How Far has *Xylella fastidiosa* Advanced in Field-Grown Southern Highbush Blueberry Plants with Different Levels of Bacterial Leaf Scorch Severity?

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Objectives

To determine whether blueberry plants infected by *Xylella fastidiosa* can be cured by practices such as late-season pruning or flail-mowing, growers need to know where in relation to bush architecture the pathogen is located in plants of different severity classes. The objective of this project was to develop this information by sampling stem sections, from the youngest growth down to the roots, in naturally infected plants and determining bacterial concentrations in these sections in relation to disease severity.

Justification

Bacterial leaf scorch caused by the xylem-limited bacterium *Xylella fastidiosa* is a lethal disease that affects several popular cultivars of southern highbush blueberry such as FL 86-19 (V1), Star, and Rebel (Brannen et al. 2008). The disease is prevalent in the major blueberry-producing states within the SRSFC, including Georgia, Florida, and North Carolina. The pathogen is transmitted by xylem-feeding leafhoppers, primarily the glassy-winged sharpshooter, *Homalodisca vitripennis* (Tertuliano et al. 2010). It infects and colonizes the xylem of the plant, obstructing water movement and ultimately causing death of the plant. There are currently no effective management options for bacterial leaf scorch in existing plantings. For new plantings, choosing cultivars with lower disease susceptibility, such as Emerald, is the best option.

Pruning has been used previously for managing diseases caused by *X. fastidiosa*. For Pierce's disease of grape (caused by another strain of *X. fastidiosa*), the date of inoculation affects within-plant movement of the bacterium in the field. 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 plants from the earlier inoculated batch recovered compared with 88% of vines from the later inoculated batch. Of the recovered vines, half had noticeably shorter new canes 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, different pruning procedures 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. 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 underwent dramatic pruning such as skeleton cut and 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, 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 become first apparent. The pathogen is thought to be inoculated into current-season growth, given that the glassy-winged sharpshooter 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 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, there is a need to determine the presence or absence in tissue sections of different age of the bacterium in plants at 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.

Methodologies

Sample collection. Three southern highbush blueberry plantings severely affected by bacterial leaf scorch were sampled for stem and root sections, utilizing mature plants of cultivars FL 86-19, Bluecrisp, and Star. In each field, 10 asymptomatic plants as well as 10 plants each with light, moderate, or severe visual symptoms of bacterial leaf scorch were selected in September or early October when symptoms are generally most severe (total of 40 plants per field). 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. 1). In addition, root segments from the same sector within the plant where the stem originated were sampled. The pruning shears used to make the stem and root sections were surface-disinfested with disinfecting wipes (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, and xylem sap was extracted from each stem or root section using physical pressure applied with a vise. Using a micropipette, the extruding xylem sap was collected into sterile microcentrifuge tubes and stored at −80^{o}C. To determine bacterial titer, total DNA was extracted from 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 TGTTGG CAT T -3 and EFTu_3 – Rev 5- AGC CTG ACCTTG ATC CAATAA -3. 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 of each primer (10 µM), and 2 µl sterile distilled deionized water. Reaction cycling conditions were 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* cells diluted 1:10 through 10^2 CFU/mL in 50 µl of xylem sap taken from disease-free plants.

Data analysis. To facilitate data presentation and analysis, 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 the 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* by real-time quantitative PCR was lowest (but non-zero) in asymptomatic plants and highest in plants with severe symptoms (Figs. 2 to 4). 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 1). In asymptomatic plants, detection was least frequent (zero in the case of Bluecrisp) in top and root sections, and highest (up to 50% in the case of FL 86-19) in middle and stem base sections. Samples from asymptomatic Star deviated slightly by having a higher detection incidence in roots (10.0%) than in stem base sections (0%), but detection in roots was based on a single positive sample (out of 10 tested) having a low bacterial density of ~85 cells/50 µl of sap.

As disease severity of the sampled plants increased, detection frequency increased in all tissue sections (Figs. 2 to 4). Even when disease severity was low (Figs. 2B and 3B), detection frequencies exceeded 50% in middle and base stem sections and were 30.0% (Bluecrisp) and 60.0% (FL 89-16) in root sections. Overall, detection frequencies were highest (≥80%) in middle, base, and root sections of plants from the moderate and severe classes. In contrast, detection frequency in the top sections of Bluecrisp and FL 86-19 remained below 50%, even in severely symptomatic plants (Figs. 2 and 3).

Bacterial titers. Bacterial population densities in xylem sap (Figs. 5 to 7) closely mirrored detection frequency data shown previously. 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. 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 the field.

Conclusions

This study suggests that pruning or flail-mowing are unlikely to be effective management options for bacterial leaf scorch in southern highbush blueberry. This was deduced from 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. Our findings further suggest that pathogen detection 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 a low disease severity level, the highest bacterial titer was 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 indicates rapid systemic spread, possibly by twitching motility which allows the pathogen to move basipetally from the inoculation site against the xylem flow (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 root tissue but not from stem sections (Chang et al. 2009).

It is not certain where *X. fastidiosa* is inoculated into blueberry plants. However, since the glassy-winged sharpshooter, the most common 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 shown in Fig. 1).

The fact that *X. fastidiosa* was detected from asymptomatic plants in this study was not surprising. All fields sampled had a high incidence of the disease, and as such it is very 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 (~50 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 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 to occur (Hill and Purcell 1995), but our results suggest otherwise, given that 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 infected vines were readily cured by pruning 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 had already established itself in the middle and base stem sections of asymptomatic plants and even further into 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 owing to the rapid establishment of the pathogen in the lower base sections and roots of the blueberry plant.

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Table 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 separate fields.

Plant severity	Detection frequency (%)		
class	Bluecrisp	FL 89-16	Star
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. 1, with ten replicates for each segment type.
[b] Low severity class not sampled for Star.

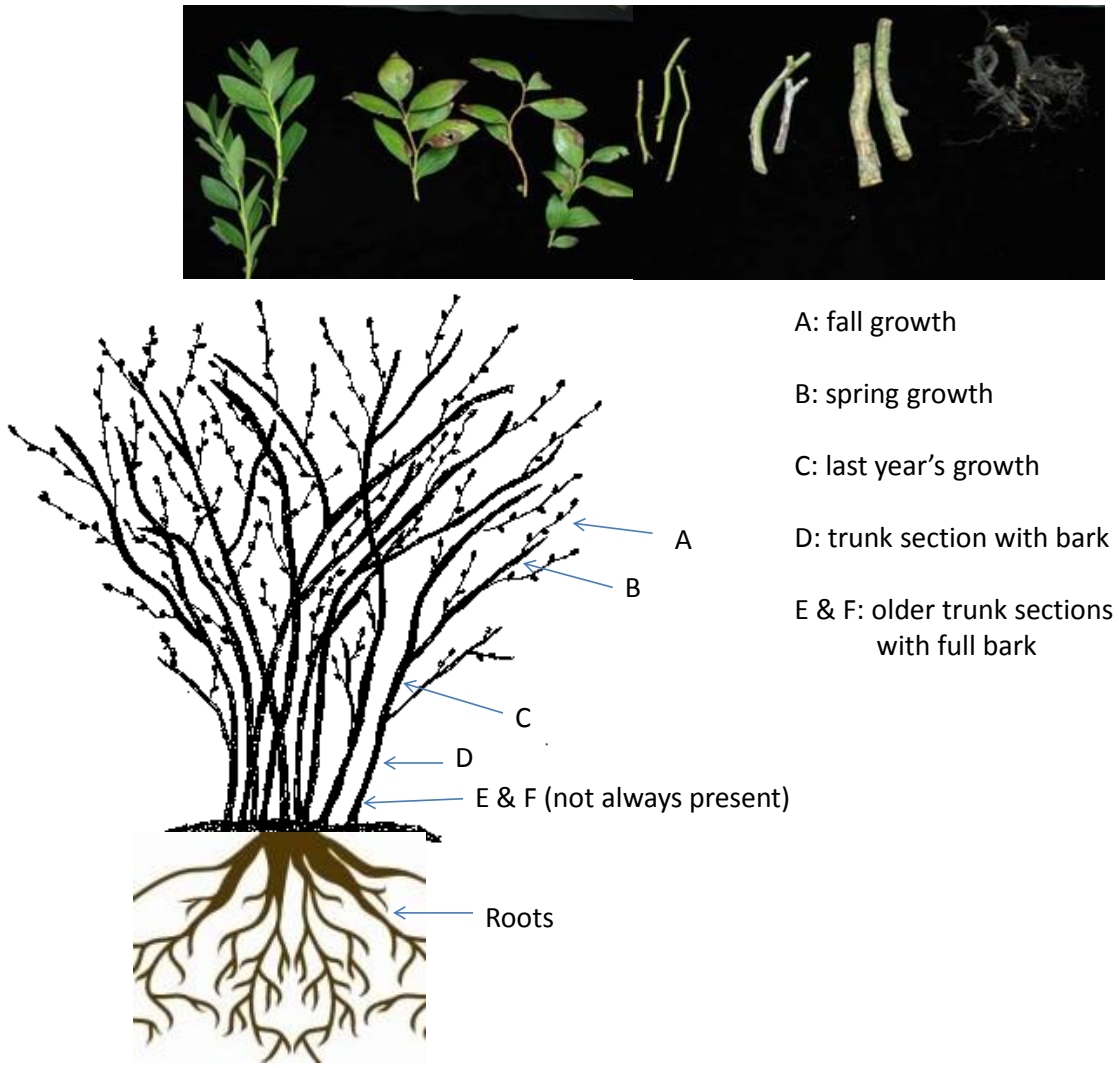

Figure 1. Stem and root sections used for xylem sap sampling to detect *Xylella fastidiosa* in naturally infected southern highbush blueberry plants in the field.

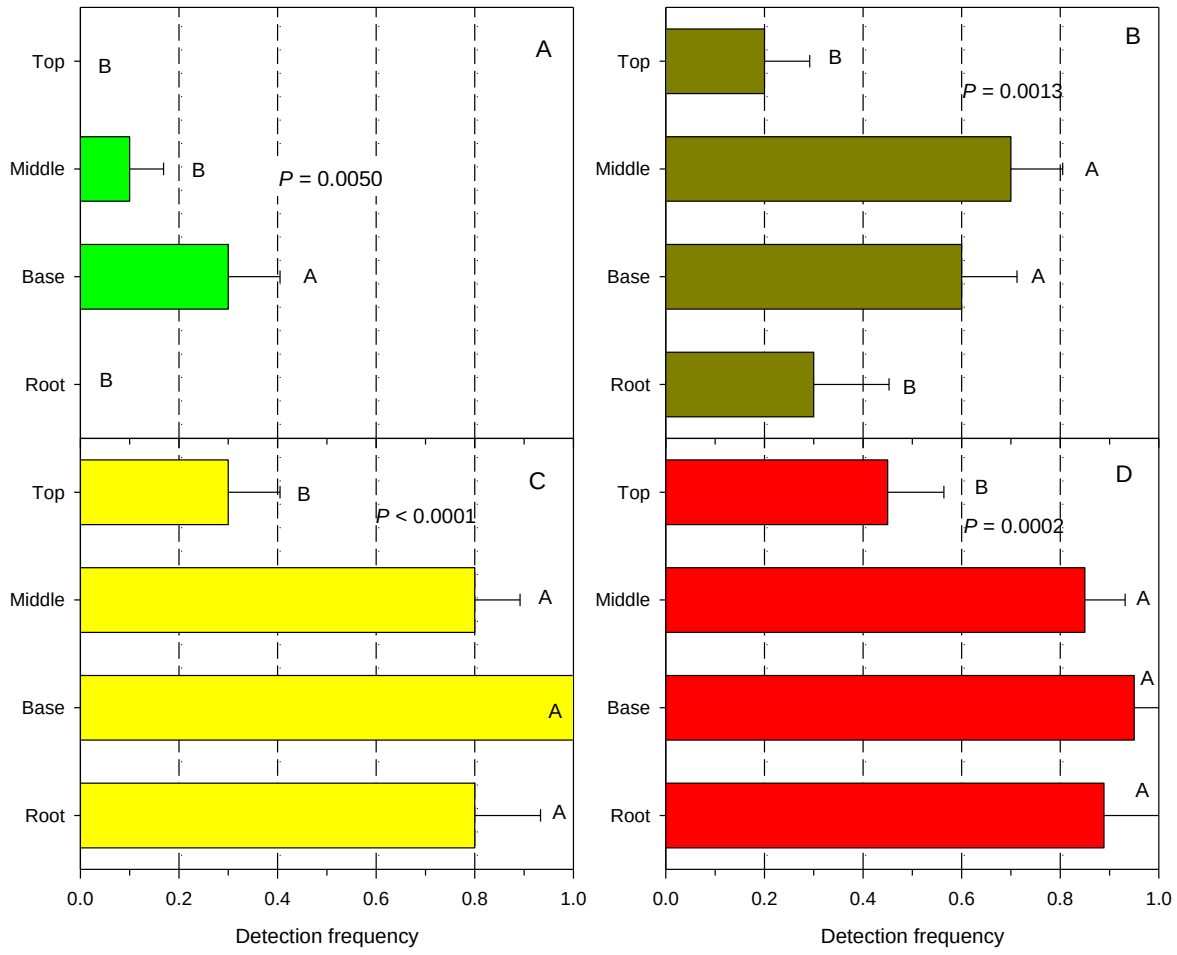

Figure 2. Detection frequency (proportion) of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of Bluecrisp southern highbush blueberry in asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms. 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 ($P > 0.05$).

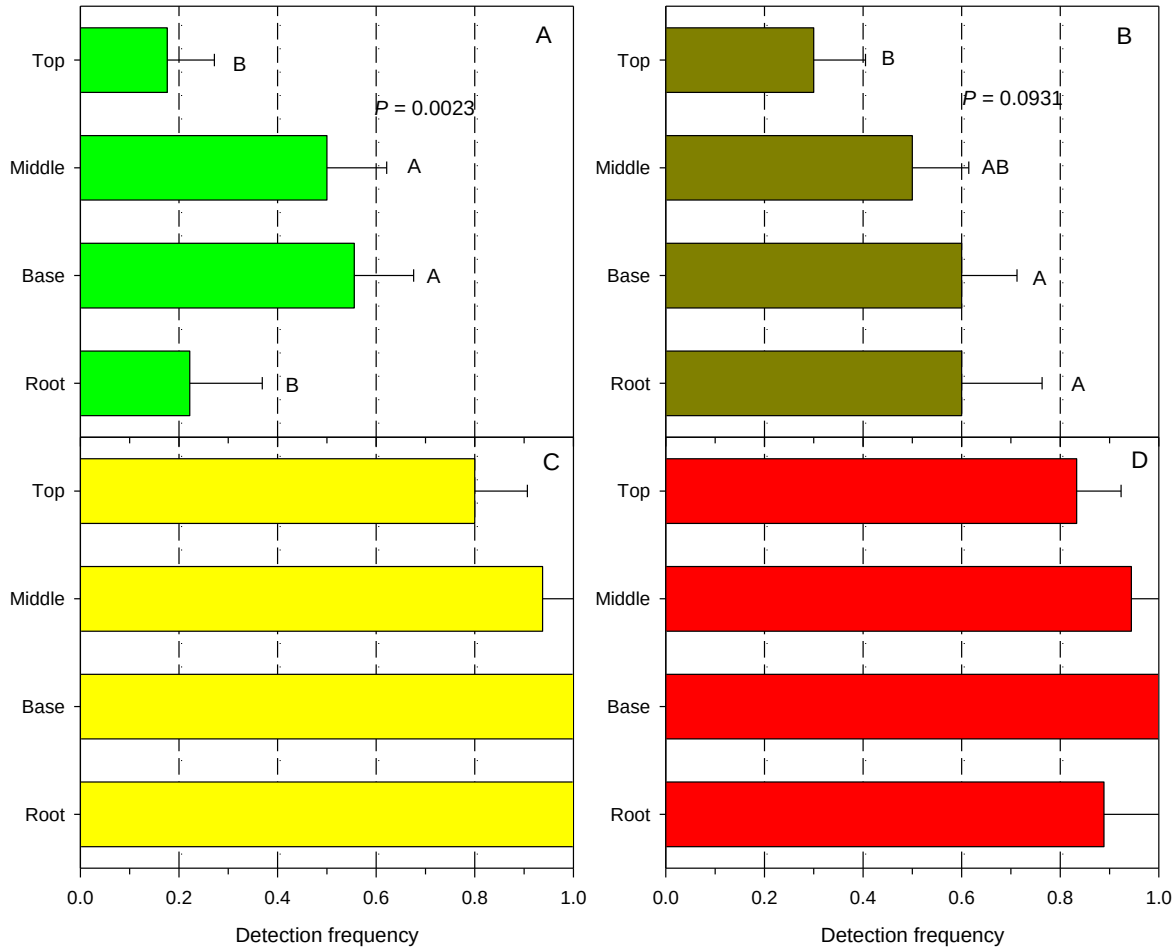

Figure 3. Detection frequency (proportion) of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of FL 89-16 southern highbush blueberry in asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms. 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 ($P > 0.05$).

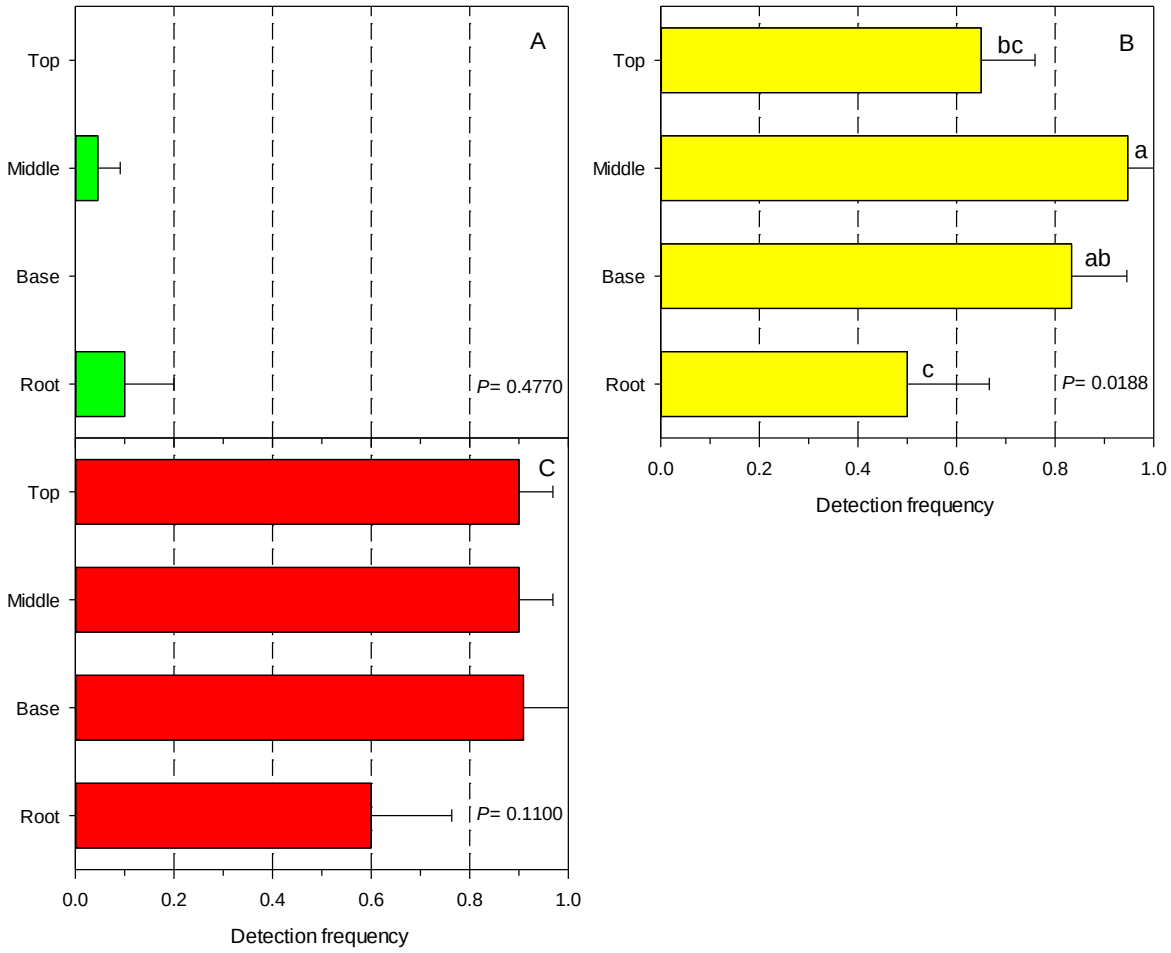

Figure 4. Detection frequency (proportion) of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of Star southern highbush blueberry in asymptomatic plants (**A**) and those having moderate (**B**) and severe (**C**) bacterial leaf scorch symptoms. 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).

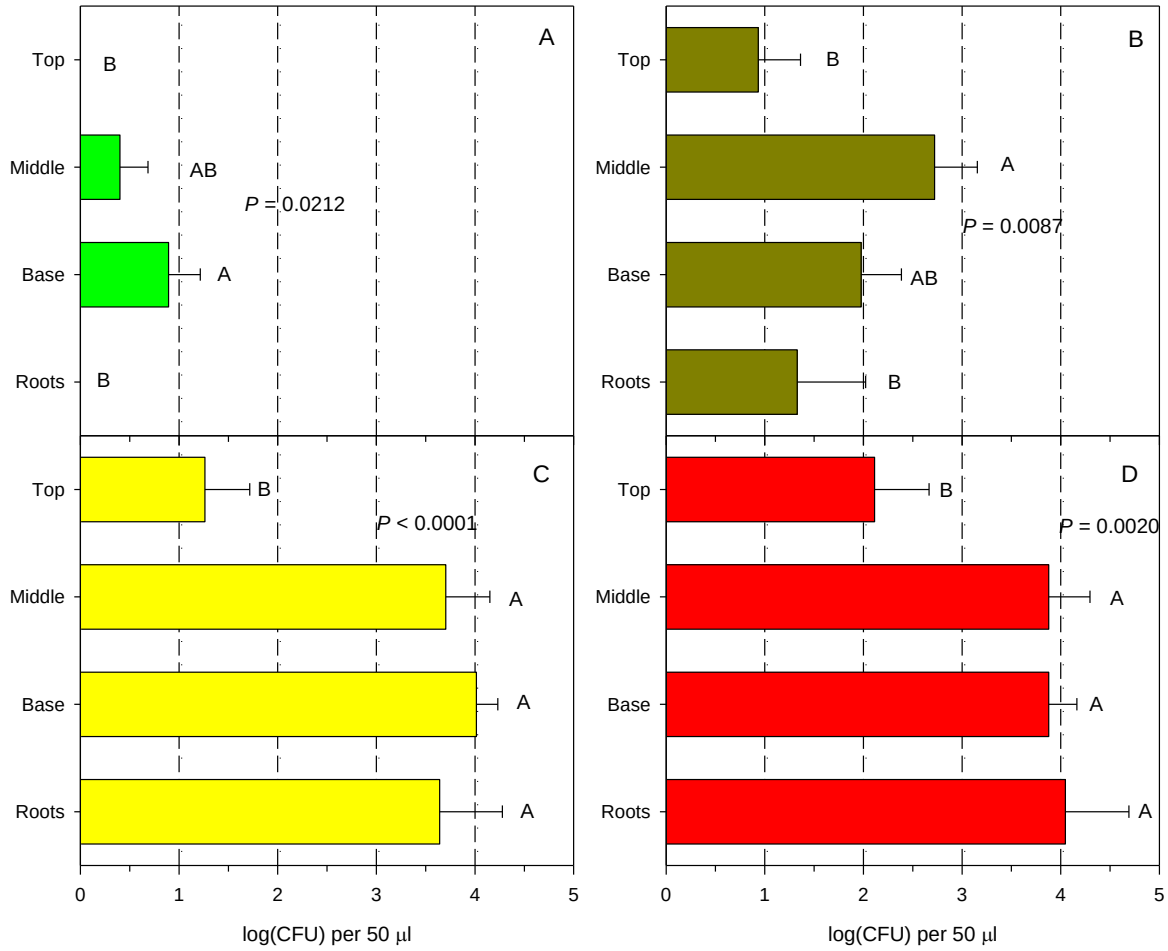

Figure 5. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of Bluecrisp southern highbush blueberry in asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms. 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 ($P > 0.05$). CFU = colony-forming units.

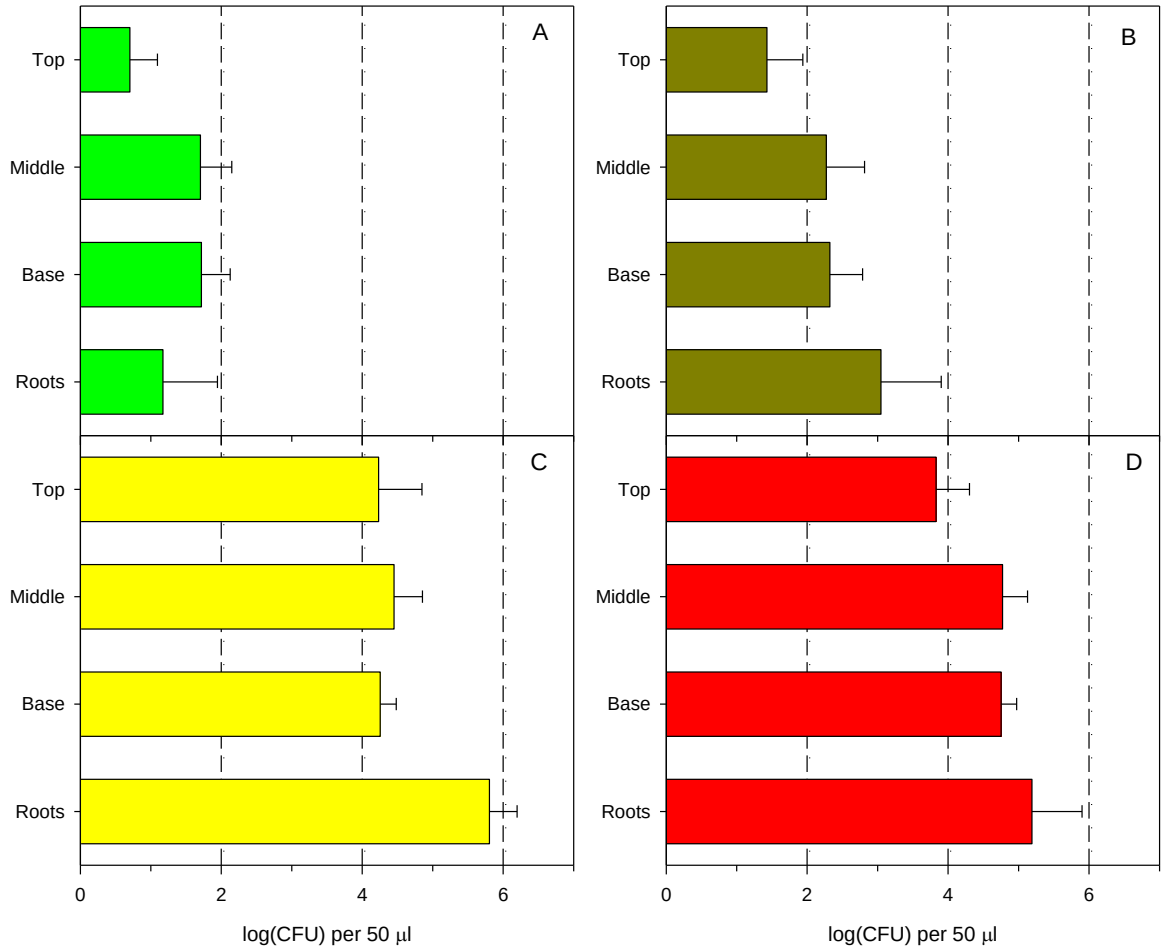

Figure 6. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of FL 89-16 southern highbush blueberry in asymptomatic plants (**A**) and those having light (**B**), moderate (**C**), and severe (**D**) bacterial leaf scorch symptoms. Values are means and standard errors. There were no statistically significant differences among sections within disease severity classes ($P > 0.05$). CFU = colony-forming units.

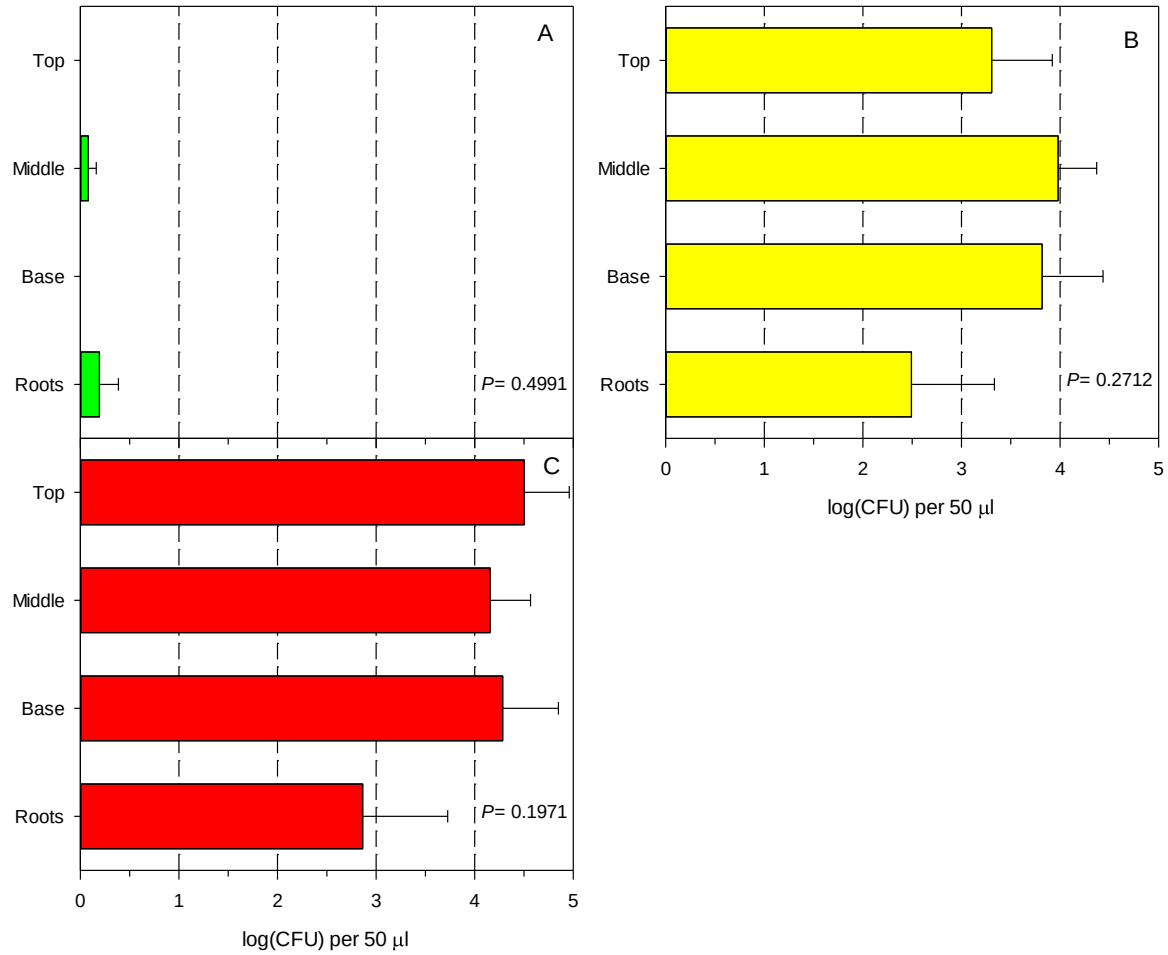

Figure 7. Titer of *Xylella fastidiosa*, as determined by real-time quantitative PCR, in xylem sap from different stem sections (top to bottom of plant) and roots of Star southern highbush blueberry in asymptomatic plants (**A**) and those having moderate (**B**) and severe (**C**) bacterial leaf scorch symptoms. Values are means and standard errors. There were no statistically significant differences among sections within disease severity classes ($P > 0.05$). CFU = colony-forming units.

Impact Statement

Distribution of the pathogen causing bacterial leaf scorch in blueberry plants with varying symptom severity

Summary

The bacterium *Xylella fastidiosa* infects the xylem (water-conducting tissue) of plants, causing bacterial leaf scorch, a lethal disease of southern highbush blueberries. Research by UGA scientists and graduate students showed that the bacterium spreads quickly within plants once the first symptoms become apparent, rendering attempts to remove infections by pruning or mowing ineffective.

Situation

Southern highbush blueberries, which produce early-maturing, high-value fruit, have been introduced relatively recently into the southeastern United States, fueling an economic boon in rural areas of the Coastal Flatwoods of Georgia. In response to the rapidly increasing acreage, several new diseases have been observed affecting this blueberry type. One of the most damaging of these diseases is bacterial leaf scorch, caused by the plant-pathogenic bacterium *Xylella fastidiosa*. This bacterium is transmitted to the plant by certain leafhopper species while they feed on blueberry shoots. The pathogen infects the xylem (water-conducting tissues) of the plant, obstructing water movement and ultimately causing death of the plant. Several of the most widely planted southern highbush cultivars are susceptible to the disease, and there are currently no reliable management options for these cultivars. 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.

Response

Three southern highbush blueberry fields naturally infected with bacterial leaf scorch were sampled to determine the distribution of *X. fastidiosa* in different tissue types. In each field, 10 asymptomatic plants as well as 10 plants each with light, moderate, or severe symptoms of bacterial leaf scorch were selected in September or October when symptoms were most pronounced. Within each plant, a representative main stem was selected and dissected into stem sections corresponding to different age groups, i.e., fall growth, spring growth, last year's growth, and lower trunk sections of different diameter classes. In addition, root segments from the same sector within the plant where the stem originated were sampled as well. Xylem sap was extracted from each stem or root segment in the laboratory, and concentrations of *X. fastidiosa* in the sap were determined using real-time quantitative PCR with species-specific primers following total DNA extraction from sap.

Results/Impacts

The bacterium was not detected in top sections (youngest growth) and roots of asymptomatic plants, but was detected at low levels in middle and base stem sections of such plants. In plants with light symptoms, the bacterium was readily detected in all stem sections (top, middle, and base) as well as in

roots, indicating that *X. fastidiosa* spreads quickly as symptoms become apparent. Bacterial concentrations were again highest in middle and base stem sections. In plants with moderate and severe symptoms, bacterial concentrations were highest in middle and base stem sections as well as in roots, indicating that the pathogen accumulates in the roots over time. Because the bacterium is already present in lower sections of the plant when symptoms become first apparent, selective pruning is not a suitable management practice for removal of bacterial leaf scorch infections. Similarly, because of the presence of the bacterium in the roots in moderately or severely affected plants, mowing of such plantings will be ineffective in eliminating the disease.