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INVESTIGATING THE *XYLELLA FASTIDIOSA* IN PIERCE'S DISEASE RESISTANT AND SUSCEPTIBLE GRAPEVINES

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Abstract. **Pierce's Disease (PD) is a major factor limiting grape production in the southeast United States. The disease is caused by *Xylella fastidiosa* Wells et al., a gram-negative bacterium that is transmitted to the xylem system of the grapevines primarily by glassy-winged sharpshooters (*Homalodisca coagulata* Say). Once it is in the xylem, the *X. fastidiosa* bacterium will use the xylem sap as a nutrient source to multiply, which may eventually cause PD in susceptible cultivars. In order to understand the fate, existence, and movement of *X. fastidiosa* in resistant, tolerant, and susceptible grapevines during the growing and dormant seasons, the presence of *X. fastidiosa* was investigated in field-grown and greenhouse-inoculated vines. *X. fastidiosa* was detected directly from xylem sap of field-grown grapevines by bacterium culture and confirmed by polymerase chain reaction. *X. fastidiosa* was detectable throughout the growing season in the PD susceptible cultivar 'Chardonnay' and the PD tolerant bunch grape cultivar 'Blanc du Bois'. The bacteria were also detectable in the dormant vines with high density in these two cultivars. *Xylella fastidiosa* was found in dormant canes of muscadine grape 'Carlos', but density of *X. fastidiosa* bacteria decreased throughout the winter months. Movement of *X. fastidiosa* in greenhouse-inoculated plants was indicated by the appearance of PD symptoms on the leaves. Symptoms of PD appeared about five weeks after inoculation in the highly susceptible cultivar 'Chardonnay', six weeks in 'Niagra', 'Concord', and the tolerant cultivar 'Lake Emerald', and the resistant muscadine cultivar 'Fry'. No difference was found in PD sensitivity among the susceptible cultivar 'Chardonnay' grafted on different rootstocks under the greenhouse conditions.**

Pierce's Disease (PD) is a major factor limiting grape (*Vitis* L.) production in the southeast United States. In Florida, it has precluded the commercial production of European grapes *V. vinifera* L. and American grapes *V. labrusca* L. The growth and development of a grape industry in Florida rests heavily upon PD resistant or tolerant muscadine grapes *V. rotundifolia* Michx. though incidence of PD has been reported in several muscadine grape cultivars (Hopkins et al., 1974; Lu et al., 2000).

PD in grapevines is caused by *Xylella fastidiosa*, which is a gram-negative, xylem-limited bacterium (Hopkins, 1989). *X. fastidiosa* is transmitted into xylem of grapevines by sharpshooters, particularly the glassy-winged sharpshooters (Hill and Purcell, 1997). Once it is in the xylem, the *X. fastidiosa* will use the xylem sap as a nutrient source to multiply, spread, and increase to concentrations that clog the xylem vascular tissue and can eventually cause PD in susceptible grape cultivars.

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Various strains of *X. fastidiosa* have been isolated from grapevines. Some strains are virulent while others are avirulent (Hopkins, 1985). The virulent strain could multiple, move systemically, and reach populations of 10^6-10^7 colony-forming units per centimeter of inoculated petioles. Avirulent strain of *X. fastidiosa* could reach only 10^4-10^5 colony-forming units per centimeter and did not move beyond the inoculated internode (Fry and Milholland, 1990). Usually, early season infection has a long period of time for the PD bacteria to multiply, reproduce, and spread throughout the vine. Late season infection has a short period of time for the bacteria to grow.

Infection of virulent strains of *X. fastidiosa* is fatal in some grape cultivars such as most *V. vinifera* grapes. Infected plants exhibit leaf marginal scorching, cluster collapse, blackened and shriveled fruits, and uneven mature of stems. Dried leaves can fall off canes/stems leaving the petioles attached to the canes. Diseased vines can die in as little as one to two years (Hopkins, 1989). However, infection of virulent *X. fastidiosa* in other grape cultivars such as Florida hybrids and *V. rotundifolia* grapes does not appear to be a serious problem until fruit maturation. These cultivars exhibit a certain degree of tolerance or resistance to the PD infection. Infected vines are able to keep the causal agent *X. fastidiosa* bacterium at sub-clinical populations and appear as symptomless or recover during the winter (Hill and Purcell, 1997).

This study was undertaken to understand the fate, existence, and movement of *X. fastidiosa* bacteria in resistant, tolerant, and susceptible grapevines during the growing and dormant seasons.

Materials and Methods

Field-grown vines. This study was conducted at the Center for Viticulture and Small Fruit Research, Florida A&M University from 2000 to 2001 and continuing in 2002. Field-grown plants from PD tolerant muscadine grape cultivar 'Carlos', PD tolerant Florida hybrid bunch grape cultivar 'Blanc du Bois', and PD susceptible *V. vinifera* grape cultivar 'Chardonnay' were used for this study. Two plants were chosen from each cultivar. Four shoots from each plant were labeled and brought into laboratory for analysis. A Plant Moisture Stress (PMS) chamber (PMS Instrument Co., Corvallis, OR) was used to extract the xylem sap unless otherwise indicated.

Xylem sap was collected monthly from shoots, petioles, and roots. Bacteria were detected directly from xylem sap by culturing on PD3 medium according to Hopkins (1988). A virulent strain of *X. fastidiosa* bacterium, which was kindly provided by Hopkins (University of Florida, Apopka), was used as the control. Polymerase chain reaction (PCR) was used for confirmation of *X. fastidiosa.*

Symptoms of PD were rated on October 25, 2001 based on a scale of 0-5 for a single plant (Fig. 1) where: 0 = no symptoms; 1 = minor symptoms up to 10% of leaves with marginal necrosis; 2 = 11-30% of leaves with marginal necrosis; 3 = 31-50% of leaves with marginal necrosis; 4 = 51-75% of leaves with marginal necrosis, or dead growing point and; 5 = over

Fig. 1. Pierce's Disease (PD) symptoms.

75% of leaves with marginal necrosis or a dead arm. The same method was used for evaluating PD severity of single shoots. Ratings of PD for a single leaf on a shoot were based on a 0-4 scale where: 0 = no symptoms; 1 = 0-25% of leaf area with marginal necrosis; 2 = 26-50% of leaf area with marginal necrosis; 3 = 51-75% of leaf area with marginal necrosis and; 4 = over 75% of leaf area with marginal necrosis.

Greenhouse-grown vine. PD susceptible *V. vinifera* grape 'Chardonnay' and *V. labrusca* grapes 'Niagra', and 'Concord', PD tolerant Florida hybrid bunch grape cultivar 'Lake Emerald', and PD resistant muscadine grape cultivar 'Fry' were used in greenhouse study. Five plants from each cultivar were used. Three of them were inoculated on 5 May 2001 with suspension culture of *X. fastidiosa* by using needle puncture technique (Hopkins, 1989). Two vines were used as controls which were inoculated with PD3 medium only. Each plant was inoculated in section A—internode between 2nd and 3rd leaves from the bottom of the plant (Fig. 2). This operation was repeated three times every 6 days in the greenhouse of Florida A&M University using a completely random design. A similar experiment was conducted to investigate the rootstock effect on PD occurrence in *V. vinifera* cultivar 'Chardonnay' grafted on different rootstocks.

Symptoms of PD appearance were recorded every week in three sections: section A, as described above; section B being the internode between the 4th and 5th leaves, and; section C being the internode between the 6th and 7th leaves. The rest of the leaves have marked as others. Observation of PD began one week after inoculation and continued every week for five months.

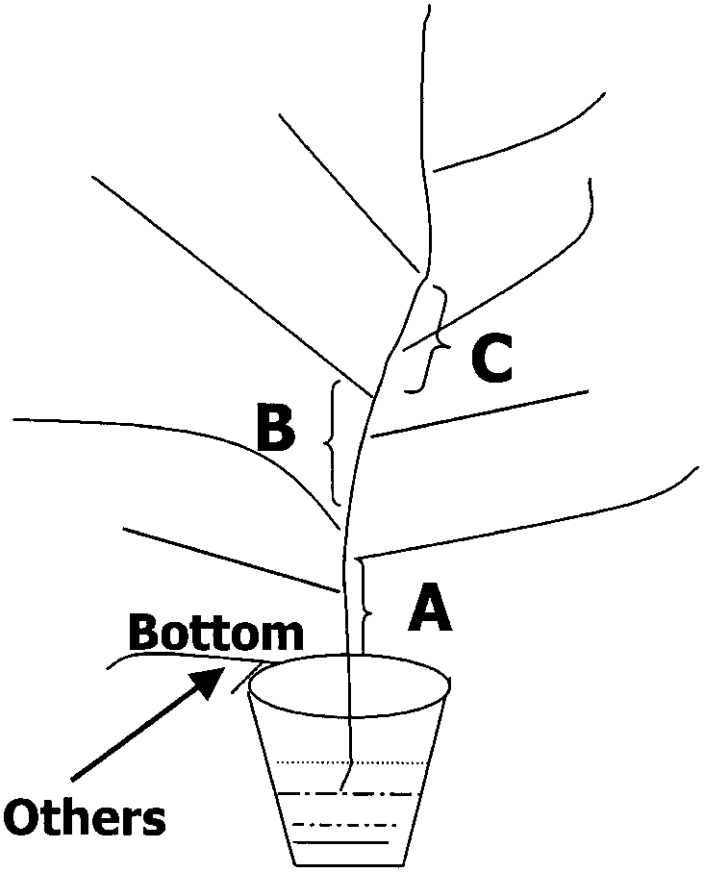

Fig. 2. Diagram of inoculation position of *X. fastidiosa* in a greenhouse-grown plant.

Results and Discussions

Isolation of bacteria from field-grown vines. The appearance of bacteria in xylem sap during the growing and dormant seasons varied among the resistant, tolerant, and susceptible grapevines when cultured on PD3 medium (Table 1). In the highly PD susceptible European cultivar 'Chardonnay', bacteria were detected in shoot xylem sap of the two selected plants throughout the year, including both the growing and dormant seasons. In the PD tolerant Florida hybrid cultivar 'Blanc du Bois', bacteria appeared in most months of the year of 2001 except July and early part of 2002. Unlike the PD susceptible 'Chardonnay', only shoots with PD ratings over 2 had bacteria growth on PD3 medium. There were relatively less bacterial colonies on the culture of xylem sap from 'Blanc du Bois' than from 'Chardonnay'. In the PD tolerant muscadine 'Carlos', bacteria were also isolated from shoots with PD symptoms during most months of 2001 and the early part of 2002. However, the number of bacterial colonies in 'Carlos' was significantly less than in 'Chardonnay' and 'Blanc du Bois'.

PCR confirmation of Xylella fastidiosa bacteria. Although bacteria were detectable in all tested grape cultivars by medium culture, not all of them were *X. fastidiosa* bacteria when they were used for amplification of the specific DNA sequence of *X. fastidiosa* bacteria by using polymerase chain reaction (Table 1). *X. fastidiosa* bacteria were consistently confirmed from the PD3 medium cultures in PD susceptible 'Chardonnay' throughout the year of 2001. However, *X. fastidiosa* bacteria were detectable in PD tolerant 'Blanc du Bois' only in January, February, August, September, October, November, and December of the year 2001. *X. fastidiosa* bacteria could not be detected in those vines from March to July 2001. Similar results were also obtained during the first half of 2002. For the PD tolerant muscadine 'Carlos', *X. fastidiosa* was not detectable until September, 2001. Positive PCR was continually confirmed for the following four months until January, 2002. For the rest of the first half of 2002, *X. fastidiosa* bacteria disappeared from the xylem sap of 'Carlos'. This result indicated that at least some *X. fastidiosa* bacteria in PD susceptible 'Chardonnay' were carried over from the previous season while others were newly introduced during the active growing season. On the other hand,

Table 1. Confirmation of *Xylella fastidiosa* in xylem sap of grape shoot tissue.

Cultivar	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Carlos												
PD3 culture	+	+	+	+	+	+	-	+	+	+	+	+
PCR	-	-	-	-	-	-	/	-	+	+	+	+
Blanc du Bois												
PD3 culture	+	+	+	+	+	+	-	+	+	+	+	+
PCR	+	+	-	-	-	-	/	+	+	+	+	+
Chardonnay												
PD3 culture	+	+	+	+	/	/	/	+	+	+	+	+
PCR	+	+	+	+	/	/	/	+	+	+	+	+

the *X. fastidiosa* bacteria in PD tolerant 'Carlos' were acquired from the sharpshooter feedings during the year.

When root xylem saps were cultured on PD3 medium, all the tested grape cultivars (PD susceptible 'Chardonnay', tolerant Florida hybrid bunch grape 'Blanc du Bois', and 'Carlos' muscadine) showed large populations of bacteria throughout 2001. However, when using PCR to confirm the status of these bacteria, no *X. fastidiosa* was detected. This result indicated that the *X. fastidiosa* bacteria may not move downward to the root systems through xylem after being introduced to the shoots by sharpshooters.

Neither *X. fastidiosa* nor other non-PD bacteria were detected in PMS chamber petiole sap extractions for any cultivar tested between May and November, 2001. Perhaps greater pressure is needed to extract *X. fastidiosa* out of petioles. No results can be reported for December, 2001 or the early months of 2002 due to the lack of petioles and leaves. Bacteria did appear on PD3 medium from August to November of 2001 from sap squeezed from the petioles after the epidermis was peeled and cultured on PD3 medium. Polymerase chain reaction confirmed the existence of *X. fastidiosa* in the petioles of the PD susceptible European cultivar 'Chardonnay' and the PD tolerant Florida hybrid 'Blanc du Bois', but not in petioles of the PD tolerant 'Carlos' muscadine.

Greenhouse-grown vines. Inoculation with *X. fastidiosa* in section A of the plants resulted in PD symptoms five weeks after the inoculation on leaves of PD susceptible *V. vinifera* cultivar 'Chardonnay'. PD symptoms appeared one week later in *V. labrusa* cultivars 'Niagra', and 'Concord', PD tolerant bunch grape cultivar 'Lake Emerald', and PD resistant muscadine cultivar 'Fry'. PD symptoms also appeared in sections B and C later among all the cultivars tested, including both resistant and susceptible grapes, even though no injections of *X. fastidiosa* were performed on those areas. This indicated that *X. fastidiosa* bacteria could move in xylem beyond the inoculation point. Table 2 shows the percentage of leaves with PD symptoms for sections A, B, and C combined. No PD symptoms were observed in control plants throughout the experiment period. The highly susceptible *V. vinifera* cultivar 'Chardonnay' and *V. labrusca* cultivar 'Niagra' had the highest percentage of leaves with PD symptoms while the PD resistant muscadine grape cultivar 'Fry' showed the lowest percentage of symptomatic leaves. In this study, both PD resistant 'Fry' and PD tolerant 'Lake Emerald' displayed moderate PD symptoms. This could indicate that the three inoculation times used in this experiment might have been excessive.

Presence of PD in grafted 'Chardonnay' vines was less severe early during the experimental period than for 'Chardonnay' grown on its own roots (Table 2). However, the severity leveled out as the experiment continued. At the end of the study, the rootstocks did not seem to provide protection from PD development under greenhouse conditions.

Table 2. PD development on greenhouse-grown plants.

Date	Cultivar														
	Chardonnay			Niagra			Concord			Lake Emerald			Fry		
	Total	PD	%	Total	PD	%	Total	PD	%	Total	PD	%	Total	PD	%
	Leaves														
06/13/01	29.7	4.0	15.4	30.7	0	0	28.7	0	0	27.3	0	0	38.3	0	0
06/20/01	30.3	5.5	18.6	22.7	1.7	7.2	28.7	1.0	3.3	27.3	2.0	7.1	38.0	2.0	5.4
06/29/01	30.8	8.5	27.8	23.0	3.7	16.1	29.7	4.0	14.0	28.3	4.3	14.8	38.7	5.7	15.1
07/06/01	31.0	11.0	35.9	23.0	6.0	25.9	30.7	7.0	22.5	28.3	4.0	14.8	40.7	7.0	17.4
07/21/01	36.0	14.0	38.4	26.0	8.0	31.0	34.0	6.7	20.1	35.0	12.0	34.3	44.7	9.7	21.6
07/28/01	37.0	16.0	44.4	26.3	11.0	41.5	34.7	8.0	24.5	36.7	14.0	38.4	45.3	10.0	22.8
08/08/01	33.5	23.0	69.0	34.7	20.0	55.8	34.3	15.0	43.4	29.3	13.0	39.9	46.0	17.0	37.8
08/16/01	34.3	25.0	73.1	39.0	27.0	65.8	36.7	19.0	51.8	30.0	14.0	43.4	46.0	18.0	38.6
08/22/01	36.0	29.2	81.3	40.3	32.3	77.4	36.7	22.3	60.1	30.0	17.0	54.0	46.0	21.3	46.5
09/05/01	28.5	23.0	81.2	40.7	33.0	80.1	39.0	25.0	62.4	32.3	20.0	60.4	49.3	27.0	54.7
09/18/01	30.5	27.0	89.0	42.3	37.0	87.0	42.0	34.0	79.6	36.0	31.0	83.6	52.3	46.0	87.3

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