TITLE OF REPORT: Final Report for CDFA Agreement number 09-0746

PROJECT TITLE: Field Evaluations of Grafted Grape Lines Expressing Polygalacturonase Inhibiting Proteins (PGIPs)

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TIME PERIOD COVERED BY THE REPORT: The results reported here are from work conducted from 1 March 2010 to 28 February 2013.

INTRODUCTION:

The project was designed to establish two field sites that would allow grape lines to be evaluated in order to assess whether polygalacturonase inhibiting proteins (PGIPs) restrict *Xylella fastidiosa* spread in grapevines.

The PI and co-PI had shown that the expansion of *X. fastidiosa* from the infection site throughout the vine, creates a systemic infection that causes Pierce's Disease (PD) and vine death (Krivanek and Walker, 2005; Labavitch 2006, 2007; Lin, 2005; Lindow, 2006, 2007a,b; Rost and Matthews, 2007). The grapevine water-conducting xylem elements are separated by pit membranes, cell wall "filters" whose meshwork is too small to permit *X. fastidiosa* passage (Labavitch et al., 2004, 2006, 2009a,b). *X. fastidiosa* uses cell wall-degrading enzymes to digest the pit membrane polysaccharides (Labavitch et al., 2009b), opening the barrier between the xylem elements and permitting systemic spread of the bacteria.

The *X. fastidiosa* genome contains a polygalacturonase (*Xf*PG) and several β-1,4-endo-glucanase (EGase) genes, whose predicted enzyme and protein products participate in the digestion of pit membrane pectin and xyloglucan polymers, thereby facilitating *X. fastidiosa* systemic movement and PD development. Labavitch et al. (2006, 2007, 2009a; Perez-Donoso et al., 2010) reported that introduction of PG and EGase into uninfected grapevines caused pit membrane breakage. Roper et al. (2006, 2007) developed an *Xf*PG-deficient *X. fastidiosa* strain and showed it was unable to cause PD symptoms, so *Xf*PG is a PD virulence factor.

The over-all research aim is to use plant proteins, PGIPs, to limit *X. fastidiosa* spread in grapevines. PGIPs are selective inhibitors of pathogen and pest PGs (Powell et al., 2000; Shackel et al., 2005; Stotz et al., 1993, 1994). Transformed grapevines expressing pear fruit PGIP (pPGIP) have reduced susceptibility to *X. fastidiosa* and transgenic PGIP is transported across the graft junction from a genetically engineered pPGIP expressing rootstock into wild-type scions (Agüero et al., 2005).

The Research Scientific Advisory Panel [RSAP 2007] review gave high priority to a PGIP-based strategy for PD control and funded proposals to identify optimal PGIPs and to optimize effective PGIP

export. The funding decisions were based on results with vines in controlled greenhouse settings, not in environments comparable to those in commercial vineyards. This project has been designed to scale up the grafted and own-rooted PGIP-expressing grapevines, plant them in field settings, and evaluate their agronomic performance and their resistance to PD in settings comparable to commercial fields.

The original funding request was to cover the expenses for 3 years to establish two vineyard field trials to evaluate grapevines grafted to rootstocks expressing pPGIP. Our portion of the field trials project has been designed to test the PD resistance and agronomic traits of grafted rootstock lines expressing pPGIP in typical vineyard settings and establishing these plantings has consumed most of the first three years of this work. Four other groups of PIs are evaluating various combinations of approaches to limit PD damage by *Xylella fastidiosa* in the same fields. The field in Solano County has been inoculated twice with *X. fastidiosa* and evaluations at the other trial site in Riverside County rely on natural infections.

OBJECTIVES:

1. Scale up the number of grafted and own-rooted pPGIP-expressing lines.
2. Plant and maintain grafted and own-rooted lines in two locations with different PD pressure.
3. Evaluate relevant agronomic traits of vines in two locations.
4. Determine PD incidence in pPGIP-expressing grafted and own-rooted lines. Test for *X. fastidiosa* presence and, if present, determine the extent of infection.

DESCRIPTION OF ACTIVITIES AND ACCOMPLISHMENTS:

Objective 1: Generate enough grafted and own-rooted grapevines for the field trial

Progress: The pPGIP-expressing 'Chardonnay' and 'Thompson Seedless' grapevines generated by Agüero et al. (2005) were maintained at the UC Davis Core Greenhouses. Vegetative cuttings of non-lignified stem sections from transgenic and control plants of both cultivars were rooted in an aeroponic cloning manifold (EZ-Clone Inc., Sacramento, CA (Figure 1)). These plants are referred to as "own-rooted plants." Rooted cuttings were transferred to soil and maintained in the greenhouse before being transferred to the field sites.

Figure 1. Grafted grapevine cuttings rooting in the EZ-Clone aeroponic device manifold.

Grafted and "transgrafted" plants were generated for the field trial and were made by green grafting rootstock stem sections with one-bud scions. "Transgrafted" plants had rootstocks from the pPGIP-expressing lines and scions that were non-transgenic. The composition of the field plot in Solano County is shown in Figure 2. A similar plot design was set up for the Riverside County site. Table 1 shows the plants that were generated and in the Solano County field as of December, 2012. Although about 50% of the plants needed for the population for the Riverside County site had been generated by the end of 2012, because of powdery mildew and mealy bug infestations in our greenhouses at UC Davis, we altered our grafting protocols to complete the population. In January 2013, David Dolan from A. Dandekar's group was added to the team to complete the generation of the grafted plants to fill out both sites. He also used wedge grafting to generate the grafted plants and used another greenhouse at UC

Davis which seems to have fewer issues. In addition, reusing the EZ-Clone tanks was problematic because the foam plugs became persistently contaminated, a new system using different media combinations was utilized beginning in mid-2011.

Figure 2. Field plan for Solano County site for Powell et al. trial.

		Chardonnay			Thompson Seedless		
	Grafting Strategy (Scion/root) Hatch – expressing pPGIP Filled – not expressing pPGIP						
Own-Rooted Plants (#)	Inoculated	17	-	(9)	8	-	9
	Non-Inoculated	8	-	(4)	4	-	5
Grafted Plants (#)	Inoculated	9	5 (4)	(9)	6 (3)	9	7 (2)
	Non-Inoculated	4	(4)	(4)	(4)	4	(4)
Grafting In Progress (#)	Potted	16	14	0	4	3	7

Table 1. Total number of grapevines planted in, and prepared for Solano County. Dashed shapes represent pPGIP expressing grapevine rootstocks and/or scions; solid shapes are null controls (no pPGIP). Own-rooted vines were inoculated on 7/21/2011 and 5/29/2012; grafted vines have not been mechanically inoculated. Grafting in progress numbers include all grafted cuttings at each checkpoint. Parentheses indicate vines that have not yet been planted in the field.

At the beginning of 2013, 44 grafted plants and an additional 8 grafts (by grafting budded scions onto rooted plants) were in 1 gal. pots in greenhouses; these are scheduled to be transplanted to the field at both sites in late May, 2013. The recently grafted plants that will complete both field trials are 14 Chardonnay trans-grafted plants, 3 Thompson Seedless transgrafted plants (Thompson seedless pPGIP expressing rootstock grafted with Thompson seedless scion not expressing pPGIP), 16 Chardonnay null control grafted plants (Chardonnay rootstock not expressing pPGIP grafted onto Chardonnay scion not expressing pPGIP), 4 Thompson seedless null control grafted plants (non-transformed Thompson seedless rootstock grafted onto Thompson seedless scion not expressing pPGIP), and 7 Thompson seedless pPGIP expressing control grafted plants (Thompson seedless expressing pPGIP rootstock grafted onto Thompson seedless expressing pPGIP scion). DNA was prepared from the vines used as source tissue for grafting and the genotypes were confirmed by PCR (Figure 3). In addition to the grafts listed previously, 31

grafted plants were generated, confirmed, and transferred to the Solano field site in May 2012 and David Dolan has generated 2x as many transgrafted plants as needed to complete both sites.

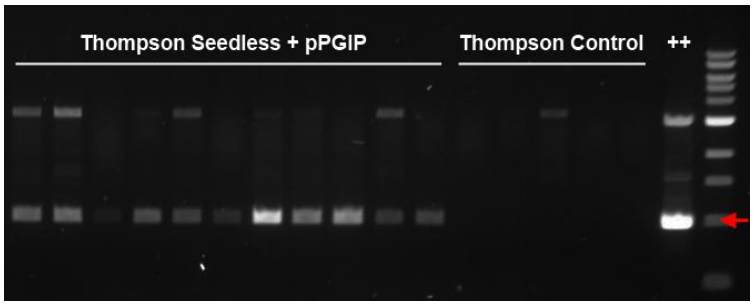

Figure 3. Sample genotyping PCR of grape leaf tissue from Thompson seedless vines expressing pPGIP and null (no pPGIP) controls used to generate the transgrafted vines planted in May, 2012. A 1 kb band (arrow) corresponding to pPGIP is expected only in samples used as rootstocks for transgrafts and pPGIP self-grafted controls. Each sample's quality was verified by amplifying a control fragment (not shown).

Results: Sufficient plants of both the Chardonnay and Thompson Seedless varieties have been self-grafted, transgrafted or propagated by self-rooting to complete the Solano and Riverside County plots designed for the trial. Not all of the vines have been transplanted to the sites but that will be completed in Spring, 2013.

Objective 2: Establish field trial sites

Progress: Two field trial sites in Solano and Riverside Counties were established to assess the PD resistance and general agronomic viability of own-rooted and grafted pPGIP-expressing grapevines. The field sites are shared by projects testing PD resistance of other transgenic grapevines from PIs, D. Gilchrist, A. Dandekar, and S. Lindow. The vines satisfying our initial PCR analysis in 2010 for our portion of the Solano County field trial were hand-planted in a randomized block design with blocks consisting of two or three individuals in the same treatment in July 2010 (Table 1). Thirty-one grafted plants, either utilizing the pPGIP-expressing material as rootstocks or the appropriate control graft combinations, were prepared as described above and hardened in a lath house for two months prior to planting in Solano County in May 2012. An additional 13 grafted vines were added to the Solano site in October 2012. Both sets of younger, grafted plants were surrounded by protective grow tubes and hand-watered every two weeks or as needed. The grapevines are planted approximately 8 ft. apart and tied to wooden stakes with trellising wires at 40 in. and 52 in. Their growth during the 2012 growing season was vigorous (Figure 4).

The vines have been pruned both to maximize potential cane number for inoculations and to establish vigorous positions for future growth. With the permit amendment granted to M. Szczerba by the BRS-USDA in March 2012, flowers and fruiting clusters were allowed to persist. All own-rooted Chardonnay vines were cordon-trained and spur-pruned and the majority of the Thompson Seedless vines were cane-pruned in an attempt to maintain proper vine balance and ensure fruit development. The Solano field site has been under weekly observation for the duration of the growing season.

Results: More than half of the Solano County site has been established with no losses of plants. The plants have been pruned and cultivated appropriately for commercial production of wine grapes (Chardonnay) and table grape (Thompson seedless). The Riverside County site should be established with existing plants in Spring, 2013.

Objective 3: Evaluate relevant agronomic traits of vines in two locations.

Progress: The grapevines planted in Solano County were monitored for general health and maintained on a weekly basis. With the permit amendment mentioned above, agronomic trait analyses were discussed among the groups and anecdotal evaluations have been used thus far.

Results: None of the vines planted in our plots have been lost due to shock or accidental mishaps. All are growing robustly and have been appropriately trained.

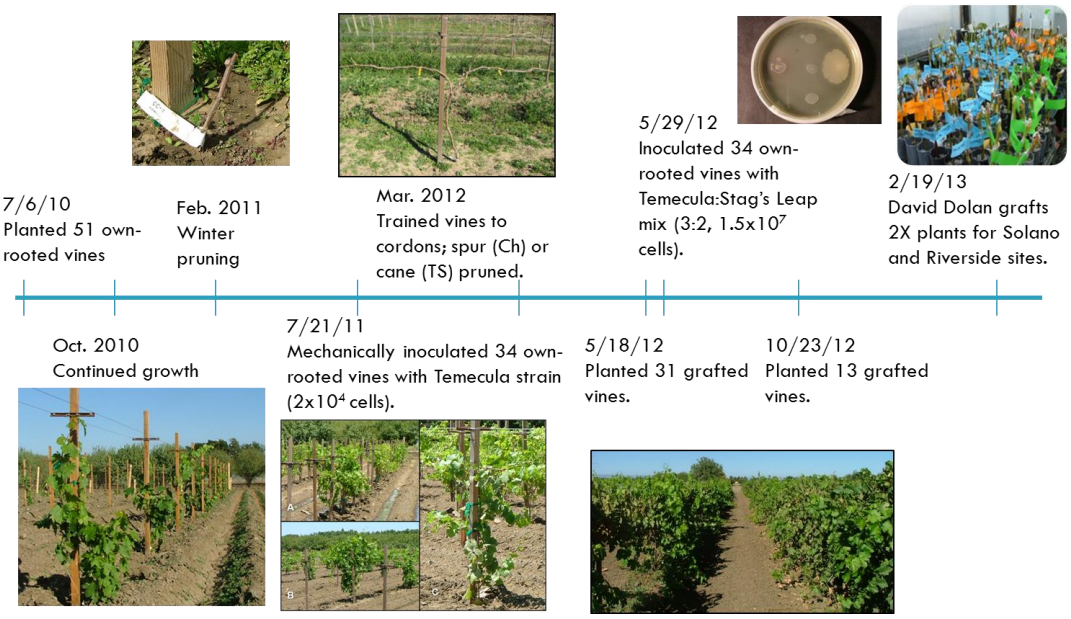

Figure 4. Images of the field throughout the three years of the field trial in Solano County.

Objective 4: Determine PD incidence in pPGIP-expressing grafted and own-rooted lines.

Progress: Two-thirds of the own-rooted vines at the Solano County site were mechanically inoculated with *X. fastidiosa* Temecula on 21 July 2011, to monitor PD incidence during the late summer 2011. No visual evidence of PD infection was observed throughout the 2011growing season or in the early 2012 months following bud break. The same 34 own-rooted vines were resubmitted to mechanical inoculations on 29 May 2012 with a mixture of *X. fastidiosa* Temecula and Stags Leap strains (3:2, v:v). Young, green tissue was chosen for inoculation with 3-4 canes chosen per plant. Mechanical inoculations were performed as in 2011 except that approximately 1.5 x 10^7 cells were used per inoculation, an increase of 750-fold over the previous year. The inoculations were performed simultaneously with the other field site collaborators with a bacterial suspension culture provided by D. Gilchrist. An example of the match-stick response seen in PD infected vines in late 2012 is shown in Figure 5. DNA was prepared

Figure 5. Typical match-stick symptoms of PD infected grape vines.

from inoculated canes and analyzed by PCR for suitability for amplification (using primers for a grape DNA sequence) and for the presence of *Xf* DNA. Several methods of isolating inoculated tissue DNA were tried in order to detect the *Xf* DNA by PCR (Figure 6).

Results: *Xf* DNA sequences were detected by PCR in the inoculated samples. No *Xf* DNA sequences were detected in uninoculated controls (Figure 6). qRT-PCR efforts to quantify the amount of *X. fastidiosa*, based on the presence of *Xf* DNA in the inoculated material continue.

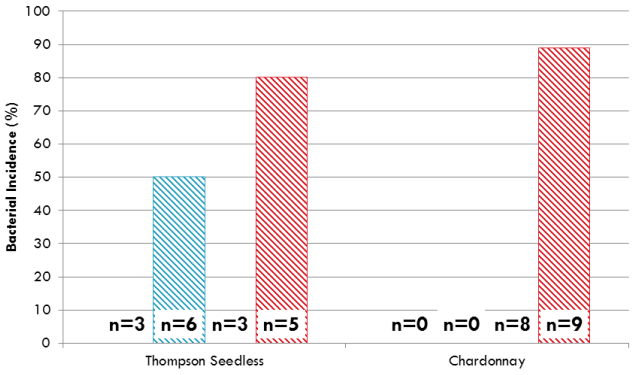

Figure 6. Results of PCR detection of *Xf* DNA sequences in inoculated vines from the Solano County site.

CONCLUSIONS:

All of the grafted plants necessary for the study in Solano County have been generated and about 50% of the plants for the site designed for Riverside County are in greenhouse pots. The genotypes of the grafted plants were confirmed by PCR analysis of DNA from the plants. An initial attempt to infect the vines in Solano County was made but no symptoms were observed. A second attempt in 2012 is being evaluated now. The results of the field evaluation will confirm that delivery of the pPGIP from rootstocks provides a means of controlling PD and *X. fastidiosa* infection in a typical vineyard setting in California. The evaluations of the performance and productivity of the plants will confirm that expression and presence of pPGIP does not affect unintentionally other characteristics of the vines. By using varieties grown for fresh fruit and for wine production in California, we are testing varieties important to California growers.

PUBLICATIONS PRODUCED:

Haroldsen VM, Szczerba MW, Aktas H, Lopez-Baltazar J, Odias MJ, Chi-Ham CL, Labavitch JM, Bennett AB and Powell ALT (2012) Mobility of transgenic nucleic acids and proteins within grafted rootstocks for agricultural improvement. *Frontiers in Plant Science* **3**:39, Published 2 March 2012.

This publication describes the use of transgrafting for agricultural plants. Examples from the work with grapes are cited.

RESEARCH RELEVANCE STATEMENT:

The results of the field evaluations now that the field trials are established will provide the means of determining whether delivery of the pPGIP from rootstocks is effective for controlling PD and *X. fastidiosa* infection in a typical vineyard setting in California. The evaluations of the performance and productivity of the plants thus far confirm that expression and presence of pPGIP does not affect unintentionally other characteristics of the vines. By using varieties grown for fresh fruit and for wine production in California, we are testing varieties important to California growers.

LAYPERSON SUMMARY OF PROJECT ACCOMPLISHMENTS:

Own-rooted, self-grafted and transgrafted Chardonnay and Thompson Seedless grapevines, including those expressing pPGIP, were generated by vegetative propagation, genotyped by PCR, and planted as part of a field trial in Solano County. Grafted vines are being generated to add to the existing site in Riverside County. The performance of the plants in the field was appropriate for commercial

settings. Mechanical inoculations with *X. fastidiosa* bacteria were done in 2011 and 2012 in Solano County and *Xf* DNA sequences have been detected in the inoculated, but not in the uninoculated, cane material. These results suggest that no false positives were encountered.

STATUS OF FUNDS:

	Amount in original budget ($)	Amount spent to 28 February 2013 ($)
Personnel		
Professional	23,075	12,739.28
SRA/Tech		
Lab Assistant	6,400	8,896.00
Other		
Employee Benefits	6,653	5,472.36
SUBTOTAL (Personnel + Benefits)	36,128	27,107.64
Supplies and Expenses	17,966	6,689.24
Equipment		
Travel	6,332	218.00
Computer Time		
Other		
Indirect Costs*		
SUBTOTAL (Supplies, Expenses, Equipment, etc.)	24,298	6,907.24
TOTAL	60,426	34,014.88

*Unspent portion was de-obligated and returned in recent new contract

INTELLECTUAL PROPERTY ASSOCIATED WITH PROJECT: This has been addressed in other projects associated with the PIs.

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