CORRELATION BETWEEN RESISTANCE TO PIERCE'S DISEASE AND *XYLELLA* STRAIN VIRULENCE USING PARTIALLY PURIFIED CULTURE FILTRATE

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ABSTRACT
Previous research at the FAMU Center for Viticulture suggested that cells of a virulent strain of *Xylella fastidiosa* (*Xf*) may produce toxic compounds that could be used to determine varietal susceptibility to Pierce's disease (PD) in grapes. I n the experiments reported here, when grape leaves were challenged with partially purified culture filtrate of *Xf* with different levels of virulence, positive correlations between the degree of leaf necrosis and (1) the virulence of the *Xf* strain and (2) the level of PD resistance were observed.

INTRODUCTION
Pierce's disease (PD), a lethal disease of grapevine, is caused by the bacterium *Xylella fastidiosa* (*Xf*) (Proteobacteria: Xanthomonadales) and is spread by leafhoppers known as sharpshooters. *Xylella fastidiosa* is native to the southeastern U.S., where it reproduces in ornamentals such as crape myrtle, eucalyptus, and hibiscus, but also in various crop plants including citrus, avocado and grapes (Blua et al. 1999). In Florida and other southeastern States, the abundance of *Xf* and vectors such as the glassy-winged sharpshooter (*Homalodisca coagulata*) has precluded commercial production of European grape varieties. The first evidence of PD infection usually is a drying or "scorching" of leaves. Typically, the leaves dry progressively over a period of days to weeks, showing a series of concentric zones of discolored and dead tissue. Vines develop symptoms as the bacteria multiply and begin to block the water conducting system and reduce the flow of water to affected leaves. However, Hopkins (1983) reported that only about 40% of the xylem vessels of infected plants have bacterial occlusions and plants with this percentage of non-functioning vessels typically do not show symptoms of water stress. The PD bacterium also has been reported to produce a phytotoxin or phytotoxins that may damage plant tissues and play an important role in disease initiation and development (Lee 1982).

OBJECTIVES
1. Determine whether partially purified culture filtrate from virulent, weakly virulent and avirulent strains of *Xf* would produce different levels of necrosis when applied to leaves of a given variety of grape.
2. Determine whether partially purified culture filtrate from a given strain of *Xf* would produce different levels of necrosis when applied to leaves from susceptible, tolerant and resistant varieties of grape.

RESULTS AND CONCLUSIONS
Cultures of virulent (PD002), weakly virulent (PD91-2) and avirulent (PD F1) strains of *Xf* were centrifuged to remove cells. The supernatant was filtered and then extracted with ethyl acetate, and the eluate was evaporated to dryness. The powder was then reconstituted in distilled water and applied to the surface of detached leaves of different grape varieties that had been wounded with a sharp needle. After 48 h, the leaves were scored based on the percentage of the leaf surface with necrotic lesions (Table 1).

In general, the mean percentage of leaf necrosis was greater when leaves were challenged with partially purified culture filtrate (PPCF) from the more virulent strains of *Xf*. For example, the leaf necrosis rating for 'Chardonnay', a highly PD susceptible variety of *V. vinifera* grape, was 1.5 for the virulent strain of *Xf*, 0.9 for the weakly virulent strain and 0.3 for the

avirulent strain. The leaf necrosis ratings for Black Beauty, a PD tolerant variety of muscadine grape, were 0.7, 0.4 and 0.1 when challenged with PPCF from the virulent, weakly virulent and avirulent strains of *Xf*, respectively.

In addition, leaves from susceptible varieties of grape generally produced greater levels of necrosis than did leaves from tolerant and resistant varieties. For example, the mean percentage of leaf necrosis for 'Chardonnay', 'Blanc du Bois' (a PD tolerant Florida hybrid bunch grape), Alachua and Noble (PD resistant muscadine grapes) were 1.5, 1.0, 0.6 and 0.0, respectively, when challenged with the PPCF from the virulent strain of *Xf*. Similar and consistent trends also were observed when using PPCF from the weakly virulent and avirulent strains of *Xf*, but as mentioned before, the leaf necrosis ratings were lower, which resulted in less overall differences between susceptible and resistant varieties.

These results suggest that *Xf* may produce extra cellular "toxin(s)" that could cause necrotic lesions when applied to grape leaves and that might have potential in screening grape germplasm and hybrids for PD resistance. The "toxins" extracted from the culture filtrate of more virulent strains of *Xf* produced more necrosis than did the "toxins" from less virulent strains. Leaves from susceptible varieties of grape also reacted more strongly to these "toxins" than did the leaves from resistant grape varieties. At this time the nature of the "toxin(s)" is not known, nor is it known whether the different strains of *Xf* produce different quantities or types of these "toxins". Future studies will attempt to answer these questions and expand the number of PD susceptible and resistant grape varieties and *Xf* strains evaluated with this test.

A

B

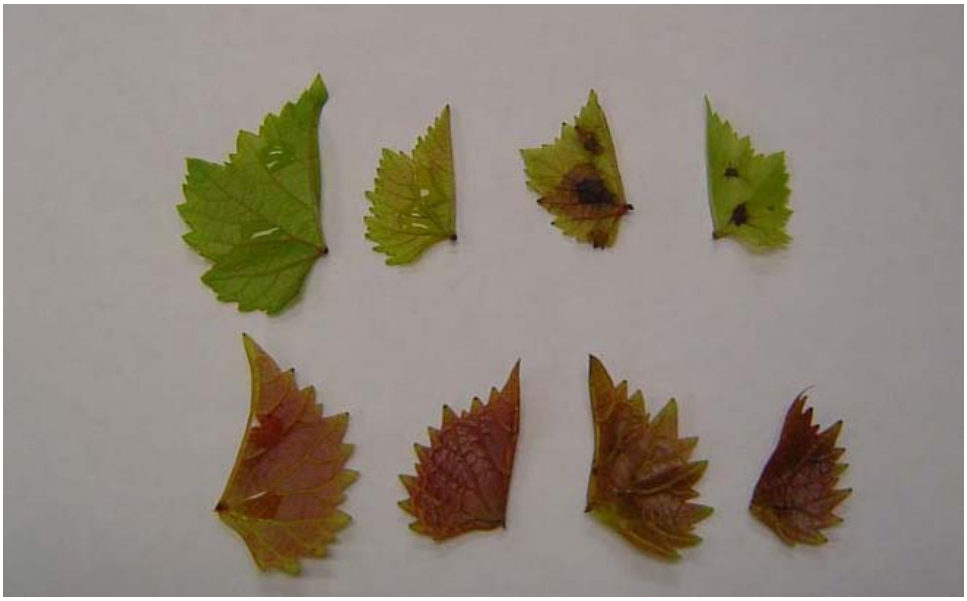

Figure 1. An example of the type of symptoms caused by *Xf* culture filtrate in young 'Chardonnay' (A, PD susceptible) and 'Noble' (B, PD resistant) grape leaves. Lanes 1 and 2 = control leaves treated with distilled water, lane 3 = leaves treated with undiluted culture filtrate from a virulent strain of *Xf* PD002, and 4 = leaves treated with diluted (1:2 vol/vol) culture filtrate of *Xf* PD002. Incubation time was 48 h.

Table 1. Response of grape leaves to partially purified culture filtrate from virulent (PD002), weakly virulent (PD91-2), and avirulent (PD-F1) strains of *Xf* as measured by the amount of necrosis produced. Leaf necrosis ratings were: 0 = no necrotic lesions; 1 = 25% or less of the leaf surface with necrotic lesions; 2 = 26-50% necrosis; 3 = 51-75% necrosis; 4 = 76-100% necrosis. The level of PD resistance: S = Susceptible, T = Tolerant and R = Resistant.

Virulent Strain (PD002)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	1	2	2	1	2	2	2	1.5	0
Blc. Bois (T)	1	1	1	1	1	1	0	1	1	2	1.0	0
Carlos (T)	0	1	0	1	1	1	2	0	1	1	0.8	0
Bl. Beauty(R)	1	1	0	1	1	1	1	1	0	0	0.7	0
Alachua (R)	1	0	1	0	1	0	1	1	0	1	0.6	0
Fry (R)	1	0	0	1	1	0	1	1	0	0	0.5	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Weakly Virulent Strain (PD91-2)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	0	0	1	1	2	1	1	0.9	0
Blc. Bois (T)	1	0	0	0	1	1	0	1	1	0	0.5	0
Carlos (T)	0	0	0	1	0	1	0	0	1	0	0.3	0
Bl. Beauty(R)	0	0	1	1	0	0	1	0	1	0	0.4	0
Alachua (R)	0	1	0	0	0	1	0	0	1	0	0.3	0
Fry (R)	0	0	1	1	0	0	0	0	0	0	0.2	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Avirulent Strain (PD-F1)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	0	1	0	0	0	0	1	0	1	0	0.3	0
Blc. Bois (T)	0	0	0	0	0	1	0	0	0	0	0.1	0
Carlos (T)	0	0	0	0	0	0	0	0	0	0	0.0	0
Bl. Beauty(R)	0	0	0	0	0	0	0	0	1	0	0.1	0
Alachua (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Fry (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Abbreviations: Chard = Chardonnay, Blc. Bois = Blanc du Bois, Bl. Beauty = Black Beauty.

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