were both flushed through healthy grape stems, 20-nm microspheres passed through pit membranes and uronic acid, a breakdown product from pectic polymers, was collected after introduction of these enzymes. This indicates that the enzymes could enlarge the size of pores, decreasing resistance to water flow (Pérez-Donoso et al. 2010).

Xylem vulnerability is generally correlated with vessel anatomy, and wider vessels are more prone to embolism (Hacke et al. 1996, Muramatsu et al. 2008). For example, trees with tracheids or narrow vessels are not as prone to water shortage or embolism during water stress induced by freeze-thaw cycles in winter (Muramatsu et al. 2008); a similar phenomenon may occur during pathogen-induced water stress. Under the same conditions, trees with wider vessels have much lower hydraulic conductance and shoot dieback attributed to embolism (Muramatsu

et al. 2008). Xylem vessels with vestured pits can reduce embolism (Muramatsu et al. 2008; Tyree and Zimmerman 2002). A larger number of shorter vessels can be present at junctions such as petiole-stem or petiole-blade and so a nonrandom distribution of vessel ends may occur, increasing susceptibility to blockage (Tyree and Zimmerman 2002).

Future research should include detailed anatomical studies on the petioles and stems of bacterial leaf scorch-susceptible and resistant southern highbush blueberry cultivars. When sapwood-specific stem conductivity (K_s) was assessed for 3,005 woody angiosperms, it was found that variation in this measurement was due to the size to number ratio of the mean vessel lumen area to the number per unit area. It was concluded that the higher the size to number ratio, the greater K_s would be, so vessel lumen area and the number of vessels per unit area would be important measurements to take (Zanne et al. 2010). Also, a closer investigation of the number of vessel ends in a junction area (petiole-stem or petiole-leaf blade) vs. a non-junction area in susceptible and resistant cultivars may help in elucidating resistance mechanism(s). Other anatomical variables that should be examined microscopically are cross-sectional lumen diameter ($\bar{A}$), the number of vessels per unit area of sapwood (N) and lumen fraction which measures relative amount of transport space (Zanne et al. 2010). Furthermore, the ratio $S = \bar{A}/N$ which measures variation in vessel composition within the space, can be used to assess vascular strategy among plant species. If values of S are high, there are few large vessels, but if values are low, then there are many small vessels (Zanne et al. 2010). Such measurements would need to be taken periodically during the season for younger, green tissues to monitor the changes that could affect susceptibility to bacterial leaf scorch.

ACKNOWLEDGEMENTS

We thank James Jacobs, Pierce County Extension Coordinator, for helping coordinate sample collection.

LITERATURE CITED

Brannen, P. M., Scherm, H., and Chang, C.J. 2008. Survey of cultivar differences in bacterial leaf scorch incidence among southern highbush blueberries. Dixie Blueberry News 8: 6-7.

Chang, C. J., Donaldson, R., Brannen, P., Krewer, G., and Boland, R. 2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. HortScience 44: 413-417.

Chatelet, D. S., Wistrom, C. M., Purcell, A. H., Rost, T. L., and Matthews, M. A. 2011. Xylem structure of four grape varieties and 12 alternative hosts to the xylem-limited bacterium *Xylella fastidiosa*. Ann. Bot. 108: 73-85.

Choat, B., Gambetta, G. A., Wada, H., Shackel, K. A., and Matthews, M. A. 2009. The effects of Pierce's disease on leaf and petiole hydraulic conductance in *Vitis vinifera* cv. Chardonnay. Physiol. Plantarum 136: 384-394.

Chouzouri, A., and Schultz, H. R. 2005. Hydraulic anatomy, cavitation susceptibility, and gas-exchange of several grapevine cultivars of different geographic origin. Acta Hortic. 689: 325-331.

Fritschi, F. B., Lin, H., and Walker, M. A. 2008. Scanning electron microscopy reveals different response pattern of four *Vitis* genotypes to *Xylella fastidiosa* infection. Plant Dis. 92: 276-286.

González-Talice, J., Yuri, J. A., Lepe, V., Hirzel, J., and del Pozo, A. 2012. Water use in three apple cultivars from the second season to sixth season in a drainage lysimeter. Sci. Hortic. 146: 131-136.

Hacke, U., and Sauter, J. 1996. Drought-induced xylem dysfunction in petioles, branches, and roots of *Populus balsamifera* L. and *Alnus gutinosa* (L.) Gaertn. Plant Physiol. 111: 413-417.

Harmon, P. F., and Hopkins, D. L. 2009. First report of bacterial leaf scorch caused by *Xylella fastidiosa* on southern highbush blueberry in Florida. Plant Dis. 93: 1220.

Heath, J., Kerstiens, G., and Tyree, M. 1997. Stem hydraulic conductance of European beech (*Fagus sylvatica* L.) and pedunculate oak (*Quercus robur* L.) grown in elevated CO_2. J. Exp. Bot. 48: 1487-1489.

Hopkins, D. L. 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27: 271-290.

Lo Gullo, M. A., Trifilò, P., and Raimondo, F. 2000. Hydraulic architecture and water relations of *Spartium junceum* branches affected by a mycoplasm disease. Plant Cell Environ. 23: 1079-1088.

McElrone, A. J., Forseth, I. N., and Sherald, J. L. 2003. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. J. Exp. Bot. 54: 419-430.

McElrone, A., Jackson, S., and Habdas, P. 2008. Hydraulic disruption and passive migration by a bacterial pathogen in oak tree xylem. J. Exp. Bot. 59: 2649-2657.

Mollenhauer, H. H., and Hopkins, D. L. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. Physiol. Plant Pathol. 9: 95-100.

Muramatsu, N., and Hiraoka, K. 2008. Hydraulic conductance and xylem anatomy in fruit tree shoots. J. Japan. Soc. Hort. Sci. 77: 122-127.

Pérez-Donoso, A. G., Sun, Q., Roper, M. C., Greve, L. C., Kirkpatrick, B., and Labavitch, J. M. 2010. Cell wall-degrading enzymes enlarge the pore size of intervessel pit membranes in healthy and *Xylella fastidiosa*-infected grapevines. Plant Physiol. 152: 1748-1759.

Saliendra, N. Z. 1991. Root-shoot interactions in the response of sugarcane to drought. M. S. thesis. University of Hawaii, Honolulu.

Solla, A., Martin, J. A., Corral, P., and Gil, L. 2005. Seasonal changes in wood formation of *Ulmus pumila* and *U. minor* and its relation with Dutch elm disease. New Phytol. 166: 1025-1034.

Tao, Y., Zong-Suo, L., Ji-Quan, X., and Shao-Zong, K. 2005. Diversity of water use efficiency in various maize varieties. Transactions of the Chinese Society of Agricultural Engineering 21: 21-25.

Tertuliano, M., Scherm, H., and Horton, D. 2010. Population dynamics and feeding preference of the glassy-winged sharpshooter [*Homalodisca vitripennis* (Germar)] (Hemiptera: Cicadellidae), vector of *Xylella fastidiosa*, on southern highbush blueberry. (Abstr.) Entomological Society of America 58th Annual Meeting, San Diego, CA.

Tertuliano, M., Srinivasan, R., and Scherm, H. 2012. Settling behavior of the glassy-winged sharpshooter, *Homalodisca vitripennis*, vector of *Xylella fastidiosa*, on southern highbush blueberry cultivars. Entomol. Exp. Appl. 143: 67-73.

Tyree, M., Patino, S., Bennink, J., and Alexander, J. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flowmeter in the laboratory and field. J. Exp. Bot. 46: 83-94.

Tyree, M. T., and Zimmermann, M. H. 2002. Xylem Structure and the Ascent of Sap. 2nd ed. Springer, Berlin.

Vale, F. X. R., Fernandes Filho, E. I., Liberato, J. R., and Zambolim, L. 2001. Quant- a software to quantify plant disease severity. Page 160 in: Proc. 8th Intl. Workshop Plant Dis. Epidemiol., Ouro Preto, Brazil.

Zanne, A. E., Westoby, M., Falster, D. S., Ackerly, D. D., Loarie, S. R., Arnold, S. E. J., and Coomes, D. A. 2010. Angiosperm wood structure: Global patterns in vessel anatomy and their relation to wood density and potential conductivity. Am. J. Bot. 97: 207-215.

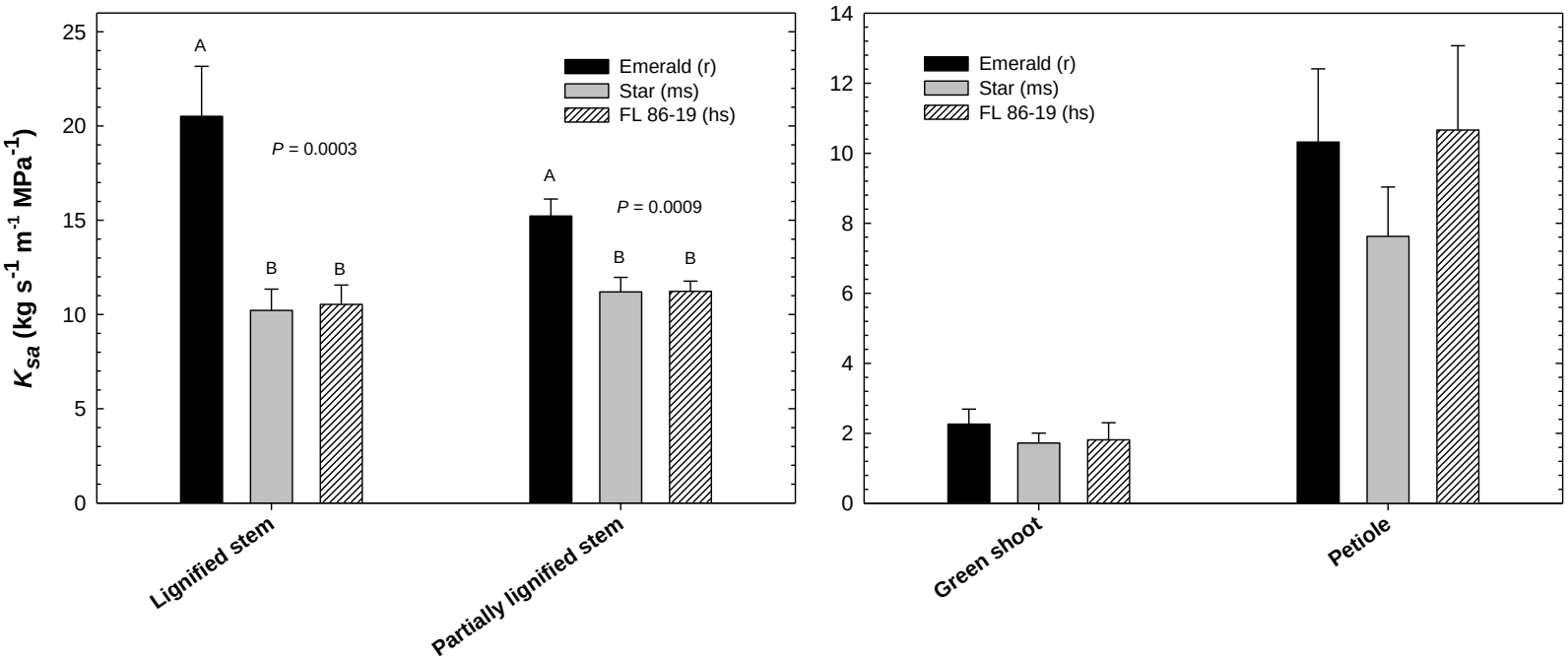

Figure 4.1. Hydraulic conductance per cross-sectional stem area (K_{sa}) of different tissue types on blueberry cultivars Emerald (resistant), Star (moderately susceptible), and FL 86-19 (highly susceptible) in 2011. Values are means and standard errors of eight replicates. For each tissue type, means followed by same letter are not significantly different according to Fisher's protected LSD test (P> 0.05).

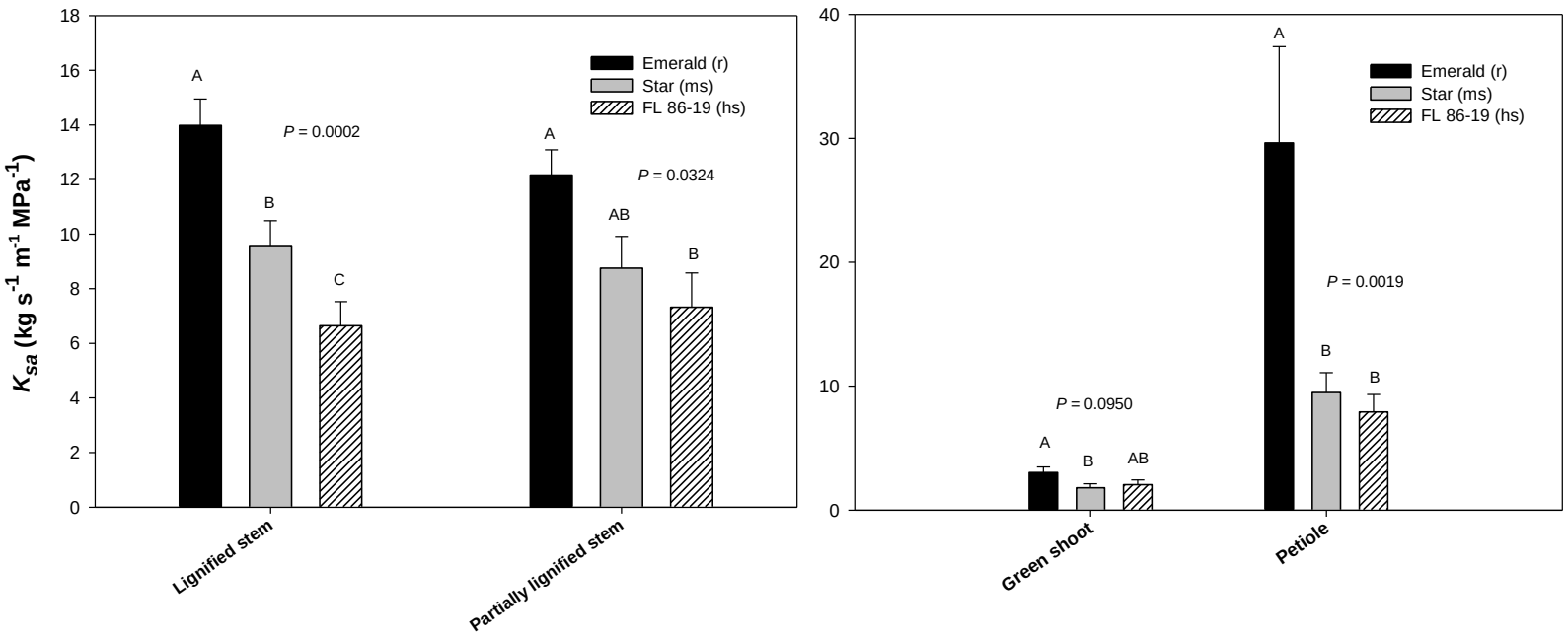

Figure 4.2. Hydraulic conductance per cross-sectional stem area (K_{sa}) of different tissue types on blueberry cultivars Emerald (resistant), Star (moderately susceptible), and FL 86-19 (highly susceptible) in 2012. Values are means and standard errors of eight replicates. For each tissue type, means followed by same letter are not significantly different according to Fisher's protected LSD test (P> 0.05).

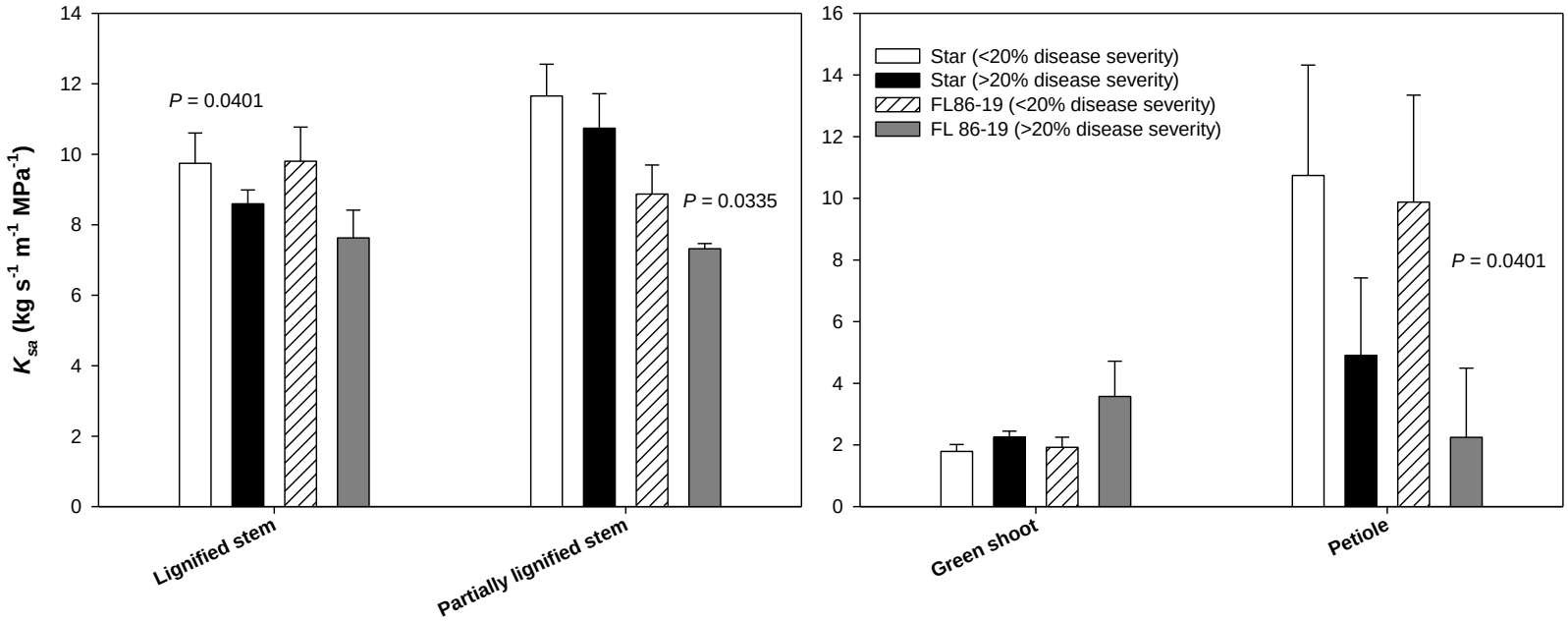

Figure 4.3. Hydraulic conductance per cross-sectional stem area (K_{sa}) of different tissue types on Star (moderately susceptible) and FL 86-19 (highly susceptible) southern highbush blueberry having either <20% or ≥20% foliar disease of bacterial leaf scorch in 2011. Values are means and standard errors. *P*-values based on pairwise comparisons between the two disease severity classes for each cultivar (median test).

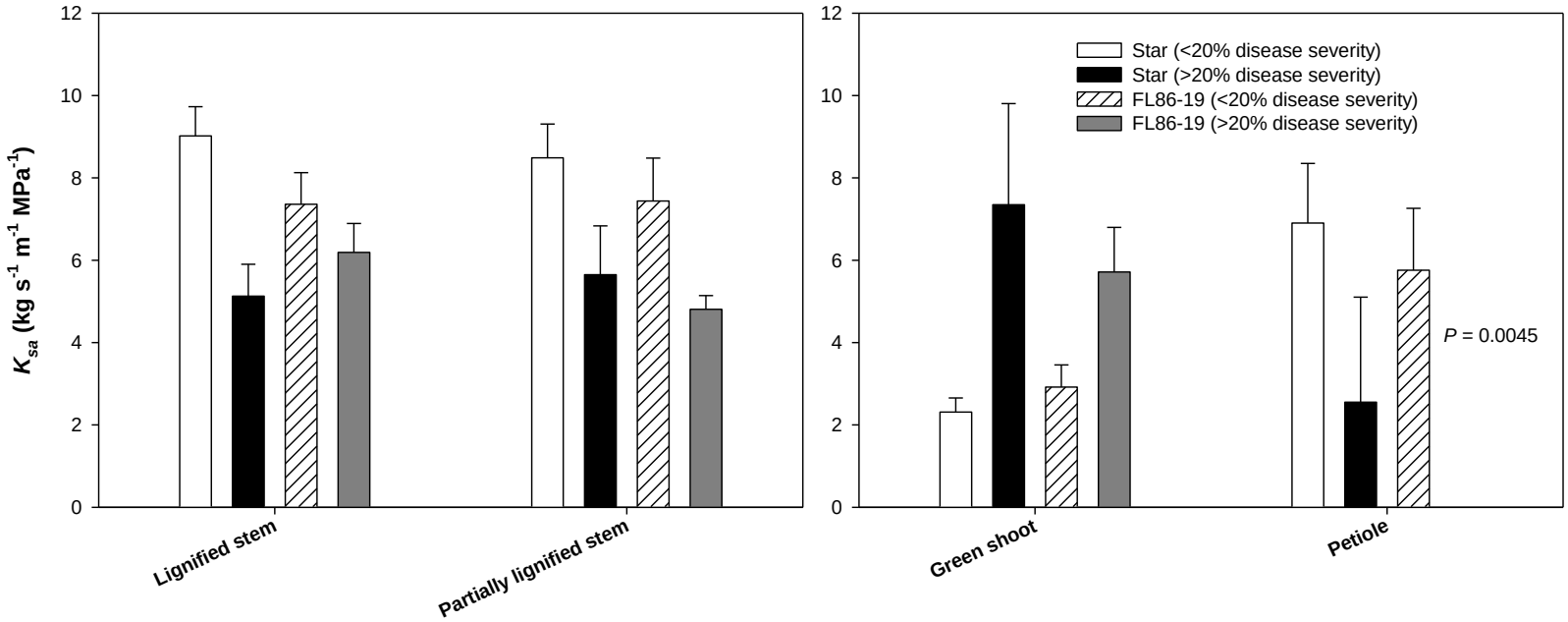

Figure 4.4. Hydraulic conductance per cross-sectional stem area (K_{sa}) of different tissue types on Star (moderately susceptible) and FL 86-19 (highly susceptible) southern highbush blueberry having either <20% or ≥20% foliar disease of bacterial leaf scorch in 2012. Values are means and standard errors. *P*-values based on pairwise comparisons between the two disease severity classes for each cultivar (median test).

CHAPTER 5

CONCLUSIONS

Research in this thesis investigated three aspects of bacterial leaf scorch in southern highbush blueberry all with direct or indirect implications for disease management options. The first study determined population densities of *Xylella fastidiosa* in stem sections taken from top to bottom as well as roots from asymptomatic, lightly, moderately, and severely symptomatic plants of susceptible southern highbush cultivars in the field. Bacterial titer was determined by extracting xylem sap from each section of the plant and testing the sap for the target pathogen using quantitative real-time PCR. Results showed that *X. fastidiosa* was detectable in the lower sections of the plant even in asymptomatic tissue, and that bacterial densities were initially low in the roots, but then increased in the roots as symptoms progressed. This demonstrated the rapid distribution of the pathogen within the plant, even at low severity levels, and thus suggested that management tactics such as pruning and mowing are unlikely to be effective because bacteria would be protected in lower stem sections and roots.

The second study assessed the likelihood of *X. fastidiosa* transmission through vegetative propagation by taking asymptomatic softwood cuttings from symptomatic plants and then conducting a series of rooting, growth, and disease assessments of the propagated plants at various ages and for both spring and fall cutting seasons. Plants suspected to be infected by *X. fastidiosa* underwent testing to confirm presence or absence of the target pathogen using real-time PCR. Even though most plants were asymptomatic at the final comprehensive disease assessment 12 to 27 months after the cuttings had been collected, >5% of plants proved to be

positive for *X. fastidiosa* during molecular testing, indicating that these plants could provide a source of inoculum in the field. We consider this level of transmission low but non-negligible. Our results suggest that precautionary measures should be taken during propagation to lessen the possibility of disease transmission.

The third study conducted as part of this thesis investigated possible resistance mechanisms related to water flow capacity in highly susceptible FL 86-19, moderately susceptible Star, and field-resistant Emerald southern highbush cultivars. Hydraulic conductance was measured and hydraulic conductance per stem cross-sectional area (K_{sa}) was calculated in different age classes of stems, green shoots, and petioles from asymptomatic and symptomatic plants collected in the field. In asymptomatic plants, In the absence of disease, Emerald had consistently the highest K_{sa}, especially in lignified and partial lignified stems, compared with moderately susceptible Star and highly susceptible FL 86-19. This high level of innate water flow capacity in Emerald may indicate a reduced propensity for xylem occlusion, thereby helping to explain its field resistance. For Star and FL 86-19, when tissues classified into different disease severity classes were compared, K_{sa} was generally lower (with the exception of the green stem sections) in plants with ≥20% foliar leaf scorch severity than in those with <20% disease severity. This difference was consistently significant in the petiole tissue of the highly susceptible FL 86-19 across both years. These differences among cultivars and between asymptomatic and symptomatic plants at the xylem functional level indicates that a detailed anatomical study is warranted to provide structural mechanism(s) for differences in hydraulic conductance and disease susceptibility among these cultivars.

In summary, starting with a resistant cultivar may be the only viable disease management option for bacterial leaf scorch of blueberry. Most likely, infections cannot be pruned or mowed

out successfully once symptoms are visually apparent. Prevention of disease transmission with clean propagation techniques is important for aiding in the management of the disease in cultivars that are at least partially susceptible. Bacterial leaf scorch will need innovative management approaches and further efforts to elucidate the mechanisms behind the high level of field-resistance in certain cultivars such as Emerald.