BIOLOGY OF THE *XYLELLA FASTIDIOSA*-VECTOR INTERFACE

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ABSTRACT
This project will build (and test) a framework to study the interactions between *Xylella fastidiosa* and insect vectors at the molecular, cellular and transmission biology levels. Understanding these interactions will lead to a better understanding of the transmission process and its biology, and potentially highlight promising strategies to disrupt pathogen retention by vectors and inoculation into plants. In addition, it will further elucidate how these genes function in infected grape and other hosts.

INTRODUCTION
Current data suggest that *Xylella fastidiosa* (*Xf*) is transmitted to plants from the precibarium of vectors where it attaches, multiplies, forms a 'carpet' of cells and eventually detaches to be injected into plants (Almeida and Purcell 2006). The details about the mechanics of an inoculation event (from the insect's probing behavior perspective) are still to be determined. In addition, there is no information on the interactions between *Xf* and vectors. To our knowledge, there is no datum on any molecular aspect of the vector-*Xf* interface, with the exception of Newman et al. (2004) who demonstrated that a cell-cell signaling mutant was not transmissible to plants by insects (which showed that signaling controls transmission, but did not identify genes associated with attachment or retention per se). This project will start to fill an essential gap in *Xf* transmission and biology research with a study on the molecular determinants of the vector-pathogen interface.

OBJECTIVES
1. Determine the effects of rpfC mutant on vector transmission.
2. Determine the transmission biology of *Xf* mutants hypothesized to be important in early and late stages of insect colonization.

RESULTS
This project is being initiated. We will first focus our studies on *Xf* attachment mutants characterized for some biological attributes by Meng et al. (2005), in addition to cell-cell signaling (rpf) mutants currently being studied by Steve Lindow's group at UC Berkeley.

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XYLEM FLUID CHEMISTRY MEDIATION OF PIERCE'S DISEASE: STIMULATION OF AGGREGATION AND BIOFILM FORMATION

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ABSTRACT
Xylella fastidiosa (Xf) is the causal agent of Pierce's disease in grapevines. The mechanisms of pathogenicity are largely due to occlusion of xylem vessels by aggregation of *Xf* and biofilm formation. Our previous work has documented the effects of xylem constituents on both *Xf* proliferation and biofilm formation. This current research utilizes 1) addition of xylem constituents to defined media *in-vitro*, and 2) exposure of *Xf* to xylem fluids of different *Vitis* germplasms to investigate effects of xylem components on *Xf* growth and biofilm formation. Xylem fluid is typically low in O_2 and our *in-vitro* studies have established the capability of *Xf* to grow under hypoxic conditions. The growth in the defined (minimal) media is often superior or equal under the oxygen-limited conditions as compared to the air-saturated media. These effects were found to be variable with *Xf* strain and media formulation indicating interactive effects between O_2 and specific xylem components. Short term (1 hour) to long term (12 days) exposure of *Xf* to *Vitis* xylem fluids showed highly significant differences in both *Xf* growth rates and biofilm formation dependent on *Vitis* genotype. *Xf* growth in *Vitis* xylem fluid was correlated to the concentration of the organic acid citric acid, many of the amino acids including glutamic acid, glutamine, histidine, valine, methionine, isoleucine and phenylalanine and inorganic ions including copper, magnesium, phosphorous and zinc. Biofilm formation was also correlated to specific xylem constituents. Our next experiments will test the growth and biofilm formation of *Xf* in response to changing the concentration of these constituents noted above in xylem fluid so that the role of each constituent can be assessed.

INTRODUCTION
We have previously established that a functional relationship exists between Pierce's disease (PD) expression and *Xylella fastidiosa (Xf)* colony growth within *Vitis* germplasms. Colony growth results from proliferation of individual *Xf* (cfu), aggregation, and biofilm formation. Both *in-vitro* and *in-vivo* experiments have shown that both *Xf* proliferation and biofilm formation may be impacted by a variety of constituents including specific inorganic ions, O_2, antioxidants, amino and organic acids and sulfhydryl groups. Newly developed defined media result in variable patterns of *Xf* colony growth (i.e. PW+ results in rapid bacterial proliferation with comparatively little biofilm formation whereas our newly defined media CHARDS provides slower bacterial growth but high biofilm formation). CHARDS media is equivalent to CHARD2 (Leite et al. 2004) except that starch at 0.2g/liter is added. These media provide an array of tools to test effects of individual compounds on *Xf* colony growth. Lastly, we can also examine *Xf* colony formation within xylem fluids of varying *Vitis* germplasms, and correlate patterns of growth to composition of the xylem fluid.

OBJECTIVES
1. Utilize defined media to examine the effects of O_2 and other xylem components on *Xf* growth.
2. Quantify the relationship between naturally occurring xylem constituents (inorganic ions, amino acids and organic acids) and *Xf* colony growth utilizing xylem fluid from a variety of *Vitis* germplasms.

Objective 1. The effects of O_2 on *Xf* growth and biofilm
Our initial work focused on the role of O_2 in *Xf* colony growth. The levels of oxygen found in xylem fluid are highly variable between almost atmospheric to anoxic levels (Gansert et al. 2001; Eklund, 2000). There may also be great variation within a plant (Dongen et al. 2003). Levels documented in xylem are generally well below atmospheric levels, and *Xf* has been defined as an obligate aerobe incapable of growth without O_2 (Wells et al. 1987). We subjected *Xf* growing in liquid PW+ media to 5 levels of O_2 ranging from atmospheric O_2 (21%) to anaerobic conditions (0%). Gas treatments were applied for 5 minutes every 24 hours. *Xf* was cultured under these conditions for 15 days at which time *Xf* growth, quantified by optical density (OD), and biofilm formation were measured. OD was measured using a Genosys 8 spectrophotometer at a wavelength of 600nm. The formation of biofilm on the surface of polypropylene tubes was assayed by the crystal violet method (Espinosa-Urgel et al. 2000). The oxygen levels were determined using a LaMotte's Dissolved Oxygen Test Kit (model EDO•code 7414) to insure the accuracy and persistence of treatment conditions.

Our results established a relationship between O_2 concentrations and *Xf* growth. For many strains tested, *Xf* growth rates were highest under atmospheric conditions and declined as O_2 levels declined. Growth comparison of *Xylella fastidiosa* pv. Pierce's disease strain 'Temecula' and *Xanthomonas campestris* under 21% oxygen and 0% oxygen headspaces, indicate that there was a discrepancy among the ability to grow under a hypoxic condition. There was no change in the optical density for *Xanthomonas* but there was continued growth for *Xylella* under the nitrogen gas treatments (0% O_2; Figure 1). This was also