- **TITLE OF REPORT**: Interim Report for CDFA Contract Number 12-0442

- **TITLE OF PROJECT: 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 October 2014 to March 2015.

- **INTRODUCTION:**

The project was designed to establish two field sites with typical vineyard practices that would allow grape lines to be evaluated in order to assess whether polygalacturonase inhibiting proteins (PGIPs) restrict *Xylella fastidiosa* (*Xf*) spread and Pierce's Disease (PD) and whether expression of pPGIP impacted the performance and attributes of the vines.

This group and others had shown that the expansion of *X. fastidiosa* from the infection site throughout the vine, creates a systemic infection that causes 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, pectin-rich cell wall "filters" whose meshwork is too small to permit movement of *Xf* (Labavitch et al., 2004, 2006, 2009a,). *Xf* produces cell wall-degrading enzymes to digest the pit membrane polysaccharides (Labavitch et al., 2009b), opening xylem connections and permitting spread of the bacteria.

The *Xf* genome contains a polygalacturonase (*XfPG*) and several □-1,4-endo-glucanase (EGase) genes, whose predicted enzyme products could participate in the digestion of pectin and xyloglucan polymers in pit membranes and, thereby, facilitate PD development by the movement of *Xf* within vines. 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 *XfPG*-deficient *X. fastidiosa* strain and showed it was unable to cause PD symptoms, so *XfPG* is a PD virulence factor.

The over-all research aim is to use plant PGIPs to limit *Xf* spread in grapevines. PGIPs are produced by plants, including in flowers and edible fruits, and are selective inhibitors of pathogen and pest PGs (Powell et al., 2000; Shackel et al., 2005; Stotz et al., 1993, 1994). Grapevines transformed by A. Dandekar's group to express the pPGIP-encoding gene from pears have reduced susceptibility to *Xf* and pPGIP is transported across the graft junction from pPGIP expressing grape and tomato rootstocks into wild-type scions (Agüero et al., 2005, Haroldsen et al., 2012).

Grafting pPGIP-producing rootstocks to scions, which do not contain an introduced pPGIP gene, is an opportunity to provide a beneficial plant fruit protein (i.e., pPGIP) without introducing a pPGIP gene into the scion itself. This project has been designed to scale up the grafted and own-rooted pPGIP expressing grapevines, plant them in field settings, and evaluate their agronomic performance and their resistance to PD in settings comparable to commercial fields.

- **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 ACTIVITES:**

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

Activities: This objective was been completed in June 2013. It is possible that there is vine death due to PD or other causes, but no plans have been made to replace the vines. Table 1 shows the number of grafted and non-grafted vines of each genotype needed to complete both fields.

Table 1.

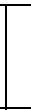
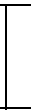
SOLANO		'Chardonnay'								'Thompson Seedless'							
	Strategy (Scion/root)																
Own-Rooted	Inoculated (2011-2013)		17								10			9			
	Non-Inoculated		8								2			5			
Grafted	Inoculated (2013, 2014)	9		8	9					9		9	9				
	Non-Inoculated	4		4	4					4		4	4				
RIVERSIDE																	
Own-Rooted	Natural Infections		13			11		6			9			12		6	
Grafted	Natural Infections	16		6	8		6		3	7		14	7		3		3

Table 1. Numbers of grapevines planted in Solano and Riverside Counties. Upper portion of the graphic is scion genotype, lower portion is rootstock phenotype; nongrafted plants have no break. Hatched fill represents pPGIP expressing rootstocks and/or scions; black fill is null-transformants (no pPGIP) controls; white fill is non-transformed controls. In Solano County, own-rooted vines were mechanically inoculated in the summers of 2011-2013; transgrafted vines were inoculated in 2013 and 2014. Vines planted in Riverside County had "natural" infections.

Objective 2: Establish field trial sites

Activities: The establishment of the field trial sites in Solano and Riverside Counties was completed in June 2013. The field plans of the Powell trial plots in Solano and Riverside Counties are shown in Figure 1.The vines satisfying our initial PCR analysis were hand-planted in a randomized block design with blocks consisting of two or three individuals in the same treatment (Table 1).

In Solano County on 25 February 2015, the vines were pruned by the PI and Mark Greenspan to maximize potential cane number for inoculations and to establish vigorous positions for future growth. The activities in 2014 and 2015 at both field sites are shown in Table 2.

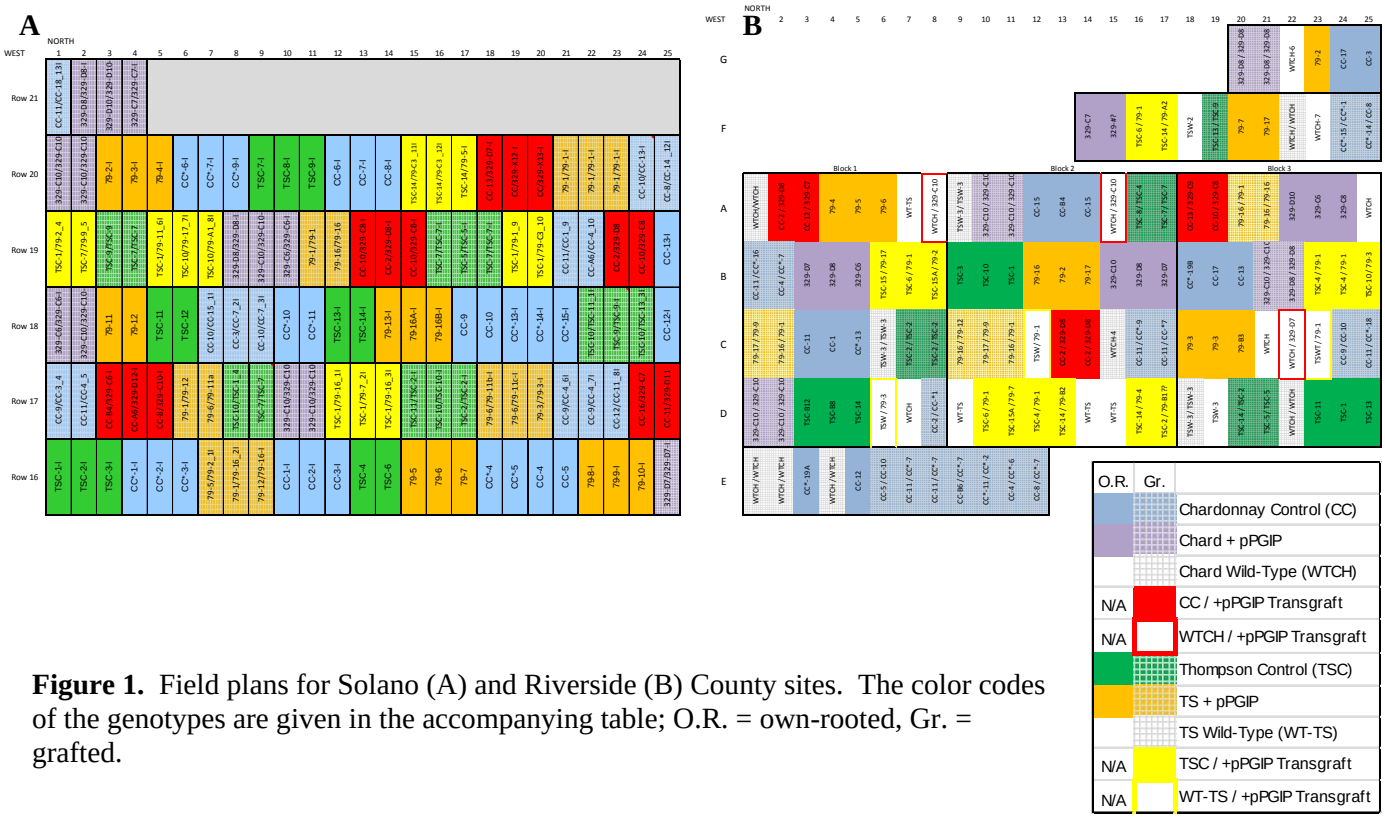


Figure 1. Field plans for Solano (A) and Riverside (B) County sites. The color codes of the genotypes are given in the accompanying table; O.R. = own-rooted, Gr. = grafted.

Table 2.

Date	Location	Activity
14 March 2014	Solano	Visual scoring of symptoms from 2011-2013 infections at each year's inoculation site on each grafted plant
19 March 2014	Solano	Visual re-scoring of symptoms from 2011-2013 infections (see above)
20 March 2014	Solano	Photos, light pruning since vines have buds that have broken; first pruning since 2013
4 April 2014	Riverside	Disease scoring of symptoms on each plant; photos taken (CJ UCD)
28 May 2014	Solano	Inoculate ca. 4 fresh canes/grafted vine for 2014; no pruning
9 July 2014	Solano	Visit field to assess disease on each plant
27 July 2014	Solano	Take cane samples of ca. 1 cane/ genotype/plot for qPCR of canes infected in 2014; prune vines again
29 July 2014	Solano	Count scorched leaves on infected canes; photos taken
3 September 2014	Solano	Disease assessment by D. Golino (UCD)
ca. 1 October 2014	Solano	Vines pruned again
6 October 2014	Riverside	Disease scoring of all plants by P. Rollhausen (PR, UCR)
9 October 2014	Solano	Count infected leaves
24 October 2014	Riverside	Disease re-scoring of all plants, photos taken by A. Powell (AP, UCD)
25 February 2015	Solano	Winter pruning of all vines by A. Powell and M. Greenspan
18 March 2015	Solano	Visual scoring and counting scorched leaves of inoculated canes (AP, UCD)
Late March 2015	Riverside	Disease scoring of all plants by P. Rollhausen (PR, UCR)

Table 2. Activities at the Solano and Riverside sites for this project in 2014.

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

Activities: Because the vines have been dormant during the winter, no assessments were made of vigor. However, during pruning in late February 2015, several dead vines were noted. Vine death will be recorded in the field assessments on 18 March 2015.

Objective 4: Determine PD incidence in pPGIP expressing grafted and own-rooted lines. Test for X. fastidiosa presence and determine the extent of infection.

Activities: At the Riverside plot, assessments of disease will be made probably the last week of March 2015.

At the Solano plot, the leaves/petioles with evidence of PD will be counted 18 March 2015 including canes which had been infected in 2011, 2012 and 2013. Infected cane material will be collected on at the same time that other groups collect their samples.

Summary:

All of the grafted plants necessary for the studies in Solano and Riverside Counties were generated, planted and inoculated according to the plans of the project. The genotypes of the grafted plants were confirmed. Initial infections in 2011 of the vines in Solano County produced no visible symptoms over a year. The second set of inoculations in Year 2 resulted in detectable *Xf* DNA in infected vines in November, 2012 and visual symptoms of PD in April, 2013. Mechanical inoculations with *X. fastidiosa* bacteria in 2011 and 2012 in Solano County resulted in the accumulation *Xf* DNA sequences only in the inoculated, but not in the uninoculated, cane material. Symptoms of PD infection were visible on the inoculated vines beginning generally in the Spring of the year following the introduction of *Xf*. Inconsistent or atypical pruning schedules have made determinations of similarities of vine phenotype and vigor to commercially propagated fields difficult, However, the over-all performance of the own-rooted 'Chardonnay' and 'Thompson Seedless' vines in the field seems to be unaffected by the expression of pPGIP either in the scion or the rootstocks. The evaluations of the leaf and cane phenotypes of the plants suggest that pPGIP expression improves resistance of vines to PD, probably more in the' Thompson Seedles's than in the 'Chardonnay' variety during natural infections in Riverside but the 'Chardonnay' vines with pPGIP had fewer PD symptoms than the 'Thompson Seedless' variety when mechanically inoculated in Solano County. By using varieties grown for fresh fruit and for wine production in California, we are comparing the impacts of these changes using varieties which grow with different habits and which are important to different segments of the community of California grape growers.

- **PUBLICATIONS AND PRESENTATIONS PRODUCED:** Results as of mid-December 2014 were presented in a poster at the Annual Pierces Disease Symposium in Sacramento by Ann Powell.

- **RESEARCH RELEVANCE STATEMENT:**

Work in this project evaluates the performance of grafted grapevine lines that produce a protein that is a candidate for control of Pierce's Disease (PD). The vines have been established in vineyards in a manner that approximates typical commercial settings in Solano and Riverside Counties with low and high PD disease pressure, respectively. The CDFA PD and GWSS Board's Research Scientific Advisory Panel had established a priority to evaluate the potential commercial use of the strategy to deliver polygalacturonase-inhibiting proteins (PGIPs) from grafted rootstocks to control PD in the scion, fruit-bearing portions of grapevines. Established transformed 'Thompson Seedless' and 'Chardonnay' grapevines expressing a PGIP from pear fruit (pPGIP) showed reduced PD incidence when inoculated with *X. fastidiosa* (Agüero *et al*., 2005). The pPGIP that was produced in the transformed rootstock was identified in samples of xylem exudate that were collected from grafted, but not transformed scions (Agüero *et al*., 2005). Therefore, cuttings from these grapevines were grafted with non-pPGIP producing scions to make comparisons of the effectiveness and outcomes between vines producing pPGIP in grafted rootstocks, those producing pPGIP throughout the vine, and vines with no pPGIP.

- **LAY PERSON SUMMARY:**

Two vineyard plots containing own-rooted and transgrafted (rootstocks expressing pPGIP grafted to fruit producing scions with no genetic modifications that, thus, do not themselves produce pPGIP) combinations of Chardonnay and Thompson Seedless grapevines were established and the identities of the genotypes were confirmed by June 2013. Mechanical inoculations with *X. fastidiosa* bacteria were done in 2011, 2012 and 2013 in Solano County and natural infections were allowed to occur in Riverside County. Data describing the agronomic and disease traits of the vines have been collected. Since this trial evaluates grape varieties grown for fresh fruit and for wine production in California, we are testing varieties important to most California grape growers; these varieties have different growth habits and products so this trial examines the efficacy of pPGIP across wine and fresh sectors of the grape industry. The initial evaluations of the performance and productivity suggest that pPGIP expression in a table grape variety (Thompson seedless) or a wine grape (Chardonnay) improves resistance of vines to PD but does not otherwise affect vine growth or berry characteristics.

- **STATUS OF FUNDS:**

	Budget TOTAL	February 1, 2013 – Feb 28, 2015	March 1, 2015 – June 30, 2016
Personnel			
Professional, 8% Ann Powell, Feb 13 – Oct 13, 16% Nov 13 – June 14 (Monthly base $7,741.67)	**35,547**	23,126.67	14,528.42
Lab Asst. I, 1 month (monthly base $2,368)	**2,368**	4355.20	0
Student Asst., 150 hrs at $10/hr	**5,100**	5823.41	0
Employee Benefits (30.3%, 33.3%)	**13,910**	8640.92	450
SUBTOTAL **(**Personnel + Benefits)	**56,925**	41,946.20	14,978.42
Supplies and Expenses	**13,907**	9,515.83	4,391.17
Equipment			
Travel	**3,000**	2,859.14	140.86
Computer Time			
Other			
Indirect Costs*			
SUBTOTAL (Supplies, Expenses, Equipment, etc.)	**16,907**	12,374.97	4,532.03
TOTAL	**73,832**	54,321.17	19,510.83

- **SUMMARY AND STATUS OF INTELLECTUAL PROPERTY ASSOCIATED WITH THE PROJECT:** None is relevant.

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