Renewal Progress report for CDFA agreement number 2011-322.

Project Title: Can Pierce's disease *PdR1* resistance introgressed into *Vitis vinifera* be translocated from a resistant rootstock to a susceptible scion?

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Cooperators: None

Time Period Covered: July 1, 2011 – March 5, 2012.

Introduction:

Pierce's Disease (PD) resistance from wild *Vitis* has been transferred into *V. vinifera* via classical (non-transgenic) breeding. However, given the extensive number of wine, raisin, and table grape varieties susceptible to PD, introgression into each will be time consuming and costly. This project describes pilot experiments designed to test the hypothesis that a Pierce's disease (PD) resistant rootstock can affect PD development in susceptible scions. The simple experimental design will determine whether or not the *PdR1* resistance factor(s) is (are) capable of systemic protection of tissues beyond the graft union to affect pathogenesis of *X. fastidiosa* in susceptible scions.

Objectives of the Proposed Research and Path to Application:

The broad goal of this proposal is to evaluate the potential of a non-transgenic, PD resistant *V. vinifera* selection used as rootstock to confer systemic resistance to PD susceptible *V. vinifera* scions.

Objective:

Objective 1: Determine effect of rootstock genetic background (+/- *PdR1*) on disease severity and *X. fastidiosa* population levels in PD-susceptible scions following challenge inoculation of scions with *X. fastidiosa*.

Description of activities conducted to accomplish each objective, and summary of accomplishments and results for each objective:

The basic experimental design evaluated Pierce's disease (PD) symptom development and *X. fastidiosa* (Xf) population levels in PD susceptible scions grafted onto rootstocks that are either resistant or susceptible to PD. (**Table 1**). The first year experiment was conducted in a greenhouse at the USDA-ARS SJVASC facility in Parlier, CA. Source of PD-susceptible plant material was the wine grape variety 'Chardonnay', known to support high populations of Xf and exhibit severe PD symptoms (Buzkan et al., 2005). Source of PD-resistant material (Ramming and Walker, 2010) was a modified backcross generation 2 raisin selection (referred to here as PDR1) with PD resistance locus *PdR1* (Krivanek et al., 2006) introgressed from 89-F0908 (*V. rupestris* X *V. arizonica*). Each treatment consisted of ~10 plants (replicates).

Table 1. Basic experimental design to evaluate effect of PD-resistant rootstocks on PD susceptible scions following challenge inoculation of scion with Xf.

Rootstock		Scion		
Variety	PD response	Variety	PD response	Expected scion response to PD
'Chardonnay'	Susceptible	'Chardonnay'	Susceptible	Susceptible
PDR1	Resistant	PDR1	Resistant	Resistant
PDR1	Resistant	'Chardonnay'	Susceptible	?
'Chardonnay'	Susceptible	PDR1	Resistant	?

The Xf strain Stag's Leap was used as challenge inoculum, as this strain was recovered from PD-symptomatic grape in California and is known to cause severe PD symptoms in inoculated plants (Hendson et al., 2001). Plants were mechanically inoculated above the graft union (e.g., scions) using a 20µl droplet of bacterial culture suspension (10^8 colony forming units/ml) placed on a partially lignified stem just above the petiole junction. Bacteria were introduced into the xylem using a 25 gauge needle pushed through the droplet, penetrating about 1/3rd of the width of the shoot. Plants were maintained in the greenhouse and evaluated visually for PD symptoms after 14 weeks. PD severity was visually assessed using a nominal 0-5 rating scale (Roper et al., 2007), where 0 corresponds to no visual symptoms and 5 corresponds to death of the plant (**Figure 1**). In all cases, the response of the scion to PD remained unaltered, regardless of rootstock genotype. 'Chardonnay' scions expressed similar levels of PD severity on both PD-susceptible (mean severity rating 3.2) and PD-resistant rootstocks (mean severity rating 3.6), whereas PDR1 scions expressed only mild symptoms on PD-susceptible (mean severity rating 1.0) or PD-resistant (mean severity rating 1.4) rootstocks. PD symptom severity ratings for mock inoculated plants of all scion/rootstock combinations had means of less than 1.0, but greater than 0, presumably due to water stress at some point post-inoculation.

Real-time PCR was used to quantify bacterial titers in stems and petiole samples collected 25 cm above the point of inoculation. DNA samples were extracted from lyophilized tissue as previously described (Ledbetter and Rogers, 2009) and used as template. Real-time PCR reactions were run in triplicate to determine technical variability and standard curves were included in each plate to facilitate normalization. Real-time PCR data were converted to the equivalent number of Xf genomes; mean population levels were compared among scion/rootstock combinations for both stem and petiole samples (**Figure 2**). Xf population levels in 'Chardonnay' scions were similar regardless of rootstock genotype (~10^7) in both stem and petiole samples. In contrast, Xf population levels were substantially lower in PDR1 scions (~10^4 – 10^5), but no significant differences were noted for PDR1 scions based on rootstock genotype.

Collectively, the results of the first year experiment indicate the tentative answer to the question posed in the project title is "No". Interestingly, PDR1 scions grafted onto 'Chardonnay' rootstocks remained resistant to PD. This observation indicates that PD-susceptibility factors present in a rootstock do not result in alteration of *PdR1*-mediated response to PD in the scion. In the second year of this project, the experiment described above will be repeated. Also, if available, an alternate source of PD resistance (SEUS germplasm) may be evaluated in the same basic experimental design.

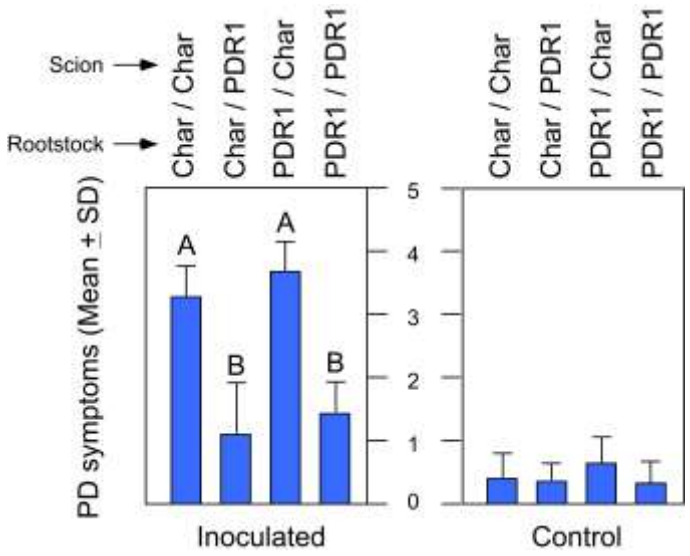

Figure 1. PD symptom ratings (0 = no disease, 5 = death) for scions at 14 weeks post inoculation. Means (+/- standard deviation) with different letters are significantly different ($P < 0.05$) based on a non-parametric rank sum test. Char ['Chardonnay'] (PD-susceptible); PDR1 (PD-resistant).

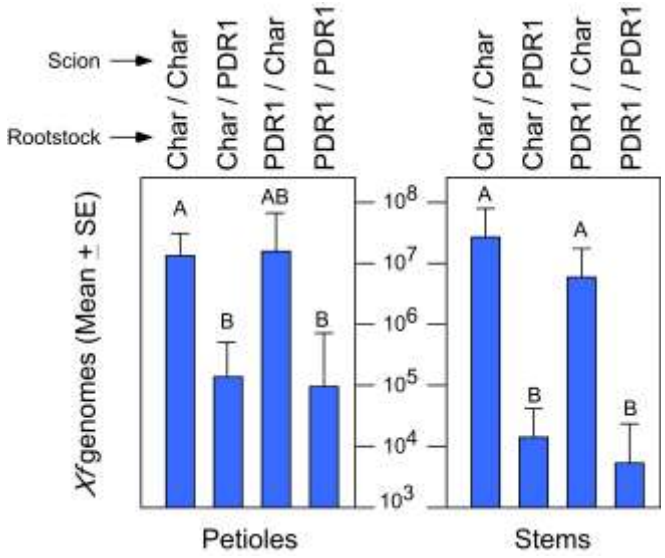

Figure 2. Estimation of *X. fastidiosa* (Xf) titer in scions 14 weeks post inoculation. Results of quantitative real time PCR are presented as the equivalent number of Xf genomes present in petiole or stem samples taken ~25 cm above the point of inoculation. Values refer to mean ± standard error for entire petioles or 2.5 cm sections of stem. Statistical analysis was by ANOVA followed by Tukey's HSD; means with different letters are significantly different ($P < 0.05$).

References:

Buzkan, N., Kocsis, L., and Walker, M. A. 2005. Detection of *Xylella fastidiosa* from resistant and susceptible grapevine tissue by sectioning and membrane entrapment immunofluorescence. *Microbiological Research* 160:225-231.

Hendson, M., Purcell, A. H., Chen, D., Smart, C., Guilhabert, M., and Kirkpatrick, B. C. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. *Applied and Environmental Microbiology* 67:895-903.

Krivanek, A. F., Riaz, S., and Walker, M. A. 2006. Identification and molecular mapping of *PdR1*, a primary resistance gene to pierce's disease in *Vitis*. *Theoretical and Applied Genetics* 112:1125-1131.

Ledbetter, C. A., and Rogers, E. E. 2009. Differential susceptibility of *Prunus* germplasm (subspecies *Amygdalus*) to a California isolate of *Xylella fastidiosa. HortScience* 44:1928-1931.

Ramming, D. W., and Walker, M.A. 2010. Breeding Pierce's disease resistant table and raisin grapes and the development of markers for additional sources of resistance. Proceedings, 2010 Pierce's Disease Research Symposium. California Department of Food and Agriculture, Sacramento, CA. pp. 243-248.

Roper, M.C., Greve, L. C., Warren, J. G., Labavitch, J. M., and Kirkpatrick, B. C. 2007. *Xylella fastidiosa* requires polygalacturonase for colonization and pathogenicity in *Vitis vinifera* grapevines. *Molecular Plant Microbe Interactions* 20:411-419.

Publications/Presentations:

Results of this research were presented in poster form at the CDFA PD symposium held in Sacramento (December 13-15, 2011). A corresponding article was published in the PD Symposium 2011 Proceedings (pp. 173-174).

Research Relevance Statement:

Based on one year of data, it is unlikely that *PdR1* germplasm will be suitable for development of commercial rootstocks that confer systemic resistance to existing PD-susceptible grape varieties used as scions. Nonetheless, *PdR1* scions remained resistant when grafted onto a PD-susceptible rootstock. Therefore, new table, raisin, and wine grape varieties bearing *PdR1*-mediated PD resistance likely may be deployed as scions onto existing PD-susceptible rootstocks. If verified, this observation may facilitate use of *PdR1* resistance, deployed as PD-resistant scions. However, low bacterial population levels (but greater than zero) in PD-resistant material used as scions could eventually allow for systemic movement of *X. fastidiosa* into PD-susceptible rootstocks. Thus, PD-resistant scions could eventually decline if susceptible rootstocks become systemically infected and develop high population levels of *X. fastidiosa* that interfere with xylem transport. Therefore, the industry may want to proceed with breeding programs aimed at developing PD-resistant rootstocks to complement efforts already in progress on development of PD-resistant scions.

Layperson Summary:

In the first year of project funding, greenhouse trials were conducted to address the hypothesis that a rootstock bearing the *PdR1* gene for Pierce's disease (PD) resistance could confer systemic resistance to a PD-susceptible scion. The mechanism hypothesized for systemic resistance (in an otherwise susceptible scion) was via soluble resistance factors transported in xylem tissue across the graft union. Results of the first year experiment indicated that response of the scion was unaffected regardless of whether or not the rootstock was resistant or susceptible to PD. This was true for both PD-susceptible and PD-resistant scions. Thus, neither resistance nor susceptibility factors were translocated in xylem across the graft union (or at least such transport had no appreciable effect on scion response to PD).

Data evaluated consisted of symptom severity ratings and measurement of *Xylella fastidiosa* (the causal agent of PD) population levels in scions 14 weeks post inoculation. In the second year of funding, the experiment will be repeated for validation of the preliminary results from the first year experiment.

The practical outcome of the research includes the following:

1. A PD-resistant rootstock bearing the *PdR1* gene will not serve as a 'magic bullet' solution for achieving field resistance to PD for the numerous PD-susceptible table, raisin, and wine cultivars in production.

2. PD-resistant scions remain resistant when grafted onto a susceptible rootstock, implying that new PD-resistant grape varieties used as scions likely may be grafted onto currently available PD-susceptible rootstocks without altering the intrinsic resistance of the scion.

3. Low bacterial population levels (but greater than zero) in PD-resistant material used as scions could eventually allow for systemic movement of *X. fastidiosa* into PD-susceptible rootstocks. Thus, PD-resistant scions could eventually decline if susceptible rootstocks become systemically infected and develop high population levels of *X. fastidiosa* that interfere with xylem transport. Therefore, the industry may want to proceed with breeding programs aimed at developing PD-resistant rootstocks to complement efforts already in progress on development of PD-resistant scions.

Status of Funds: Total FY12 budget awarded was $14,112. As of February 2, 2012: $10,767 committed, $3,345 available. Salaries: $10,161 planned, $9,301 committed, balance of $860 available. Supplies: $3,951 planned, $1,467 committed, $2,484 available. FY13 funds requested in original proposal: total $14,366.

Intellectual Property:
The raisin selection used as rootstock in these experiments is derived from the collaborative breeding projects of David W. Ramming (USDA-ARS) and M. Andrew Walker (University of California) and is currently being developed (through additional backcrosses) into a raisin variety, ultimately to be released, licensed, and patented as per standard USDA-ARS policies described in "ARS Policies and Procedures - Technology Transfer in ARS (141.2-ARS)" at: http://www.afm.ars.usda.gov/ppweb/PDF/141-02.pdf.