Progress Report for PD Project 2009-223
March 1, 2010

I. Project title: Influence of Host Xylem Chemistry on Regulation of *Xylella fastidiosa* virulence Genes and Host Specificity

II. Principal investigators and cooperators:

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III. List of objectives and description of activities conducted to accomplish each objective:

This project continues our efforts to understand the regulation of virulence genes in *Xylella fastidiosa* to develop strategies for reducing their function in susceptible host plants for disease control. Following our recent finding (Cooksey, 2008; Shi et al., 2010) that genes that are known or expected to be involved in virulence in a Pierce's disease (PD) strain were differentially regulated in the xylem fluid of a susceptible plant (grape) vs. a resistant plant (citrus), we propose to test whether this is a general trend, using other susceptible, resistant, and tolerant plant combinations with strains of *X. fastidiosa* that have different host range specificities. We also propose to identify the components of xylem fluid that are responsible for this differential expression.

Objective 1. Assess virulence gene expression of several different host-range strains of X. fastidiosa in the xylem fluid of a comment set of plant hosts.

Objective 2. Assess the influence of specific components of plant xylem fluids on the expression of virulence genes of X. fastidiosa.

IV. Summary of major research accomplishments and results for each objective:

Since the project was submitted, we have completed writing up and published our finding of differential regulation of genes of *Xylella fastidiosa* when grown in xylem fluid of citrus vs. grapevine (Shi et al., 2010). This publication included further data, collected since the proposal was submitted, showing that the grape strain does grow in xylem fluid from citrus, so the lower expression of virulence genes was not due to simple inactivity of the bacterium in citrus xylem fluid. Since funding was obtained in late fall of 2009, we have collected 30-50 ml of xylem fluid from all of the hosts we plan to test with different strain/host combinations proposed for this project. More fluid will be collected starting in April. A postdoctoral researcher was identified to begin working on the gene expression studies for the project in early 2010, but that individual recently decided to seek employment in another state due to spousal employment. We are close to identifying another postdoctoral researcher to begin soon on the gene expression studies.

Table 1. Strain/host combinations to be tested in assessing the influence of different xylem fluids on regulation of virulence genes of *X. fastidiosa*

Source of xylem fluid	Strains of *X. fastidiosa* to be tested and known host reaction			
	Temecula (PD strain)	Ann 1 (oleander strain)	Dixon (almond strain)	MLS024 (mulberry strain)
Grape (Pinot Noir)	Susceptible	Resistant	Susceptible	Susceptible
Oleander	Resistant	Susceptible	Resistant	Resistant
Almond	Tolerant	Resistant	Susceptible	Resistant
Mulberry	Resistant	Resistant	Resistant	Susceptible
Citrus	Resistant	Resistant	Resistant	Resistant

V. Publications or reports resulting from the project:

Shi, X. Y., J. Bi, J. G. Morse, N. C. Toscano, and D. A. Cooksey. 2010. Differential expression of genes of *Xylella fastidiosa* in xylem fluid of citrus and grapevine. FEMS Microbiol. Lett. 304:82-88.

Cooksey, D. A. 2009. Influence of Host Xylem Chemistry on Regulation of *Xylella fastidiosa* virulence Genes and Host Specificity. Page 72 In: Pierce's Disease Research Symposium, 2009, CDFA.

VI. Presentations on research:

Poster presentation at CDFA Pierce's Disease Research Symposium, Sacramento, CA, December 9-11, 2009.

VII. Research relevance statement:

Understanding the basis for differential regulation of virulence genes in response to differences in plant xylem could lead to strategies to reduce virulence gene expression for practical control. For example, the reduced expression of virulence genes that we observed in a PD strain in citrus xylem fluid may be due to substances that repress expression of these genes. Such substances could then be directly tested for controlling disease expression in susceptible grapevine cultivars with the substance used much like a pesticide or antimicrobial drug (Zhong et al., 2008). Alternatively, there may be substances in susceptible grapevine xylem fluid that induce the expression of virulence of the PD strain but are lacking in citrus. These substances may also be useful, since over expression of some virulence factors could result in reduced disease symptoms. For example, synthesis of a diffusible signaling factor by the virulence gene *rpfF* in *X. fastidiosa* appears to reduce virulence (Chatterjee et al., 2008). The steps to achieving field application of such strategies are to 1) determine whether differential regulation of virulence genes in xylem fluid from susceptible vs. resistant and tolerant hosts is a general trend for different strains of *X. fastidiosa*, 2) determine what specific components of xylem fluid are

responsible for these differences, 3) test the application of such substances for reducing disease expression on grapevine in greenhouse and field assays. Our goal is to accomplish the first two steps with funding from this project. The proposed research addresses PD/GWSS Research Priority of "exploiting *X. fasidiosa* virulence factors to control Pierce's disease."

VIII. Lay summary of current year's results:

Since this project was proposed, we have published the results from our previous work showing that a number of genes expected to be important for disease expression in a Pierce's disease (grape) strain of *Xylella fastidiosa* were expressed at higher levels in xylem fluid extracted from grapevines than in xylem fluid extracted from citrus (resistant to this strain). This publication included further data, collected since the proposal was submitted, showing that the grape strain does grow in xylem fluid from citrus, so the lower expression of some virulence genes was not due to simple inactivity of the bacterium in citrus xylem fluid. Since funding was received in late fall of 2009, we have been collecting xylem fluid from the different hosts that we plan to include for broader gene expression studies with several bacterial strain/host combinations this year.

IX. Status of funds:

Funding for the project was received in late fall of 2009. We have not yet hired a postdoctoral researcher for this project but began xylem collection using state Agricultural Experiment Station funds supporting part of the salary of an existing Staff Research Associate in the Cooksey lab. As of 1/31/2010 (the date of our last UCR financial statement), we had not expended any funds from the UC PD project account, so at that time, the balance was $58,544.58.

X. Summary and status of intellectual property produced during this research project:

Results obtained from this research will be in the public domain.

Literature Cited:

Chatterjee, S., Newman, K.L., and Lindow, S. E. 2008. Cell-to-cell signaling in *Xylella fastidiosa* suppresses movement and xylem vessel colonization in grape *Mol. Plant-Microbe Interact.* 21:1309- 1315.

Cooksey, D. A. 2008. Differential expression of genes of *Xylella fastidiosa*in xylem fluid of citrus and grapes. Pages 97-110 In: Pierce's Disease Research Symposium, 2008, CDFA.

Zhong, Z., X. Yu, and J. Zhu. 2008. Red bayberry extract inhibits growth and virulence gene expression of the human pathogen *Vibrio cholerae*. *J. Antimicrobial Chemotherapy* doi:10.1093/jac/dkm540.