IMPACT OF HOST PLANT XYLEM FLUID ON *XYLELLA FASTIDIOSA* MULTIPLICATION, AGGREGATION, AND ATTACHMENT

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ABSTRACT

Research in Temecula Valley indicated that the proximity of citrus groves to vineyards has influenced the incidence and severity of Pierce's disease (PD), *Xylella fastidiosa (Xf)*, in grapes. Although the glassy-winged sharpshooter (GWSS) feeds on and moves back and forth between Temecula citrus groves and vineyards, there are no visible *Xylella fastidiosa (Xf)* symptoms in the citrus. This implies that citrus trees are resistant or tolerant to the *Xf* but may be a reservoir to harbor the pathogen for GWSS acquisition while grape vines are susceptible. We investigated the mechanisms of host plant resistance/susceptibility by examining the impact of xylem fluid of grapefruit, orange, lemon and grape on *Xf* multiplication, aggregation and attachment as well as the related xylem fluid chemistry. Our laboratory experiments revealed that xylem fluid of grapefruit, orange and lemon caused an aggregation of Temecula PD cells to form large white clumps while grape xylem fluid did not cause visible clumping, but created a visible thick biofilm. The numbers of *Xf* cells in grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in grape xylem fluid treatment. The numbers of *Xf* cells in orange or lemon xylem fluid tests were generally lower than those in grape xylem fluid treatment. Citrus xylem fluid significantly inhibited *Xf* biofilm formation compared to grape xylem fluid. The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid. Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid. Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid. Our results indicate that the differences between citrus and grape plants in their responses to *Xylella* may be due to differences in their xylem fluid chemistry.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-limited, plant pathogenic bacterium that causes Pierce's disease (PD) in grapes (Purcell, 1981). *Xf* is mainly vectored by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* in Southern California. Although a comprehensive list of suitable hosts for the GWSS has been identified, comprising 75 plant species in 35 families (Turner and Pollard, 1959), the major crop hosts in Temecula Valley are citrus and grapes. Previous studies in California have identified 94 plant species in more than 28 of plant families as host of *Xf* (Freitg, 1951; Raju et al, 1983; Raju et al., 1980). Most identified *Xf* hosts show no symptoms but serve as inoculum sources of *Xf* for vector acquisition. Perring et al (2001) studied the incidence of PD in the Temecula Valley and found that proximity of citrus groves to vineyards has influenced the incidence and severity of PD in grapes. The PD infection is most severe when the grape vines are adjacent to citrus, and that the damage declines as one moves away from citrus (Perring et al., 2001). Although the GWSS feeds on and moves back and forth between citrus trees and grape vines, there is generally no *Xf* caused disease symptom in citrus in the area. This implies that citrus trees are resistant or tolerant to the *Xf*, but may be a reservoir to harbor the pathogen for GWSS acquisition and transmission while grape vines are susceptible. Little is known about the biochemical mechanisms involved in host plant resistance/susceptibility to *Xf* in the system. Additional information is required to determine if citrus can be suitable reservoirs for *Xf*. Elucidation of the biochemical mechanisms may be useful for developing host plant resistance in grapes as a sustainable component of integrated pest management program.

Xf aggregates to form biofilm inside its host plants and insect vectors. The biofilm formation is considered as a major virulence factor of PD (Marques and Ceri, 2002). Biofilm is defined as structured communities of sessile microbial aggregates enclosed in a self produced polymeric matrix and attached to a surface (Costerton et al., 1995). It was recently reported that a defined medium with some components based on susceptible grape cultivar "Chardonnay" xylem fluid chemistry better supports *Xf* growth and stimulates *Xf* aggregation and biofilm formation in vitro (Leite et al. 2004). However, the effect of citrus xylem fluid on *Xf* multiplication, aggregation and biofilm formation remains unknown.

Xf is a nutritionally fastidious bacterium (Wells et al. 1987). In defined medium certain amino acids are essential for *Xf* growth, glucose stimulates the growth while fructose and sucrose have inhibiting effect (Wells et al. 1987; Chang and Donaldson, 2000). It is not known whether differences in contents of amino acids and the sugars in the xylem fluid of citrus

and grape may differentially affect growth of *Xf*. Redox status also likely affects the tendency for *Xf* aggregation and biofilm formation. Adding reducing agents such as glutathione to artificial medium promotes *Xf* aggregation and biofilm formation (Leite et al., 2004). It was reported that thiols mediate the aggregation and adhesion of *Xf* (Leite et al., 2002). Thiol-containing compounds in xylem fluid include cysteine, methionine and glutathione. The redox status in citrus and grape xylem fluid and its role in *Xf* aggregation and biofilm formation, and host plant resistance/susceptibility to *Xf* need to be further investigated.

OBJECTIVES

1. Investigate the effect of host plant xylem fluid on *Xf* multiplication, aggregation and attachment.
2. Determine the biochemical mechanisms of host xylem fluid influence on *Xf* multiplication, aggregation and attachment.

RESULTS

Commercial citrus (lemon, orange and grapefruit) groves in proximity to vineyards were selected in the Temecula Valley, California. Three blocks of 30 citrus and 30 grape vines were used. A minimum of 15 citrus trees and 15 vines were randomly selected from each block (making a total of 15 trees or vines from each plant species) to extract xylem fluid. Terminal shoots from each plant were used for xylem extraction with a pressure bomb apparatus (Anderson et al., 1989). Upon collection, the xylem fluid was immediately placed on dry ice before final storage in a -80 ^{o}C freezer. The samples were used to test the impact of these xylem fluid on *Xf* resistance and chemical analyses of soluble carbohydrates, free amino acids, and redox status.

Effects of xylem fluid of each plant species on *Xf* attachment were evaluated on the biofilm formation. Formation of biofilm on the abiotic surfaces was assessed as described by Espinosa-Urgel et al. (2000). The analyses of *Xf* multiplication and aggregation were based on the fact that optical density (540 nm) is correlated with bacterial cell numbers and aggregation state as described by Burdman et al. (2000).

Our data indicated that, when the xylem fluid of grapefruit, orange and lemon was added to the PD Temecula strain of *Xf* in PD3 medium in glass culture tubes, there were heavy *Xf* cell aggregations to form large white clumps in suspension of the culture and the culture fluid was clear with no significant turbidity; in contrast, grape xylem fluid added to the same *Xf* culture did not cause visible clumping, but rather a visible thick biofilm was formed on the surface of glass tube and the culture was turbid (Figure 1). After homogenization of the culture, we found that the numbers of *Xf* cells in the grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in the grape xylem fluid treatment (Figure 2). The numbers of *Xf* cells in orange or lemon xylem fluid treatments were generally lower than those in grape xylem fluid treatment (Figure 3). These data suggest that the citrus species, especially grapefruit, are suitable hosts for *Xf* growth and may serve as a great reservoir of the pathogen for GWSS acquisition. Our assay results revealed that xylem fluid of the citrus species significantly inhibited *Xf* biofilm formation compared to that of grape (Figure 4). Our attempt to investigate the biochemical mechanisms likely to be involved indicated that 96% of amino acids in grape xylem fluid was comprised of glutamine, while 47% of amino acids in grape fruit xylem fluid was proline (Figure 5). The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid (Figure 5). Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid (Figure 6). Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid (Figures 7 and 8).

CONCLUSIONS

Xylem fluid of grapefruit, orange and lemon caused PD Temecula strain of *Xf* cells to aggregate and form large white clumps but inhibited the attachment. In contrast, grape xylem fluid did not cause visible clumping but led to heavy attachment. Grapefruit xylem fluid significantly increased multiplication of *Xf* cells compared with grape xylem fluid. Citrus species, especially grapefruit, appear to be suitable hosts for *Xf* growth and may serve as a reservoir of the pathogen for GWSS acquisition and transmission to grape vines. Further research is underway to elucidate the biochemical mechanisms.

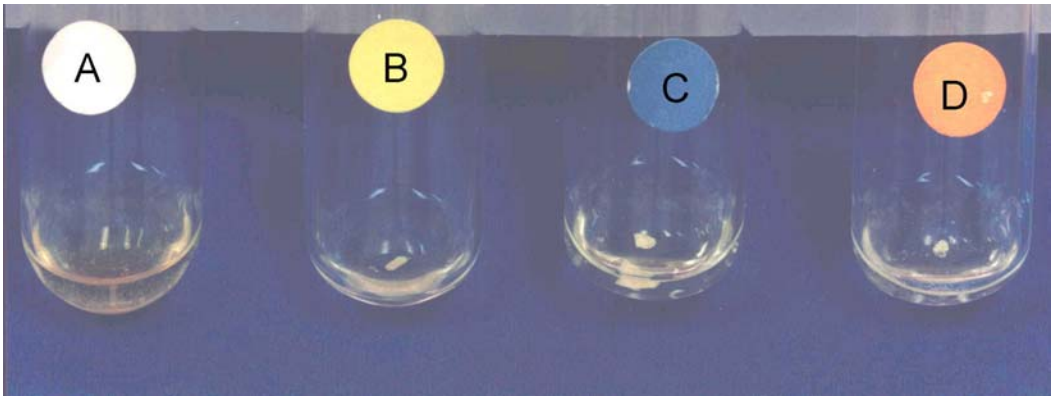

Figure 1. Effect of host plant xylem fluid on *Xf* aggregation. A, treatment with grape xylem fluid. B, treatment with grapefruit xylem fluid. C, treatment with orange xylem fluid. D, treatment with lemon xylem fluid. Note that white clumps of *Xf* aggregates are formed in the grapefruit, orange and lemon xylem fluid treatments.

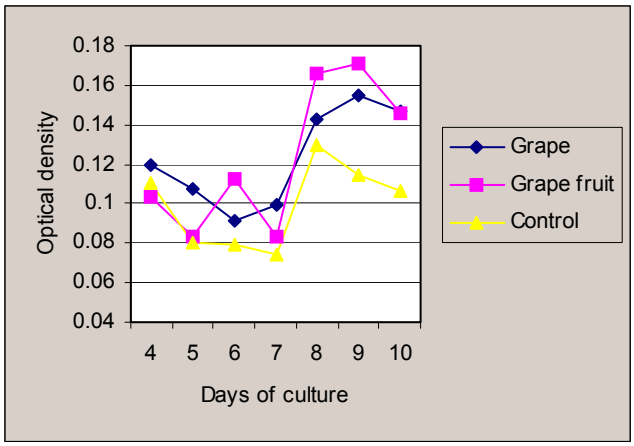

Figure 2. Effect of host plant xylem fluid on *Xf* growth.

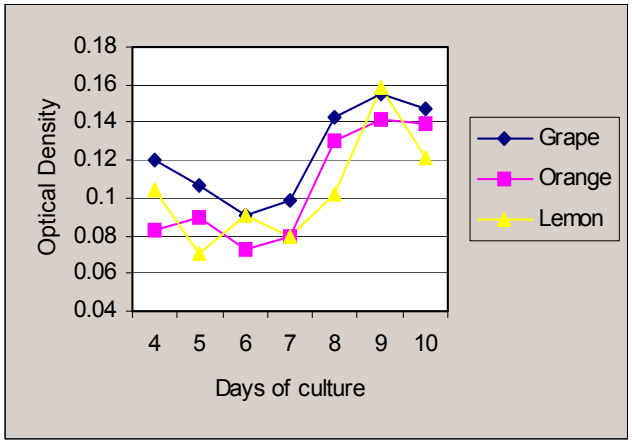

Figure 3. Effect of host plant xylem fluid on *Xf* growth.

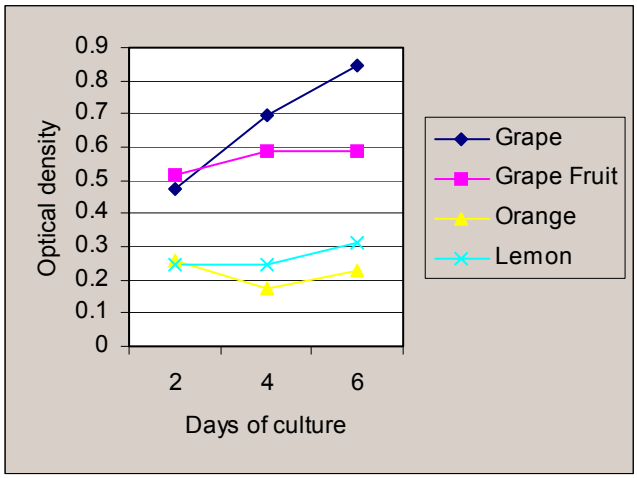

Figure 4. Effect of host plant xylem fluid on *Xf* biofilm formation.

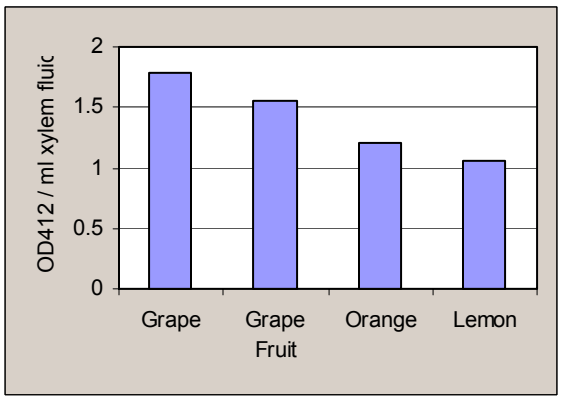

Figure 8. Total thiol contents in host xylem fluid.

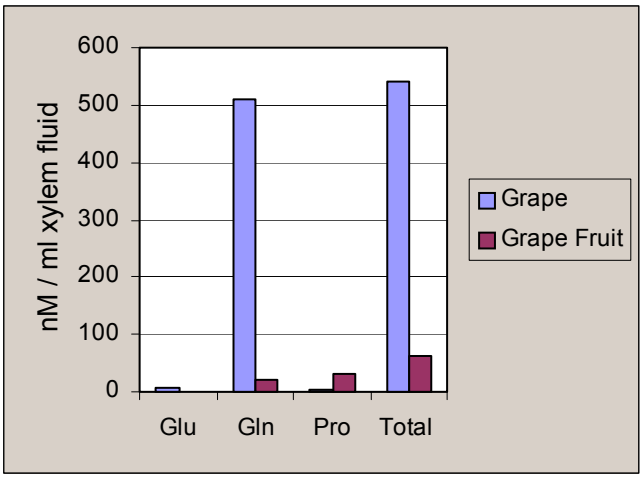

Figure 5. Some amino acid contents in grape and grape fruit xylem fluid.

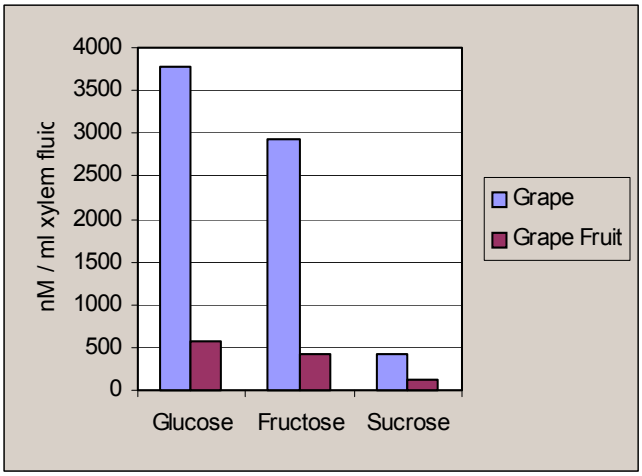

Figure 6. Sugar contents in grape and grape fruit xylem fluid.

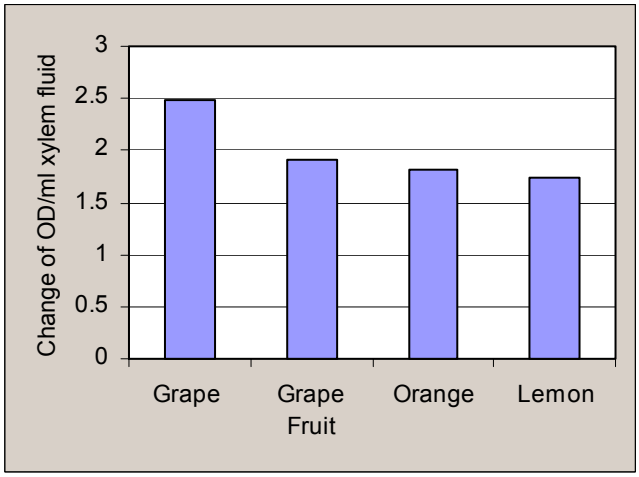

Figure 7. Peroxidase levels in host xylem fluid.

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