INTERIM REPORT FOR CDFA AGREEMENT NUMBER 18-0328-000-SA: CHARACTERIZATION OF THE LIPOPOLYSACCHARIDE-MEDIATED RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION IN GRAPEVINE

Principal Investigator:
Caroline Roper, Ph.D.
Department of Microbiology and Plant Pathology
University of California
Riverside, CA 92521
mcroper@ucr.edu

Co-principal Investigator:
Dario Cantu, Ph.D.
Department of Viticulture and Enology
University of California
Davis, California 95616
dacantu@ucdavis.edu

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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). *Xf* is limited to the xylem of the plant host and is transmitted by xylem-feeding insects, mainly sharpshooters. Extensive xylem vessel blockage occurs in infected vines (Sun *et al*., 2013), and symptoms include leaf scorch, raisining of berries, stunting, and vine death. PD has devastated some viticulture areas in California.

Our previous study confirm that lipopolysaccharide (LPS) is a major virulence factor for *Xf.* LPS comprises approximately 70% of the Gram-negative bacterial cell surface, making it the most dominant macromolecule displayed on the cell surface (Caroff & Karibian, 2003). LPS is a tripartite glycolipid that is generally comprised of a highly-conserved lipid A, an oligosaccharide core, and a variable O antigen polysaccharide (Whitfield, 1995) (**Fig. 1**). We demonstrated the *Xf* O antigen is a linear α1-2 linked rhamnan and compositional alterations to the O antigen significantly affected the adhesive properties of *Xf,* consequently affecting biofilm formation and virulence (Clifford *et al*., 2013). In addition, we demonstrated that truncation of the LPS molecule severely compromises insect acquisition of *Xf* (Rapicavoli *et al.,* 2015). We coupled these studies with quantification of the electrostatic properties of the sharpshooter foregut to better understand the interface between the *Xf* cell and the insect. We then sought to test our additional hypothesis that the *Xf* LPS molecule acts as a Pathogen-Associated Molecular Pattern (PAMP), and the long chain O antigen serves to shield *Xf* from host recognition, thereby modulating the host's perception of *Xf* infection (Rapicavoli *et al*., 2018).

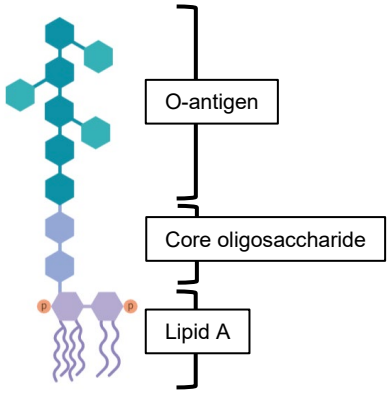

Figure 1. Schematic of a single LPS molecule containing lipid A, core oligosaccharide, and the O-antigen.

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 PAMP. PAMPs, also known as Microbe-Associated Molecular Patterns (MAMPs), are conserved molecular signatures that are often structural components of the pathogen (i.e. LPS, flagellin, fungal chitin, etc.). PAMPs are recognized by the host as "non-self" and can be potent elicitors of basal defense responses. 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 LPS 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, we observe profound changes that occur in the xylem that are linked to presence of *Xf.* These include an oxidative burst and suberin

deposition, as well as tyloses production (Rapicavoli *et al*, 2018). Interestingly, we also observe significant defense response to *Xf* in the phloem tissue, a tissue historically overlooked in the context of this xylem dwelling pathogen that mainfest in the form of callose deposition. 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. Clearly, *Xf* has evolved a mechanism to circumvent the host basal defense response as it successfully colonizes and causes serious disease in grapevine. We tested our hypothesis that 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 basal defense responses and establish itself in the host (Rapicavoli *et al.*, 2018).

To explore the role of LPS as a shield against basal defense responses in grapevine, we investigated elicitation of an oxidative burst, an early marker of basal defense responses, *ex vivo* in *V. vinifera* 'Cabernet Sauvignon' leaf disks exposed to either wild type *Xf* or *wzy* mutant cells. When we examined ROS production in response to whole cells, *wzy* mutant cells (in which lipid A-core is exposed) induced a stronger and more prolonged oxidative burst in grapevine leaf disks than did wild type *Xf.* Specifically, ROS production peaked at around 12 minutes and lasted nearly 90 minutes. Wild type *Xf* cells (in which lipid A-core would be shielded by O antigen) failed to produce a sharp peak as compared with the *wzy* mutant, and ROS production plateaued much sooner (around 60 minutes) (data shown in Rapicavoli *et al*, 2018).

In addition to the role of LPS in promoting bacterial infection, 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 plant defense priming and stimulates the plant to initiate a faster and/or stronger response against future invading pathogens (Conrath, 2011, Newman *et al.*, 2000). We demonstrate that pre-treatment with LPS isolated from *Xf* would result in an increase in the grapevine's tolerance to *Xf* by stimulating the host basal defense response. Our *ex vivo* data showing that both wild type *and wzy* mutant LPS elicit an oxidative burst, an early marker of defense that can potentiate into systemic resistance, in grapevine leaf disks support this hypothesis. To determine if the primed state affects the development of PD symptoms, we documented disease progress in plants that were pre-treated with either wild type or *wzy* LPS and then challenged with *Xf* either 4 or 24 hours later. Notably, we observed a decrease in PD severity in vines pre-treated with *Xf* LPS and then challenged with *Xf* **(Fig. 2) (**Rapicavoli *et al*, 2018).

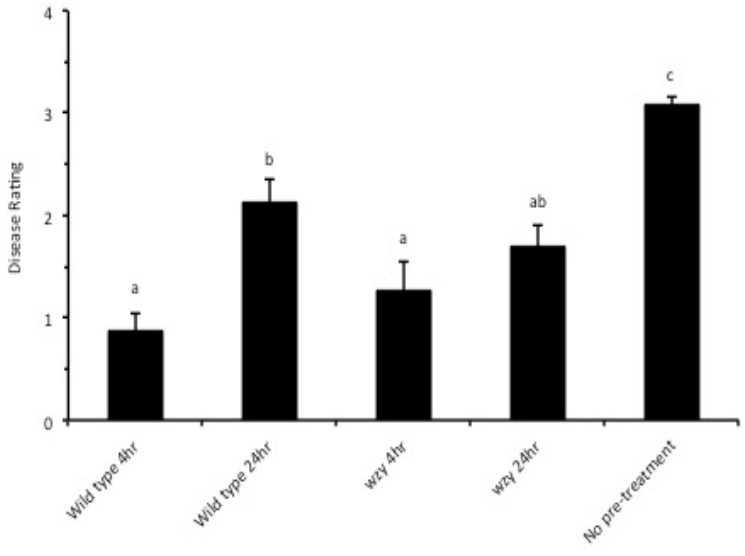

Figure 2. Pierce's Disease symptom severity in grapevines primed with purified *Xf* LPS. Average disease ratings of *V. vinifera* 'Cabernet Sauvignon' grapevines pre-treated with wild type or *wzy* mutant LPS (50µg/mL), then challenged at 4 h or 24 h post-LPS treatment with *Xf* cells. Disease ratings were taken at 12 weeks post-challenge. The LPS pre-treated plants are significantly attenuated in symptom development, compared with plants that did not receive pre-treatment ($P < 0.05$). Graph represents the mean of 24 samples per treatment. Bars indicate standard error of the mean.

Previously, we completed a global RNA-seq-based transcriptome profile where we sequenced the transcriptomes of grapevines treated with wild type, *wzy* mutant cells, or 1XPBS buffer (Rapicavoli *et al*, 2018). The goal was to identify genes that are differentially expressed when plants are inoculated with either wild type or the *wzy* mutant while using mock-inoculated plants as the controls. PTI usually causes major transcriptional reprogramming of the plant cells within hours after perception (Dow *et al.*, 2000; Tao *et al.*, 2003), so our initial experiments were targeted toward early time points during the infection process (0, 8, and 24 hours post-inoculation). The RNA-seq data demonstrate that the grapevine is activating defense responses that are distinct to each treatment and time point **(Fig. 3A)**. For example, enrichment analysis of *wzy*-responsive genes at 8 hpi identified predominant biological processes associated with cellular responses to biotic stimulus and oxidative stress **(Fig. 3B)**. This included a significant increase in the production of thioredoxins, glutaredoxins, and other ROS-scavenging enzymes involved in antioxidant defense. In addition, there was high expression of genes involved in the production of phytoalexins (e.g. stilbene synthase), antimicrobial peptides (e.g. thaumatin), and PR genes. In contrast, wild type-responsive genes in this time point were enriched primarily in responses to abiotic or general stresses (i.e., drought, oxidative, temperature, and wounding stresses) and were not directly related to immune responses **(Fig. 3B)**. Notably, by 24 h post-inoculation, overall transcriptional profiles of both *wzy* and wild type-inoculated vines shifted dramatically. Grape genes in *wzy* mutant-inoculated vines were no longer enriched for immune-specific responses, and we speculate that this is due to the effective O antigen-modulated oxidative burst. In contrast, genes of wild type-inoculated plants were strongly enriched for immune responses **(Fig. 3C)**. We hypothesize that at 8 h, the high molecular weight O antigen is still effectively shielding wild type cells, therefore causing a delay in plant immune recognition. However, by 24 h post-inoculation, the production of ethylene-induced plant cell wall modifications, compounded by progressing bacterial colonization and the potential release of DAMPs via bacterial enzymatic degradation of plant cell walls, has triggered grapevine immune responses, and the plant is now fighting an active infection. This indicates that the O antigen does, indeed, serve to shield the cells from host recognition, allowing them to establish an infection (Rapicavoli *et al*., 2018). Complete RNAseq data can be found in the supplementary information in Rapicavoli *et al*, 2018. *Nature Communications*, 9 (1): 390.

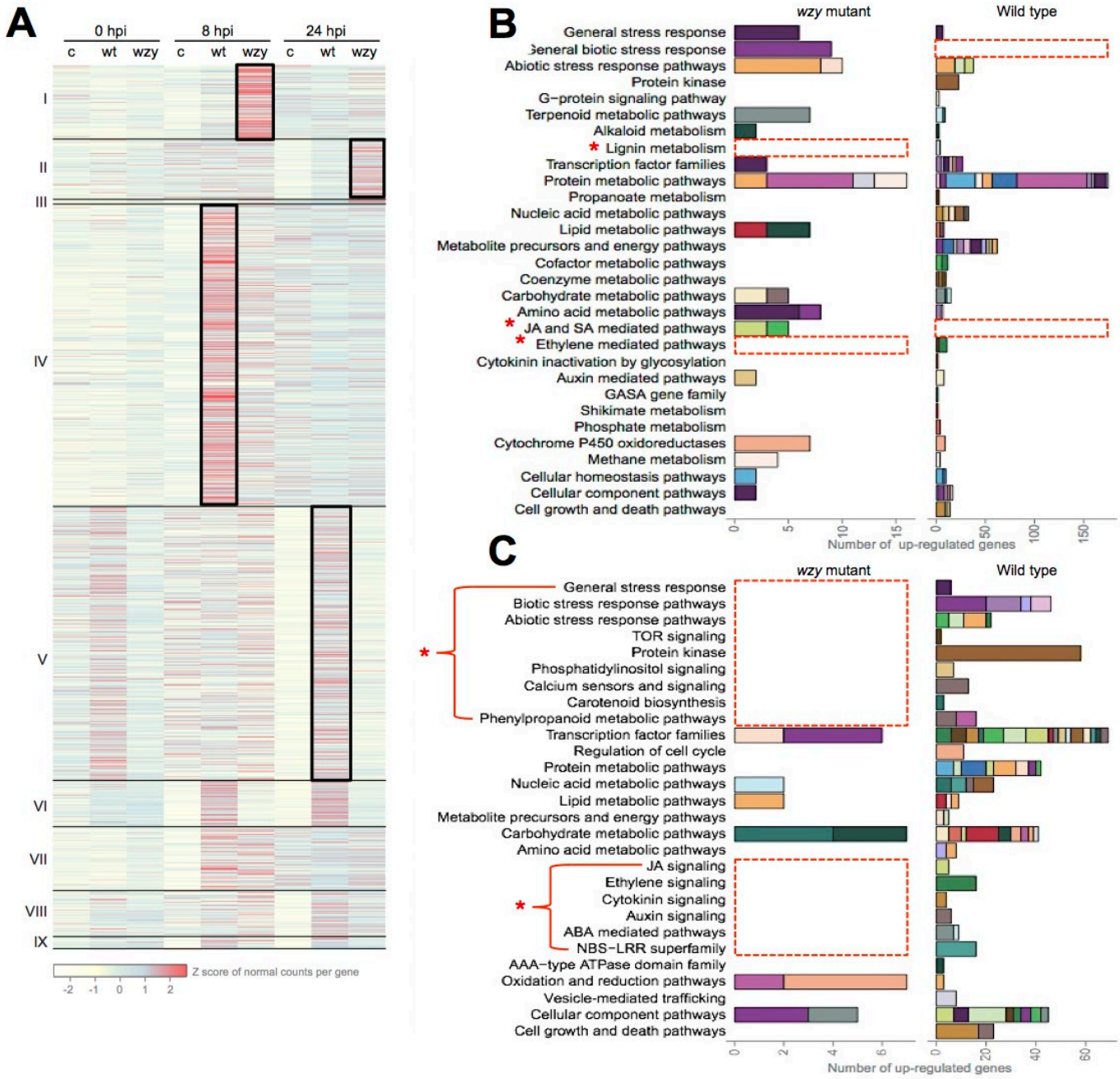

Figure 3. Grapevine responses to early infections by *wzy* mutant and wild type *Xf*. (A) Up-regulated grape genes ($P < 0.05$) in response to *wzy* mutant or wild type bacteria at 8 and 24 hours post-inoculation (hpi) when compared to the wounded control (c). Genes are classified into nine groups (I - IX) based on their expression pattern. The colors in the heat map represent the Z score of the normal counts per gene, and black boxes represent gene groups in each treatment that exhibited the most pronounced differences in expression at each time point. **(B)** Enriched grape functional pathways ($P < 0.05$) among genes up-regulated during *wzy* (Group I) or wt (Group IV) infections at 8 hpi. **(C)** Enriched grape functional subcategories ($P < 0.05$) among genes up-regulated during wzy (Group II) or wt (Group V) infections at 24 hpi. Colored stacked bars represent individual pathways. Red boxes highlight functions of interest (*) that are enriched in one treatment, but not enriched in the other at each time point.

In addition to exploring early defense response, we also characterized the transcriptional response at systemic locations distal to the POI and at longer time points: 48 h, 1 week, and 4 weeks. This tested our hypotheses that (i) truncated *Xf* O antigen is more readily perceived by the grapevine immune system, allowing the plant to mount an

effective defense response to *Xf* and (ii) that the initial perception of the truncated LPS, belonging to the *wzy* mutant, is propagated into a prolonged and systemic response. Local tissue of *wzy*-infected plants induced genes enriched in cell wall metabolism pathways, specifically pectin modification, at 4 weeks post-inoculation **(Fig. 6A)**. This is a stark contrast with wild type-inoculated vines, in which these pathways were up-regulated as early as 8 h post-inoculation. This likely explains why this pathway is not enriched in local tissue of wild type-inoculated vines at these later time points. The induction of SA-mediated signaling pathways in *wzy*-inoculated vines was further supported by the presence of 4 genes, including two Enhanced disease susceptibility 1 (EDS1) genes. EDS genes are known defense genes associated with the SA pathway and have been implicated in grapevine defenses against powdery mildew. The consistent enrichment and up-regulation of SA-associated genes (and thus, the maintenance of the signal), including the presence of PR-1 and other salicylic acid-responsive genes at 8 h post-inoculation, strongly suggests that the plant is preventing the development of infections by *wzy* cells via a SA-dependent pathway. In wild type vines, consistent enrichment of JA-associated genes was further supported by the presence of 9 genes functioning in the metabolism of alpha-linolenic acid, which serves as an important precursor in the biosynthesis of JA **(Fig. 4A)**.

Enrichment analyses of *wzy*-responsive genes in systemic tissue included drought stress response pathways, namely genes enriched in ABA signaling (seen at 48 h post-inoculation) **(Fig. 4B)**. Subsequently at 1 week post-inoculation, the enrichment of lignin metabolism genes is likely part of the vine's stepwise response to this abiotic stress. This is in contrast with wild type-inoculated vines in which these pathways were enriched at 8 h post-inoculation. Enrichment analysis of wild type-responsive genes in systemic tissue included regulation and signaling pathways, including MAPK and G protein signaling **(Fig. 4B)**. Furthermore, genes enriched in ERF transcription factors were up-regulated at 4 weeks post-inoculation, demonstrating that activation of ethylene-mediating signaling is perpetuated during the infection process. Notably, beginning at 1 week, genes enriched in JA-mediated signaling pathways were up-regulated in systemic tissue, and expression continued to increase at 4 weeks post-inoculation. This consistent enrichment and up-regulation provides further support for the role of JA in grapevine responses to wild type *Xf*. Our findings establish that this phytohormone pathway is initiated within the first 24 h post-inoculation, and the signal is consistently maintained in both local and systemic tissue. A total of 7 genes enriched in callose biosynthesis were up-regulated at 4 weeks post-inoculation, in response to wild type cells, which is over half of the total callose-related genes in the genome. The consistent up-regulation of these genes (beginning at 24 h post-inoculation) establishes this structural barrier as an important plant defense response to *Xf* infection. Overall, the RNAseq data strongly indicate that during the days and weeks post-inoculation with *wzy* mutant cells, grapevines are no longer fighting an active infection. We hypothesize that the intense *wzy*-induced oxidative burst during the first 24 h post-inoculation, in combination with other pathogenesis-related responses, had a profound antimicrobial effect on invading *wzy* cells. These responses likely eliminated a large majority of *wzy* mutant populations, and the plant no longer sensed these cells as a biotic threat. In contrast, following recognition of wild type *Xf* cells at 24 h post-inoculation, grapevines began responding to an active threat and initiated defense responses, such as the production of phytoalexins and other antimicrobial compounds. Furthermore, these vines were actively trying to prevent systemic spread of the pathogen through the production of structural barriers, such as tyloses and callose.

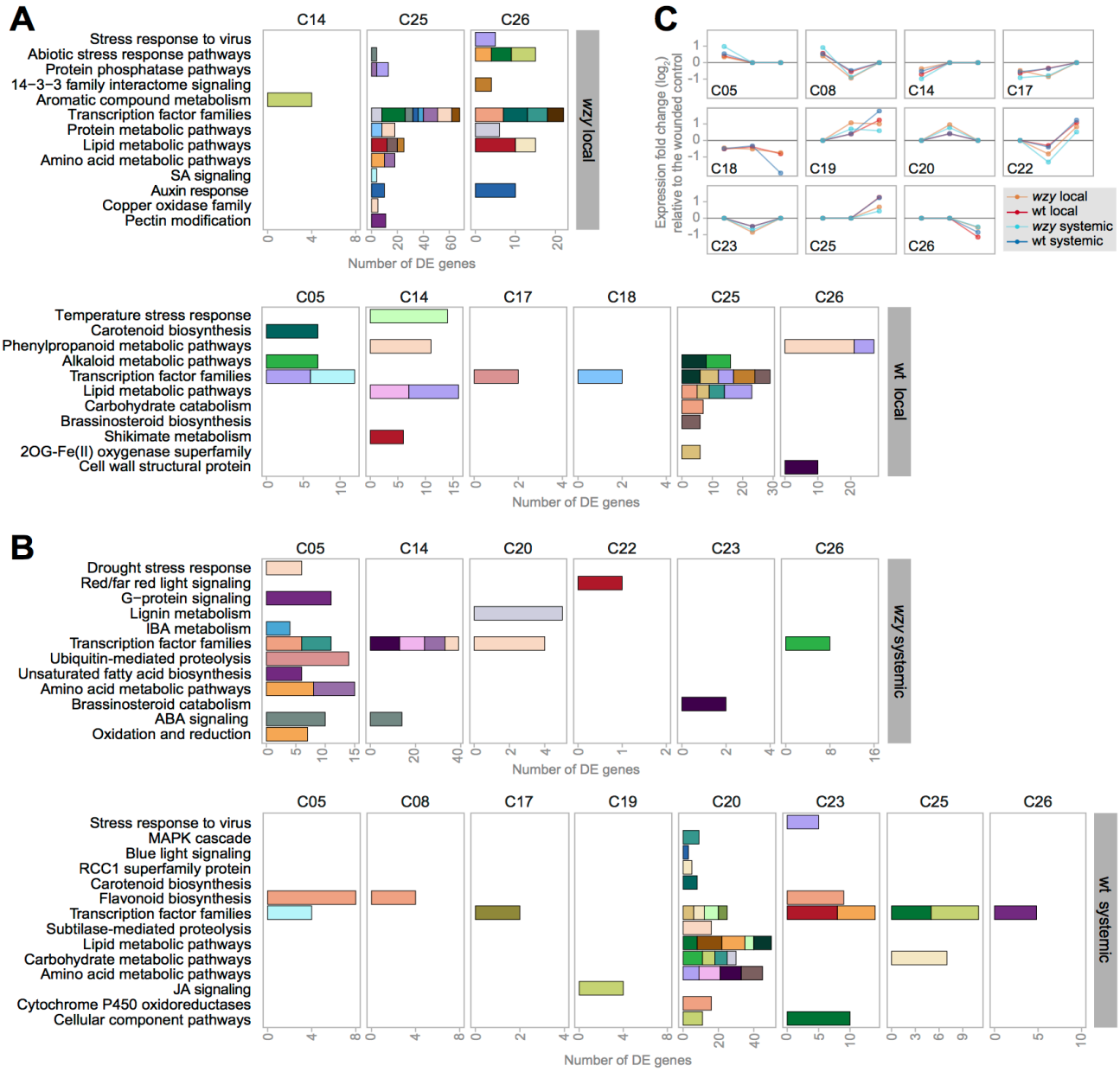

Figure 4. Transcriptomic analysis of late grapevine responses to *Xf* wild type and *wzy* mutant strains in local and systemic tissue. Enriched grape functional pathways ($P < 0.05$) in differentially expressed (DE) gene clusters representing local **(A)** or systemic **(B)** responses to *Xf* inoculation. Only enriched pathways related to grapevine immune responses and that were unique to wild type (wt) or *wzy* mutant inoculations are depicted. Colored stacked bars represent individual pathways. **(C)** Patterns of expression of gene clusters enriched in functional pathways with biological relevance. Lines represent the medoids for each cluster. Dots represent expression fold changes of each medoid (log2) at a given time point post-inoculation (in order: 48 h, 1 week, and 4 weeks) when compared to the wounded control.

OBJECTIVES

1. Characterization of the temporal aspects of the primed state in grapevine
2. Characterization of the molecular mechanisms underlying the grapevine immune response to *Xf*
3. Functional genomics of grapevine immunity to *Xf*

RESULTS AND DISCUSSION

Objective 1: Characterization of the temporal aspects of the primed state in grapevine

We previously showed treating grapevines with LPS before inoculating with *Xf* reduces PD symptoms at 12 weeks post-inoculation (**Fig. 2**) (Rapicavoli *et al.*, 2018). To explore if the primed state can be extended over time, we have tested if an additional LPS application following elicitation of the plant defense priming can

increase PD tolerance. Grapevines were treated with wild type LPS and challenged with *Xf* four hours later. After 48 hours or 1 week, grapevines received an additional LPS treatment. Appropriate controls received diH_2O instead of LPS and 1X PBS instead of *Xf* cells. All plants were scored for PD symptom development using a disease rating scale of 0 to 5 where 0 is a healthy vine and 5 is a dead vine (Guilhabert & Kirkpatrick, 2005). To assess the overall performance of the grapevines during the disease development trial, we used the 'area under the disease progression curve' (AUDPC) method as a measure of disease development. The p-values for the 1 week LPS application AUDPC scores and titer did not show any significant difference between an additional LPS application or water treatments. The AUDPC values for primed vines that received an additional LPS application at 48 hrs. were significantly lower than primed vines that did not receive an additional LPS application (**Fig. 5A**). It appears that an additional LPS dose at 48 hrs. increases the reduced symptom phenotype observed in primed plants. However, no significant difference was found between titer values of these two treatments (**Fig. 5B**). The petioles sampled to measure *Xf* titer were collected 13 weeks post-inoculation. It may be that collecting petioles at this point is too late to observe any significant titer differences between treatments.

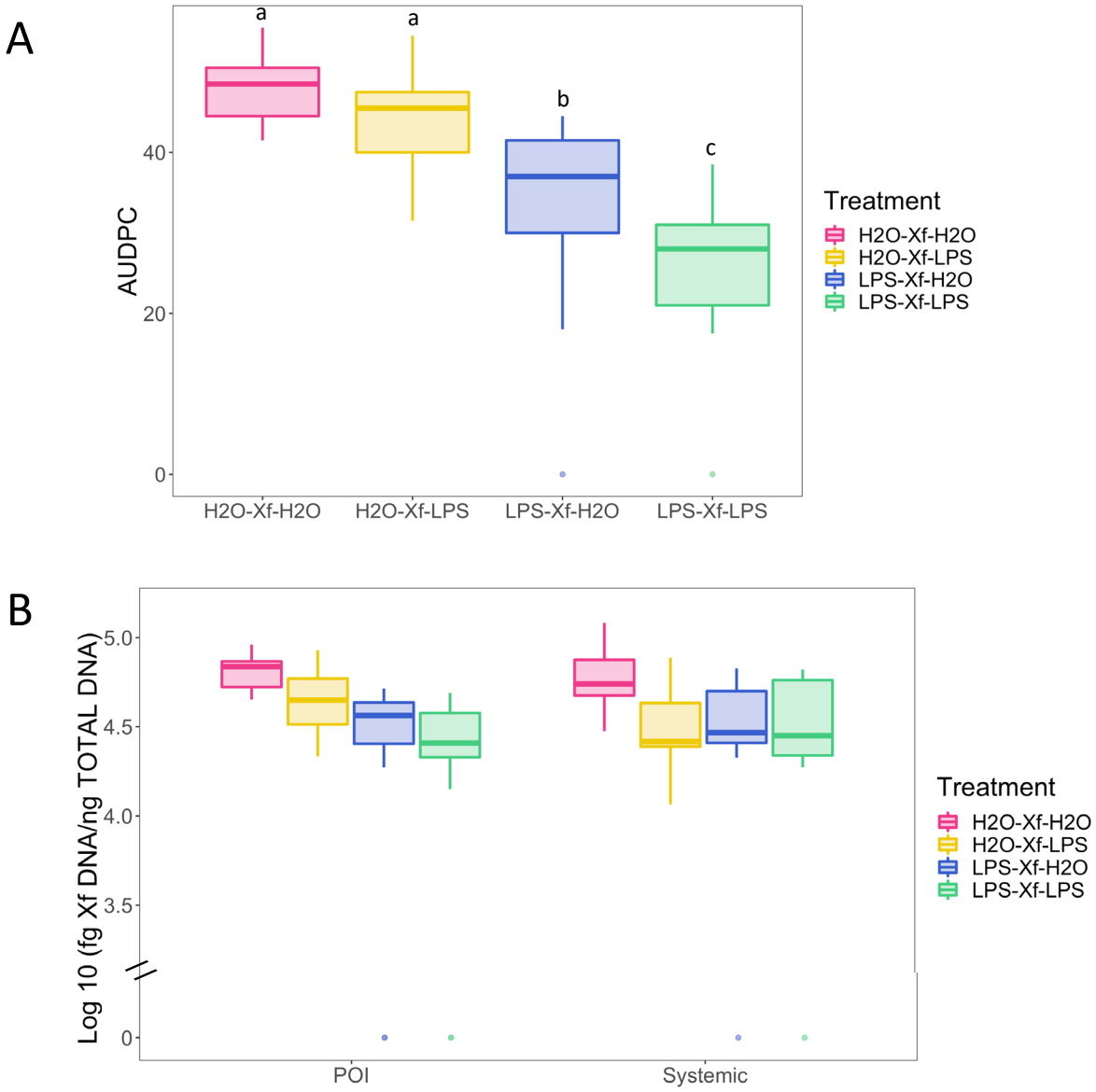

Figure 5. An additional LPS application enhances the reduced symptom phenotype observed in primed vines. *Vitis vinifera* 'Cabernet Sauvignon' grapevines received an additional LPS application 48 hours after being inoculated with *Xf* wild type LPS or water and challenged with *Xf*. **A)** Area under the disease progression curve (AUDPC) values for primed (vines that received LPS pre-treatment) and naïve (vines that received water instead of LPS) vines treated with an additional LPS application. Grapevines were scored on a weekly basis using a PD rating scale of 0 to 5 ($P < 0.05$, Wilcoxon test, n = 13). **B)** Quantification of *Xf* DNA (Log10 fg *Xf* DNA per ng of total DNA) in petioles of primed and naïve grapevines with an additional LPS dose at the point of inoculation (POI) and 20 nodes above (systemic) using qPCR.

Objective 2: Characterization of the molecular mechanisms underlying the grapevine immune response to *Xf*

The molecular mechanisms underlying defense priming and its importance in enabling heightened immunity to counteract pathogens are poorly understood. To better understand the changes occurring in gene expression patterns that potentiate the priming phenotype in grapevine, we will perform a series of RNA-Seq experiments that will highlight genes and pathways induced during priming in both local and systemic tissue. We previously showed treating grapevines with LPS before inoculating with *Xf* reduces PD symptoms at 12 weeks post-inoculation (Rpicavoli *et al.*, 2018). We repeated this experiment and monitored plants for PD symptom development and harvested petioles from these plants for RNA-seq analysis. Grapevines were treated with wild type LPS and challenged with *Xf* cells 4 hours later. Petioles for RNA-Seq were harvested at 4 h, 24 h, and 48 h post-*Xf* challenge from the point of inoculation and 20 nodes above the point of inoculation. RNAseq analysis is under preparation. To assess the overall performance of vines treated with LPS over the entire disease development trial, we used the 'area under the disease progression curve' (AUDPC) method as a measure of disease development. The AUDPC values showed primed plants are significantly lower in disease severity compared to the naïve plants which did not receive LPS. Our results indicate LPS treatment primes the immune system before *Xf* challenge leading to reduced symptom development and significantly lower bacterial titer in grapevines (**Fig. 6**).

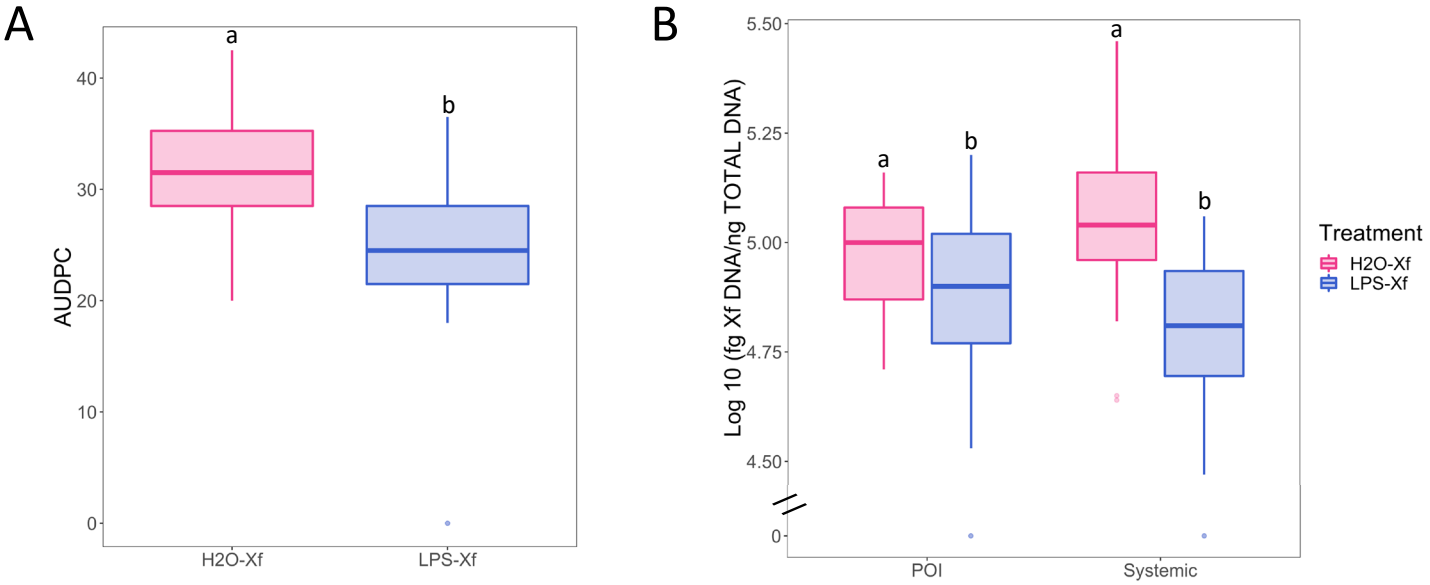

Figure 6. Pre-treatment with lipopolysaccharide reduces PD symptom development and bacterial titer in grapevines infected with *X. fastidiosa*. *Vitis vinifera* 'Cabernet Sauvignon' grapevines were inoculated with *Xf* wild type LPS or water 4 hours prior to inoculation with *Xf*. **A)** Area under the disease progression curve (AUDPC) values for primed (vines that received LPS pre-treatment) and naïve (vines that did not receive an LPS pre-treatment and received water instead) vines. Grapevines were scored on a weekly basis using a PD rating scale of 0 to 5. ($P < 0.0001$, Wilcoxon test, n = 26, n = 25). **B)** Quantification of *Xf* DNA (Log10 fg *Xf* DNA per ng of total DNA) in petioles of primed and naïve grapevines at the point of inoculation (POI) and 20 nodes above (systemic) using qPCR. Appropriate controls received water instead of LPS and 1X PBS instead of bacterial cells and showed no symptoms and no *Xf* was detected. (POI: $P < 0.05$, Systemic: $P < 0.0001$, Wilcoxon test, n = 26, n = 25).

Objective 3: Functional genomics of grapevine immunity to *Xf*

We have determined that LPS-mediated early elicitation of the basal defense response leads to systemic and prolonged activation of defense pathways related to *Xf* perception in grapevine. Our experiments identified several genes involved in plant defense that were enriched in response to *wzy* cells (Rapicavoli *et al*, 2018). For this objective, our goal is to generate transgenic Thompson Seedless grapevines overexpressing these genes and test resistance to *Xf* in the field and greenhouse. We will also incorporate candidate genes from our transcriptome analysis results in objective 2. Several of these candidate genes have been cloned into the pCambiaK-APS vector for gene overexpression and transformed into *Agrobacterium tumefaciens*. These constructs will be used to transform Thompson Seedless grapevines at the Plant Transformation Facility in University of California - Davis.

PUBLICATIONS AND PRESENTATIONS

Publications:

Rapicavoli, J. N., Blanco-Ulate, B., Muszyński, A., Figueroa-Balderas, R., Morales-Cruz, A., Azadi, P., Dobruchowska, J., Castro, C., Cantu, D., and Roper, M. C. (2018) Lipopolysaccharide O-antigen Delays Plant Innate Immune Recognition of *Xylella fastidiosa*. *Nature Communications*, 9 (1): 390.

Rapicavoli, J. N., Kinsinger, N., Perring, T. M., Backus, E. A., Shugart, H. J., Walker, S., & Roper, M. C. (2015) O Antigen Modulates Insect Vector Acquisition of the Bacterial Plant Pathogen *Xylella fastidiosa*. *Applied and Environmental Microbiology*, *81*(23): 8145-8154 **(AEM Spotlight and Journal Cover Photo)**

Clifford, J. C., Rapicavoli, J. N., and Roper, M.C. (2013) A Rhamnose-Rich O-Antigen Mediates Adhesion, Virulence, and Host Colonization for the Xylem-Limited Phytopathogen *Xylella fastidiosa*. *Molecular Plant-Microbe Interactions: MPMI,* 26 (6): 676-85.

Oral Presentations:

Caroline Roper: O-antigen acts as a shield to delay early plant immune recognition of *Xylella fastidiosa*, International Society of Molecular Plant Microbe Interactions Congress, Glasgow, Scotland (July 2019).

Jeannette Rapicavoli. "O antigen functions as a shield during the *Xylella fastidiosa*-grapevine interaction." American Phytopathological Society Annual Meeting 2016, Tampa, FL (August 2016)

Jeannette Rapicavoli. "Zeta potential: utilizing surface charge to explore host-pathogen interactions." Center for Plant Cell Biology Symposium, UC Riverside (December 2015)

Poster Presentations:

Claudia A. Castro and M. Caroline Roper. Lipopolysaccharide-induced plant defense priming against *Xylella fastidiosa* infection in *Vitis vinifera* grapevines (2019). International Society for Molecular Plant-Microbe Interactions Congress, Glasgow, UK.

Jeannette N. Rapicavoli, Barbara Blanco-Ulate, Rosa Figueroa-Balderas, Abraham Morales-Cruz, Dario Cantu, and M. Caroline Roper. Contribution of cell surface carbohydrates to the *Xylella fastidiosa*-grapevine interaction (2016). International Society for Molecular Plant-Microbe Interactions Congress, Portland, OR

Jeannette N Rapicavoli, Nichola Kinsinger, Thomas M. Perring, Crystal M. Johnston, Sharon Walker, and M. Caroline Roper. Lipopolysaccharide modulates the vector-pathogen interface of the bacterial phytopathogen, *Xylella fastidiosa* (2015). American Phytopathological Society Annual Meeting, Pasadena, CA.

RELEVANCE STATEMENT

Our ongoing work demonstrates that pre-treatment with purified LPS primes the grapevine immune system which results in reduced PD severity and lower bacterial titer when these plants are infected with *Xf*. We will also conduct an in-depth transcriptome analysis of LPS-primed grapevines to better understand mechanisms of defense against *Xf*. The overall outcome will result in fundamental knowledge about grapevine immune responses at the molecular level that we will utilize to test novel gene targets for creating PD-resistant grapevines.

LAYPERSON SUMMARY

Plants have developed complex mechanisms to defend themselves from constant biotic and abiotic challenges presented by a fluctuating environment. One of these mechanisms, called plant defense priming, is a tool that exploits plant 'memory' to counteract pathogens and abiotic stress. This 'memory' allows plants to quickly recognize pathogens and activate strong immune responses that result in disease resistance or tolerance. Successful plant pathogens, like *Xf*, must overcome plant immune responses to establish themselves and cause disease. We have shown *Xf* utilizes the prominent O antigen surface carbohydrate found in its LPS molecule to shield itself from being recognized by the grapevine immune system, effectively delaying its detection by the plant. However, if we isolate its LPS and inject it directly into the plant like a vaccine, it elicits strong immune responses and conditions grapevines for enhanced defense against *Xf*. We will employ this knowledge to better understand the mechanism of this enhanced response, test if we can maintain the primed state, and apply these results to create PD-resistant grapevines.

STATUS OF FUNDS

The funding for this project is going towards supporting a graduate student, Claudia Castro, in the Roper laboratory. In the Cantu laboratory, funds for this project are supporting the salary and benefits for a postdoctoral researcher. We anticipate spending the remainder of the salary, supply, services, and greenhouse recharge funds associated with this project.

INTELLECTUAL PROPERTY

To date, there is no intellectual property associated with this project. If this research leads to materials or procedures that will be subject to intellectual property restrictions, 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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