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PROJECT TITLE: Characterization of the lipopolysaccharide-mediated response to *Xylella fastidiosa* infection in grapevine.

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REPORTING PERIOD: The results reported here are from work conducted March 2014 to present (July 2014).

INTRODUCTION: *Xylella fastidiosa* (*Xf*), a gram-negative, fastidious bacterium, is the causal agent of Pierce's Disease (PD) of grapevine (*Vitis vinifera*) and several other economically important diseases (Chatterjee *et al.*, 2008; Varela, 2001). PD has devastated some viticulture areas in California and there are currently no effective control measures available to growers targeted towards the bacterium itself. Lipopolysaccharide (LPS) is a tripartite glycolipid molecule that is an integral part of the Gram-negative bacterial outer membrane. It is primarily displayed on the outer surface of the cell, thereby mediating interactions between the bacterial cell wall and its environment. LPS plays diverse roles for the bacterial cell. It provides structural integrity to the cells and can act as a permeability barrier to toxic antimicrobial substances (Kotra *et al.*, 1999; Raetz & Whitfield, 2002). Because of its location in the outer membrane, it is also a key contributor to the initial adhesion to a surface or host cell (Goldberg & Pler, 1996; Walker *et al.*, 2004). We have been exploring the roles of LPS in the Pierce's Disease cycle and in the Plant-Microbe-Insect (PMI) interactions of *Xf*. We targeted our studies towards the outermost exposed region of the LPS molecule, the O-antigen. By mutating a key O-antigen polymerase, *wzy* (XP0836), in the Temecula1 (Pierce's Disease) isolate, we have demonstrated that severe truncation of the O-antigen, and subsequent termination in the synthesis of rhamnose-rich subunits, alters the adhesive and aggregative properties of the cell considerably, thus causing a marked defect in biofilm formation. Furthermore, the resulting mutation of the O-antigen caused increased sensitivity of the bacterium to hydrogen peroxide stress *in vitro* and resulted in a significantly less virulent pathogen that is severely impaired in host colonization (Clifford *et al.*, 2013). It has long been speculated that *Xf* surface polysaccharides play a role in the host-pathogen interaction with grapevine and our ongoing studies confirm that LPS is a major virulence factor for this important agricultural pathogen. We have also determined that the O-antigen plays an important role in the insect transmission of *Xf* by an efficient vector, the Blue-Green Sharpshooter (*Graphocephala atropunctata*). In collaboration with Dr. Thomas Perring (University of California-Riverside, Dept. of Entomology) and Dr. Elaine Backus (United States Department of Agriculture, Agricultural Research Service, Parlier, CA) we have conducted intricate transmission assays and determined that alterations in O-antigen structure and composition, indeed, result in a change in the adhesive properties of *Xf* within the insect as well.

Contrary to the role of LPS in promoting bacterial survival *in planta*, the immune systems of plants have also evolved to recognize the LPS structure and mount a basal defense response to counteract bacterial invasion (Dow *et al.*, 2000; Newman *et al.*, 2000). LPS is considered a Pathogen Associated Molecular Pattern (PAMP). PAMPs, also known as Microbial Associated

Molecular Patterns (MAMPs), are conserved molecular signatures that are often structural components of the pathogen (ie. LPS, flagellin, fungal chitin, etc.). These PAMPs are recognized by the host as "non-self" and can be potent elicitors of the basal defense response. This line of defense against invading pathogens is referred to as PAMP triggered immunity (PTI) and represents the initial layer of defense against pathogen ingress (Nicaise *et al.*, 2009). PTI is well studied in both mammalian and plant hosts. However, little is known about the mechanisms involved in perception of the LPS PAMP in grapevine, particularly the *Xf* LPS PAMP. *Xf* is introduced by its insect vector directly into the xylem, a non-living tissue, which cannot mount a defense response on its own. However, in other systems, profound changes do occur in the adjacent living parenchyma cells upon infection suggesting that these cells communicate with the xylem and are capable of recognizing the presence of a pathogen (Hilaire *et al.*, 2001). The plant immune system can recognize several regions of the LPS structure, including the conserved lipid A and core polysaccharide components (Newman *et al.*, 2007; Silipo *et al.*, 2005). Bacteria can also circumvent the host's immune system by altering the structure of their LPS molecule. Specifically, bacteria can display different O-antigen profiles by varying the extent of polymerization or by completely abolishing synthesis of the O-antigen depending on the environment and developmental phase of the cell (Bergman *et al.*, 2006; Guerry *et al.*, 2002; Lerouge & Vanderleyden, 2002). We speculate that during the interaction between *Xf* and a susceptible grapevine host, the bacterium's long chain, rhamnose-rich O-antigen shields the conserved lipid A and core-oligosaccharide regions of the LPS molecule from being recognized by the grapevine immune system, providing an opportunity for it to subvert the basal defense response and establish itself in the host. A similar scenario occurs in *E. coli*, where truncation of the O-antigen caused an increased sensitivity to serum suggesting the full length O-antigen provides a masking effect towards the host immune system (Duerr *et al.*, 2009; Guo *et al.*, 2005). *Salmonella enterica* subsp. *enterica* sv. (S.) Typhimurium also possesses an O-antigen that aids in evasion of the murine immune system (Duerr *et al.*, 2009).

Our main aim is to further explore the role of LPS, specifically focusing on the O-antigen moiety, in the interaction between *Xf* and the grapevine host and to use this information to develop and evaluate an environmentally sound preventative application for PD. We hypothesize that the LPS molecule contributes not only to biofilm formation but also modulates the host's perception of *Xf* infection. The *Xf* O-antigen mutant we currently have, and the additional ones we propose to construct in this study, provide a unique platform designed to test this hypothesis. The fundamental goal is to elucidate the mechanism(s) that *Xf* uses to infect the grapevine host and exploit this knowledge to evaluate the use of LPS structural variants as a preventative treatment for control of PD.

OBJECTIVES OF PROPOSED RESEARCH

Objective 1: Characterization of *Xf* LPS mutants *in vitro* and *in planta*.

Objective 2: Examination of the LPS-mediated response to *Xf* infection.

Objective 3: Evaluation of structural variants of LPS as a preventative treatment for PD.

SUMMARY OF ACCOMPLISHMENTS AND RESULTS

Objective 1: Characterization of *Xf* LPS mutants *in vitro* and *in planta*.

We have determined that the wild-type *Xf* O-antigen is composed primarily of 2-linked rhamnose with smaller amounts of glucose, ribose, xylose and mannose (Clifford *et al.*, 2013). Most importantly, we demonstrated that mutation of the O-antigen polymerase, *Wzy*, results in a severely truncated O-antigen resulting from a depletion of the majority of the 2-linked rhamnose. This change was confirmed both electrophoretically and biochemically utilizing gas

chromatography and mass spectrometry (GC/MS) techniques in collaboration with the Complex Carbohydrate Research Center (CCRC) at the University of Georgia. Notably, the depletion of rhamnose led to a marked reduction in virulence and host colonization (Clifford et al, 2013). This indicates that the process of rhamnose biosynthesis and its incorporation into the O-antigen is a vulnerable step in the *Xf* LPS biosynthetic pathway that could be exploited for disease control. Therefore, in this objective we are building on our current knowledge and continuing our studies by focusing on the process of rhamnose biosynthesis in *Xf*. We are presently creating mutants that we hypothesize will be unable to synthesize rhamnose, and we will structurally characterize the O-antigen from these mutants in collaboration with the CCRC. Following this, we will define the biological impact of these mutations by conducting virulence and colonization studies in grapevine. We will also determine the effect of these mutations *in vitro* using substrate attachment, cell-cell aggregation, and visualized biofilm studies that reflect host colonization behaviors.

Using comparative genomics, we have identified five genes with high homology to those involved in rhamnose biosynthesis in other bacterial systems. The genes are designated *rmIB*₁ (XP0208), *rmIA* (XP0209), *rmIC* (XP0210) and *rmID* (XP0211) (in map order) that encode proteins involved in the conversion of glucose-1-phosphate into dTDP-rhamnose (Jiang *et al.*, 1991; Koplín *et al.*, 1993; Rahim *et al.*, 2001). The first step is catalyzed by RmlA (dTDP-glucose synthase). Subsequent reactions are catalyzed by RmlB (dTDP-D-glucose-4,6-dehydratase), RmlC (dTDP-L-rhamnose synthetase) and RmlD (dTDP-4-dehydrorhamnose reductase) (Koplín *et al.*, 1993). The resulting dTDP-L-rhamnose is then incorporated into the O-antigen. The *rml* genes are usually clustered within a single locus and our *in silico* analysis demonstrates the presence of a *rml* locus in *Xf*. We also identified an additional, unlinked copy of *rmIB*, designated *rmIB*₂ (XP1617).

Mutations in the *rml* locus in *Xanthomonas campestris* pv. *campestris*, a close relative of *Xf*, resulted in a loss of rhamnose only in the O-antigen with no change in the sugars comprising the core oligosaccharide (Koplín *et al.*, 1993). Similarly, in *Stenotrophomonas maltophilia*, *rml* mutants were lacking O-antigen (Huang *et al.*, 2006). We hypothesize that *rml* mutants in *Xf* will be similarly affected and be significantly depleted of O-antigen. Caveat: In *P. aeruginosa*, mutations in *rmIC* did affect the core polysaccharide. However, the core was then unable to act as an attachment point for O-antigen resulting in a LPS structure composed only of lipid A + truncated core oligosaccharide and no O-antigen (Rahim *et al.*, 2001). It is currently unknown if the *Xf* core polysaccharide contains significant amounts of rhamnose. However, the creation of the *rml* mutants combined with our biochemical analyses will aid in determining that.

Mutant construction: We are currently constructing the *Xf* Δrml mutants using site-directed mutagenesis, and we have completed constructs for the $\Delta rmlAB_1CD$ mutant. Our next step is to make an *rmIB*₁*CD/rmIB*₂ double mutant. In the event that deletion of the entire locus has pleiotropic effects (ie. affects exopolysaccharide production), we will knock out each gene individually. We will begin with *rmIA*, which encodes the enzyme that catalyzes the first step in pathway of rhamnose biosynthesis. If needed, we will then sequentially knock out *rmIB*₁, *rmIC* and *rmID* also create a $\Delta rmlB_2$ mutant. All mutants constructed in this study will be complemented with the wild type copy of the gene, including the native promoter, using the complementation vectors now available for *Xf* developed by the Igo lab at UC-Davis (Matsumoto *et al.*, 2009). Once we have obtained these mutants and their complements, we will begin biochemically characterizing their O-antigen. We predict that the O-antigen in the *rml* mutants will be completely devoid of rhamnose. We will confirm this by conducting glycosyl composition and linkage analyses in collaboration with the Complex Carbohydrate Research Center

(University of Georgia). This Center routinely performs carbohydrate linkage analysis studies on a recharge basis.

LPS isolation and O-antigen purification: We have isolated LPS from the *Xf* wild type and our previously constructed O-antigen mutant strains. LPS from the *rml* mutant strain (from at least three biological replicates for each strain) will be isolated once complemented strains are made. Purified LPS from the wild type and O-antigen mutant strains were lyophilized and sent to the CCRC for NMR analysis in June 2014. We are currently waiting on those results. LPS was purified from whole cells using a modification of the hot phenol extraction method (Marolda, 2006). Briefly, cells were harvested from plated cultures, adjusted to a final optical density (OD_{600})=0.5 in 1.5mL of 1X PBS. Cell pellets were washed three times to remove residual EPS and stored overnight at -80 °C prior to hot phenol treatment. The resulting LPS fraction was desalted and concentrated to dryness using a lyophilizer (**Figure 1**). Aliquots of purified LPS were run on a 12% discontinuous Tricine-PAGE gel (Schägger, 2006) and silver stained (Tsai & Frasch, 1982). LPS from *rml* mutant strains and their complements will also be sent to the CCRC for structural characterization. O-antigen will be isolated from the total LPS fraction by mild acid hydrolysis in 1% acetic acid for 4 hours at 100° C, followed by centrifugation at 8,000 rpm for 30 minutes. The supernatant will be removed and reserved for glycosyl composition and linkage analysis.



Figure 1. Lyophilized LPS sent to the CCRC.

LPS glycosyl composition and linkage analysis: Glycosyl composition analysis will be performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-O-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis as previously described (Merkle & Poppe, 1994). GC/MS analysis of the TMS methyl glycosides will be performed on an Agilent 7890N GC interfaced to a 5975C MSD, using a Supelco EC-1 fused silica capillary column (30m × 0.25 mm ID). For glycosyl linkage analysis, the sample will be permethylated, depolymerized, reduced, and acetylated; and the resultant partially methylated alditol acetates will be analyzed by GC-MS (York, 1985). These techniques will allow us to deduce a preliminary structure and determine any differences between the wild type and mutant strains.

Surface attachment, aggregation and biofilm studies: Once we have the LPS structural data, we will begin to link *Xf* LPS structure to function using *in vitro* assays. Attachment to a surface is the first step in successful biofilm formation and because of the location and abundance of LPS in the outer membrane we hypothesized that LPS plays a key role in mediating initial attachment to the cellulose and chitin substrates *Xf* encounters in the plant and insect, respectively. We previously demonstrated that a mutant in the Wzy polymerase was deficient in cell-cell aggregation and hyperattached to surfaces, which led to a defect in biofilm formation (Clifford et al., 2013). We will similarly test the *rmlAB₁CD* and *rmlAB₁CD/rmlB₂* (and single *rml* mutants if necessary) mutants for these behaviors to determine if the inability to synthesize rhamnose results in defective biofilm formation.

Virulence and host colonization assays: Once we have obtained both the *rml* mutants and their complemented strains, we will mechanically inoculate *Vitis vinifera* 'Cabernet Sauvignon' vines using the pin-prick method (Hill & Purcell, 1995). The Roper lab has extensive experience with this technique. Each plant will be inoculated twice with a 20 µL drop of a 10⁸ CFU/mL

suspension of either wild type *Xf* or the *rml* mutants constructed in this objective. We will inoculate 10 plants/mutant and repeat each experiment 3 times. Plants inoculated with 1x PBS will be used as negative controls. All plants will be rated on a disease scale of 0-5 with 0 being healthy and 5 being dead (Guilhabert & Kirkpatrick, 2005). We will also assess the *Xf* populations in the plants by isolating cells from the petioles at the point of inoculation and 25 cm above the point of inoculation to assess the ability of the *rml* mutants to systemically colonize the host. Isolations will be performed at five and fourteen weeks post-inoculation. Petioles will be surface sterilized and ground in 2 mL of sterile 1x PBS. The resulting suspension will be diluted and plated on solid PD3 medium and colonies will be counted and normalized to tissue weight.

Objective 2: Examination of the LPS-mediated response to *Xf* infection.

In grapevine, recognition of PAMPs other than LPS, such as the *Botrytis cinerea* endopolygalacturonase BcPG1 and β -glucans, trigger a cascade of signaling events including calcium ion influxes, reactive oxygen radical accumulations, and activation of protein kinases, that coordinate the transcriptional activation of defense genes (Aziz *et al.*, 2003; Aziz *et al.*, 2007; Poinssot *et al.*, 2003). The LPS PAMP can induce similar responses in other plant species, but these studies have been performed primarily in model systems, such as *Arabidopsis thaliana* or tobacco (Desaki *et al.*, 2006; Zeidler *et al.*, 2004). There is limited knowledge about the grapevine response to the LPS PAMP, particularly on the transcriptional level. However, one study demonstrated that a rhamnolipid MAMP from *P. aeruginosa* could induce defense-related responses in grapevine cell suspensions (Varnier *et al.*, 2009).

The defense reactions activated upon PAMP recognition involve intricate networks of transcriptional regulators and phytohormone signaling. Genome-wide transcriptional profiling is a logical starting point to begin understanding this complex process in the *Xf*-grape pathosystem (Jones & Dangl, 2006). We speculate that mutated *Xf* LPS (deplete of O-antigen) recognition elicits a transcriptional response that results in the deployment of specific defense reactions in grape that results in less disease and host colonization. We hypothesize that the grapevine is recognizing the conserved core/lipid A portions of the *Xf* LPS molecule and that the long chain O-antigen serves to camouflage the rest of the LPS PAMP (the core-lipid A complex) from being recognized by the host innate immune system. Thus, we expect an increase in expression of defense-related genes in plants inoculated with the O-antigen mutants (*wzy::kan*, *rmlAB₁CD* and *rmlAB₁CD/rmlB₂*) that are deplete of O-antigen as compared to wild type *Xf*. The studies detailed below are designed to test our hypothesis that loss of the rhamnose-rich O-antigen allows the grapevine to more readily perceive the *Xf* LPS molecule and that this recognition leads to elicitation of a specific transcriptional response associated with defense.

LPS-induced oxidative burst in grapevine: To explore the role of LPS as an elicitor of basal defense responses in grapevine, we first investigated ROS production *ex vivo* using a luminol assay. In this assay, 0.125 inch *V. vinifera* 'Cabernet Sauvignon' leaf disks were placed into wells of a 96-well plate and soaked in water overnight, allowing any damage-associated ROS to leach from the disks. The following day, water was removed and replaced with a solution containing Luminol (100nM), Horseradish Peroxidase (1mg/100 μ L), and purified wild type or *wzy* mutant LPS (50 μ g/mL), water negative control, or flg22 (10 μ M) positive control. ROS production was measured through the chemiluminescence of luminol and monitored over 60 minutes. Due to the increased exposure of the Lipid A-Core Oligosaccharide region of the *wzy* mutant, we hypothesized that we would see a quicker, stronger oxidative burst, compared with wild type. As shown in **Figure 2A**, both wild type and *wzy* mutant LPS induced an oxidative burst in grapevine leaf disks. However, the burst induced by *wzy* mutant LPS was stronger and

more prolonged than wild type, peaking around 5 minutes and returning to near-basal levels around 32 minutes post-elicitation.

ROS production in response to live *Xf* cells: Once we established that *X. fastidiosa* LPS induced an oxidative burst in *V. vinifera* 'Cabernet Sauvignon' leaf disks, we then turned our attention to ROS produced in response to living *X. fastidiosa* wild type and *wzy* mutant live culture. LPS O-antigen moieties can adopt numerous conformations, sometimes bending to shield the cell from recognition. This is the case with species of *Salmonella*, where O-antigen-mediated evasion of innate immune activation significantly enhances bacterial survival *in vitro* and *in vivo* (Duerr *et al.*, 2009). Our working hypothesis is that wild type O-antigen helps mask *Xf* from recognition by the grapevine host, thereby facilitating *Xf* establishment *in planta*. Luminol assays were conducted in the same manner as described previously, except that suspensions of wild type or *wzy* mutant culture (at 1×10^8 CFU/mL) were added to each well. As shown in **Figure 2B**, the *wzy* mutant culture induced a strong response from grapevine leaf disks. ROS production peaked at around 12 minutes and lasted nearly 100 minutes. Wild type culture failed to produce a sharp peak, as compared with the *wzy* mutant, and ROS production plateaued around 60 minutes.

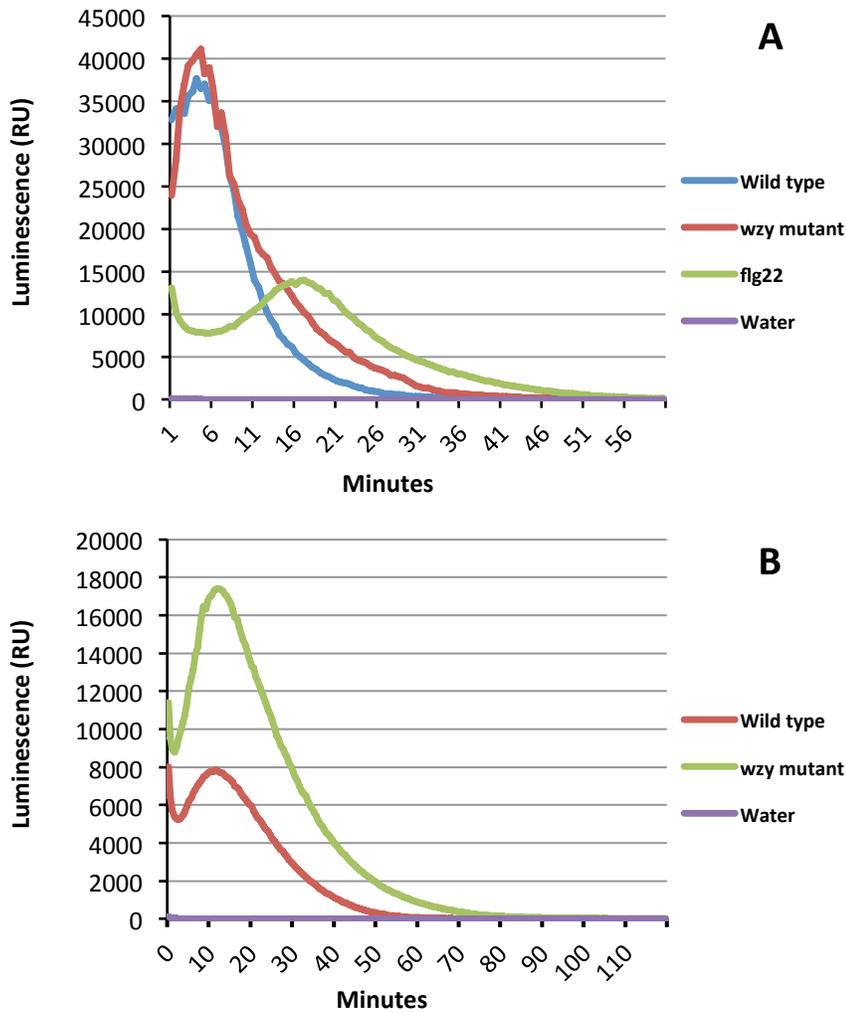


Figure 2. A) LPS-induced ROS production in grapevine.

Presence of an oxidative burst was determined through the chemiluminescence of luminol. The *wzy* mutant LPS elicits a stronger, more prolonged response in grapevine leaf disks, compared with *Xf* wild type LPS. **B) ROS production in response to live *Xf* cells.** Suspensions of wild type or *wzy* mutant culture were added to grapevine leaf disks, and ROS production was monitored through the chemiluminescence of luminol. The O-antigen mutant culture induced a strong response from grapevine leaf disks, peaking at around 12 minutes and plateauing around 100 minutes. Wild type culture failed to produce a sharp peak, as compared with *wzy* mutant cells, and ROS production plateaued at around 60 minutes.

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Transcriptome profiling: High-throughput sequencing technologies provide a relatively inexpensive means to profile the expression of nearly all genes in a tissue simultaneously. The application of transcriptome profiling approaches using next generation RNA sequencing (RNA-seq) will

allow us to monitor the activation or suppression of specific defense pathways at the genome scale. In early July of 2014, individual vines of *V. vinifera* 'Cabernet Sauvignon' were inoculated with *Xf* wild type or *wzy::kan* live culture. We inoculated 9 vines for each treatment. Vines inoculated with 1xPBS buffer alone served as the negative controls for the experiment. Using the pin-prick method described previously, each vine was inoculated 2x with a 20 μ L drop of a 10^8 CFU/mL suspension of either wild type *Xf* or the *wzy* mutant. The inoculum was immediately drawn into the petiole due to the negative pressure in the xylem. PTI usually causes major transcriptional reprogramming of the plant cells within hours after perception (Dow *et al.*, 2000; Tao *et al.*, 2003). Thus, petioles were harvested at the following four time points: 0, 1, 8, and 24 hours post-inoculation. To stabilize transcripts, petioles were submerged into liquid Nitrogen immediately after harvesting and stored at -80°C until RNA extraction. Currently, RNA extraction protocols are being optimized to ensure that yields are sufficient prior to the downstream applications. Before construction of the RnaSeq libraries is conducted, we plan to first use qPCR to fine-tune the timing of the transcriptional profiling. This will be done through monitoring the induction of known LPS-induced genes in *A. thaliana* (*PR-1, 2, 3, 4, 5*) at varying time points following inoculation. Using this information, we will finalize the time points we will use for the genome-wide transcriptional profiling. Sequencing libraries will be generated from the polyadenylated plant messenger RNA and sequenced using the Illumina HiSeq 2000 platform. Transcript expression levels will be determined by alignment of the sequencing reads onto a non-redundant set of functionally annotated reference genes generated in the Cantu lab that are based on a combination of the publically available grapevine datasets (Jaillon *et al.*, 2007; Velasco *et al.*, 2007) (<http://www.ncbi.nlm.nih.gov/unigene>). Statistical inference will be applied to determine with confidence the subset of genes that are up- or down-regulated upon inoculation with live culture (Cantu *et al.*, 2011b). Additionally, PAMPs are known to induce systemic resistance (i.e. in distant plants organs) (Erbs & Newman, 2003; Mishina & Zeier, 2007). We will assess if *Xf* LPS recognition results in the activation of defense responses in distal parts of the grapevines, thereby, providing resistance at the whole vine level. We will collect and sequence RNA as described above from petioles 25 cm above the site of original inoculation at 24 and 48 hours after inoculation when transcriptional modifications associated with the induction and maintenance of systemic acquired resistance can be detected (Maleck *et al.*, 2000; Wang *et al.*, 2006).

We will perform all of our studies in the *Cabernet sauvignon* variety because it performs well in the greenhouse in Riverside, CA where the plant studies will be carried out. In addition, because we have chosen a variety other than the sequenced variety (Pinot noir), the RNA-seq transcriptome analysis will provide valuable information on the transcriptome of an additional grape variety. Moreover, a genome wide approach will tell us the array of defense or stress-associated genes induced by the LPS from a xylem-dwelling pathogen, an area of research where there is limited knowledge.

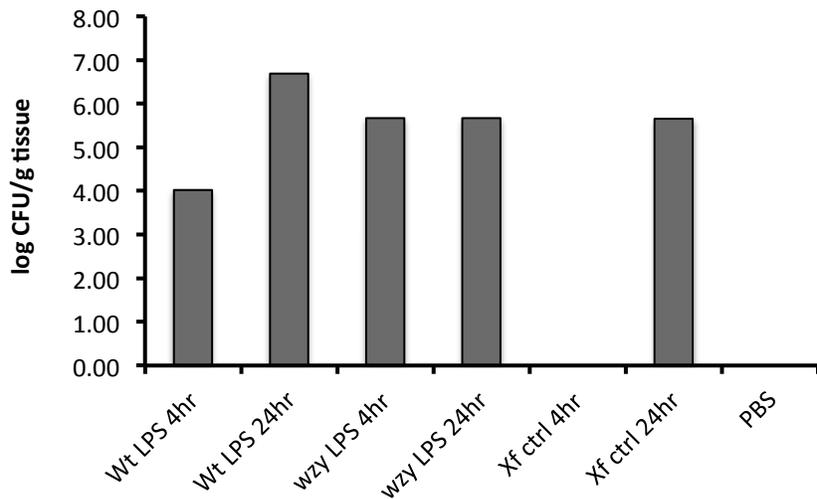
Objective 3: Evaluation of structural variants of LPS as a preventative treatment for PD.

In some systems, treatment with LPS alone does not induce a measurable difference in gene expression. However, it does potentiate a more robust and measurable defense response following challenge with a pathogen. Pre-treatment of plants with LPS can prime the defense system resulting in an enhanced response to subsequent pathogen attack. This defense-related "memory" is called priming and stimulates the plant to initiate a faster and/or stronger response against future invading pathogens (Conrath, 2011). Priming often results in rapid and robust activation of defense responses such as the oxidative burst, nitric oxide synthesis and expression of defense-related genes (Erbs & Newman, 2003; Newman *et al.*, 2000). The LPS PAMP has been specifically implicated in priming in the *X. campestris* pv. *vesicatoria* pathosystem. Pepper leaves pre-treated with LPS isolated from incompatible (non-virulent)

xanthomonads had enhanced expression of several PR proteins after being challenged with virulent *X. campestris* pv. *vesicatoria* (Newman *et al.*, 2000). In this objective, we hypothesize that pre-treatment with LPS isolated from *Xf* O-antigen mutants results in a difference in the grapevine's tolerance to *Xf* by stimulating the host basal defense response.

Priming assays: A pilot study of this experiment is currently in progress, and preliminary data is shown below in **Figure 3**. Grapevine petioles were pre-treated with 40 μ L of either wild type or *wzy* mutant LPS (50 μ g/mL). 1xPBS served as the negative control. After we mechanically inoculated the vines with LPS, we challenged with an inoculation of live wild type *Xf* cells (40 μ L of a 1x10⁸ CFU/mL suspension). These inoculations were performed at 4 and 24 hours after the original inoculation with the LPS. These time points were established based on previously described assays (Newman *et al.*, 2002). We inoculated 24 vines/treatment/LPS concentration/time point. Once we have established that we can induce the primed state in grapevine, we will then begin assessing how long the temporal window of the primed state lasts by increasing the amount of time between the inoculation with the LPS and the challenge with live *Xf* cells. We will begin with a one-week interval in between the two inoculations and increase by one-week intervals until we no longer see that the vines are in the primed state. Once we have established the time frame of the primed state, we can then determine if it is necessary to have a continuous supply of LPS either from a modified strain of *Xf* or by multiple exogenous applications to extend the primed state.

Bacterial growth *in planta*: Pre-treatment of LPS can also restrict *in planta* growth (Erbs & Newman, 2003; Newman *et al.*, 2002). Notably, we have shown that removal of the rhamnose-rich O-antigen from the *Xf*



LPS results in a more adhesive pathogen for the *wzy::kan* mutant. Therefore, we reason that in addition to potentiating the defense response, purified *Xf* LPS depleted of the O-antigen could behave as a cellular glue that essentially hinders live *Xf* cells from moving systemically throughout the plant. The goal is to cause *Xf* to be less invasive, thereby decreasing the chance that that bacterium will systemically colonize the trunk of the vine creating a chronic infection that leads to the eventual death of the vine. To determine if pre-treatment of plants with mutated *Xf*

Figure 3. Bacterial colonization *in planta*. Individual vines of *Vitis vinifera* 'Cabernet sauvignon' were pre-treated with wild type or *wzy* mutant LPS (50 μ g/mL) and followed with an inoculation of live wild type *Xf* cells at 4 or 24 hours post-inoculation with LPS. Isolations were performed at 2 weeks post-inoculation. Data are means of 24 replicates per strain.

LPS does, in fact, lead to a decrease in bacterial growth, we isolated *Xf* populations at the point of inoculation. Plants were pre-treated with the same concentration of LPS as described above and then challenged with an inoculum dose of 40 μ L of a 1x10⁸ CFU/mL suspension of *Xf*. We have quantified bacterial populations at two weeks post-inoculation with the *Xf* cells. We will

also quantify bacterial populations at 6 weeks post-inoculation to determine if the primed state potentiates over time. *Xf* is a slow growing organism, which is why we chose these long time points. Our working hypothesis is that for plants treated with *wzy* mutant LPS, whose conserved portions are more exposed, plant basal defense responses will be faster and more aggressive than for plants treated with wild type LPS, therefore resulting in overall reduced bacterial titer *in planta*. As shown in **Figure 3**, which represents bacterial isolations performed at 2 weeks post-inoculation, there was no significant difference in bacterial titer amongst the treatments. We believe this to be a result of isolating at such an early time point. As stated previously, *Xf* is a slow growing organism, so we anticipate that isolations at 6 weeks post-inoculation will be more informative. Once we have established that the primed state can restrict *in planta* growth, we will also investigate if the primed state affects movement *in planta*. We plan to quantify bacterial populations at 25cm above the point of inoculation.

We plan to conduct the experiments outlined in Objective 3 in year 2 of the project (2014-2015)

PUBLICATIONS PRODUCED AND PRESENTATIONS MADE

Oral Presentation:

Jeannette N. Rapicavoli, Holly J. Shugart, Crystal M. Johnston, Thomas M. Perring, Abraham Morales-Cruz, Dario Cantu, and M. Caroline Roper. 2014. Implication of lipopolysaccharide in the vector-microbe and plant-microbe interactions of the bacterial phytopathogen, *Xylella fastidiosa*. Hemipteran-Plant Interactions Symposium, Riverside, CA.

Poster Presentations:

Jeannette N. Rapicavoli, Holly J. Shugart, Crystal M. Johnston, Thomas M. Perring, Abraham Morales-Cruz, Dario Cantu, and M. Caroline Roper. 2014. The role of lipopolysaccharide in the vector-microbe and plant-microbe interfaces of the bacterial phytopathogen, *Xylella fastidiosa*. American Phytopathological Society Annual Meeting, Minneapolis, MN.

RESEARCH RELEVANCE STATEMENT

The proposed project will address a key aspect of the interaction of *Xf* with its grapevine host. In addition, it will provide knowledge about basal resistance to disease in grapevines and plant hosts in general. Notably, we will also test a potential preventative measure for PD. Information gleaned from this project could also help guide traditional breeding programs aimed at disease resistance by identifying potential resistance markers. The overall outcome will result in a foundation of fundamental knowledge about PD at the molecular level that we will use to develop an innovative and environmentally sound approach to controlling this disease.

LAYPERSON SUMMARY

Xylella fastidiosa (*Xf*), a bacterial pathogen, is the causal agent of Pierce's disease (PD) of grapevine and poses a serious threat to the viticulture industry. We have demonstrated that truncation of the O-antigen portion of the lipopolysaccharide (LPS) entity alters the adhesive properties of the cell, leading to a defect in mature biofilm formation. Furthermore, depletion of the O-antigen results in a significantly less virulent pathogen that is severely compromised in host colonization. Additionally, LPS is a Pathogen-Associated Molecular Pattern (PAMP) that potentially triggers the basal immune response. We hypothesize that the long chain O-antigen also allows *Xf* to circumvent the innate immune system by masking the conserved core and lipid A portions of the LPS chain from the host immune system. The goal of the proposed work is to further explore the role of LPS, specifically focusing on the O-antigen moiety, in the interaction between *Xf* and the grapevine and to use this information to develop and evaluate an

environmentally sound preventative application for PD.

STATUS OF FUNDS

The funding for this project is largely going towards supporting a Ph.D. graduate student, Mrs. Jeannette Ropicavoli. The Cantu lab has hired a computational biologist (Dr. Katherine Amrine), who will be leading the transcriptomics data analysis of this project, and she will be supported by funds for this project starting in April 2014. We anticipate spending the remainder of the salary, supply, services and greenhouse recharge money associated with this project as it progresses. This project is the main focus of her Ph.D. dissertation.

INTELLECTUAL PROPERTY

We do not anticipate that this research will lead to materials or procedures that will be subject to intellectual property restrictions. However, if it does, their availability and use will be subject to the policies of the University of California for managing intellectual property (<http://www.ucop.edu/ott/pdf/consult.pdf>).

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