- **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 1 November 2015 - 29 February 2016.

- **INTRODUCTION:**

The project was designed to establish two typical vineyard sites to assess whether polygalacturonase inhibiting proteins (PGIPs) restrict *Xylella fastidiosa* (*Xf*) spread and Pierce's Disease (PD) symptoms and whether expression of pPGIP impacted the performance and attributes of table and wine grapevines.

This group and others had shown that the expansion of *X. fastidiosa* from the infection site throughout the vine creates systemic infections that cause 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* polygalacturonase (*XfPG*) and several β-1,4-endo-glucanase (EGase) 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, demonstrating that *XfPG* is a PD virulence factor.

The over-all research aim of this project is to use plant PGIPs to limit *Xf* spread in grapevines. PGIPs are produced in flowers and edible fruits and are induced by contact with pathogens. PGIPs 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 expressed the pPGIP-encoding gene from pear fruit and these vines have reduced susceptibility to *Xf*. pPGIP is transported from pPGIP expressing grape and tomato rootstocks across the graft junction into wild-type scions (Agüero et al., 2005, Haroldsen et al., 2012).

Grafting pPGIP-producing rootstocks to non-pPGIP expression scions, because the scions 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. Results presented in Objectives 3 and 4 show that there is vine death over the past 3 years due to PD andto other causes, in a very small number of cases; no plans have been made to replace the dead vines. Table 1 shows the number of grafted and non-grafted vines of each genotype that were planted by June 2013.

Results: Sufficient plants of both the 'Chardonnay' and 'Thompson seedless' varieties have been self-grafted, transgrafted or propagated by own rooting to complete the Solano and Riverside County plots. The genotypes of the plants were verified. All of the vines have been transplanted to the sites.

Table 1. Field Inventory

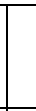
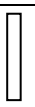
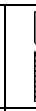
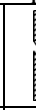
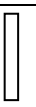
SOLANO		'Chardonnay'								'Thompson Seedless'							
	Strategy (Scion/root)																
Own-Rooted	Inoculated (2011-2013)		17								8			9			
	Non-Inoculated		8								4			5			
Grafted	Inoculated (2013, 2014, 2015)	9		11	9	0				9		9	9				
	Non-Inoculated	4		4	4	2				4		4	4				
RIVERSIDE																	
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. Total numbers of grapevines planted by 2013 in Solano and Riverside counties. The upper portion of the graphic is scion genotype, the lower part of the graphic is rootstock phenotype; nongrafted plants have break between the upper and lower parts of the graphics. 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 2011-2015; transgrafted vines were inoculated in 2013, 2014 and 2015. Vines planted in Riverside County had "natural" infections.

Objective 2: Establish field trial sites

Activities: 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 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. The young plants were placed in protective grow tubes and hand-watered every two weeks in Solano County or as needed. In Riverside County, the plants were watered by drip irrigation. In Riverside, the plot is at the bottom of a small hill and the soil is very sandy and porous; irrigation water accumulates in the lowest row (Row E). At both sites, grapevines were planted approximately 8 ft. apart and tied to wooden stakes with trellising wires at 40 and 52 inches.

In Solano County, the vines were pruned by the PI and the field crews to maximize potential cane number for inoculations and to establish vigorous positions for future growth. The pruning schedule and method was non-conventional but was done in a manner to try to standardize vine growth in our plots with the practices by the other PIs with plots in the same field and to be able to preserve the inoculated vines for observations and sampling. With the permit amendment granted by the BRS-USDA in 2012, flowers and fruiting clusters were allowed to persist. Initially, all of the 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 in our field in Solano site. Subsequent prunings have not taken into account varietal differences. The vines at the Riverside site were pruned according to the schedule established at UCR and varietal differences were not addressed. The Solano site has been under observed approximately monthly in the 2015 growing season. The vines in Riverside County established themselves well and were monitored by UCR staff and the PI twice during the 2015 season. The activities through at both field sites are shown in Table 2.

Results: Since 3 June, 2013, both the Riverside and Solano County sites have been established with all the planned plantings for this project. A consistent pruning regime remains a goal for this plot so comparisons can be made with other evaluators. In 2014, thirteen evaluations were made of the plots (10 in Solano and 3 in Riverside); 9 were made by the PI. In 2015, nine evaluations were made of the plots (6 in Solano and 3 in Riverside); 8 evaluations were made by the PI. The vines at the Riverside site were removed in late 2015 because evaluations at that site had been completed. No evaluations of the Solano field have been made in 2016 due to wet weather.

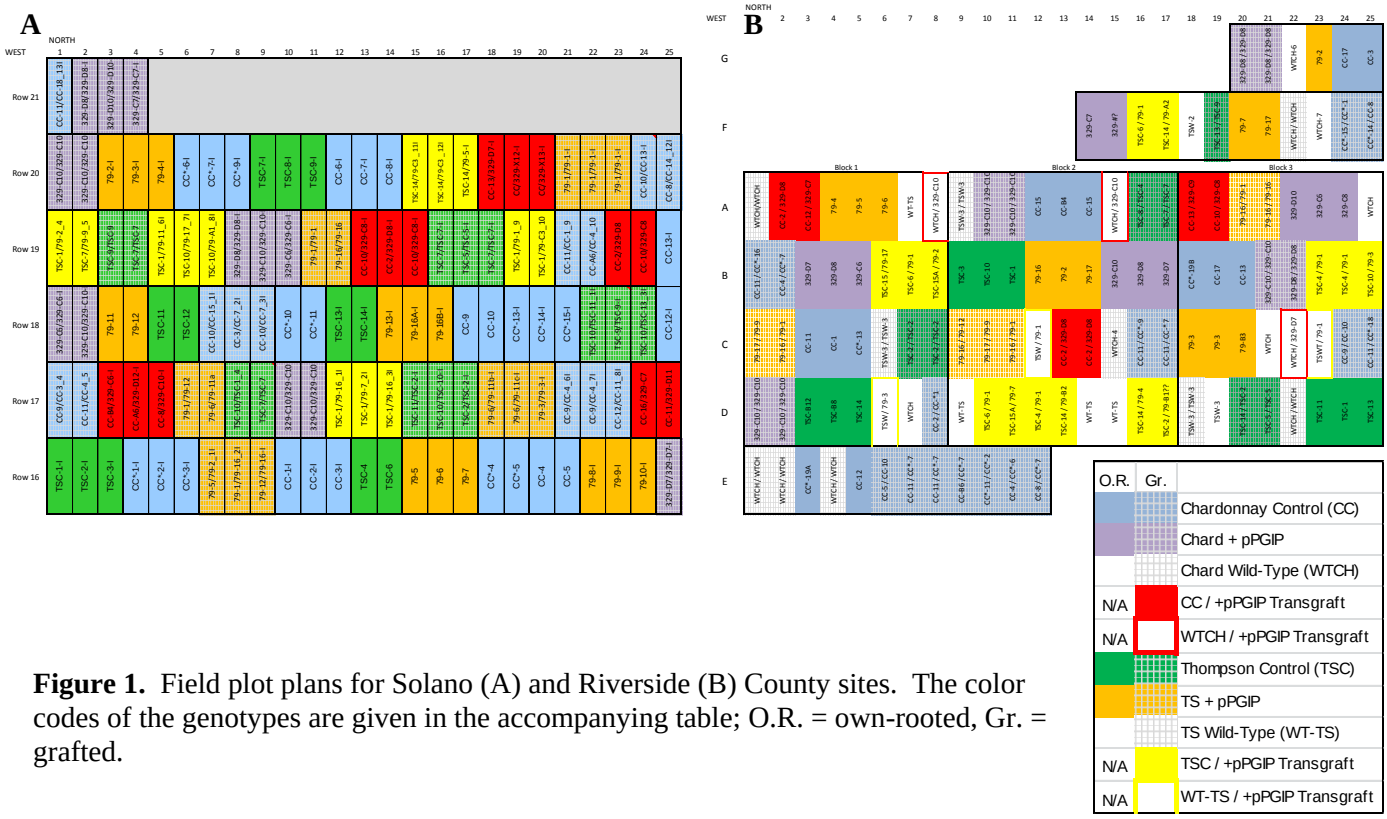


Figure 1. Field plot plans for Solano (A) and Riverside (B) County sites. The color codes of the genotypes are given in the accompanying table; O.R. = own-rooted, Gr. = grafted.

Table 2. Site activities

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. Rolshausen (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)
15 February 2015	Solano	Prune vines assisted by M. Greenspan while other groups were also pruning (AP UCD)
25 March 2015	Solano	Score plants for scorching, late growth, death, take photos (AP, UCD)
19 May 2015	UCD	Meet with other PIs to consider future of the project
26 May 2015	Solano	Prune vines to conform with other groups (AP UCD)
27 May 2015	Solano	Inoculate at least 4 canes per grafted plant with inoculum provided by D.

		Gilchrist. Tag with yellow/orange pull tags (AP, BN, TL, KP UCD)
2 June 2015	Riverside	Vine assessments and photos taken with P. Rolshausen (AP UCD, PR UCR
17 June 2015	Riverside	UCR staff (Peggy Mauk) evaluated vines (PM UCR)
Late June 2015	Riverside	Plantings removed
7 August 2015	Solano	Scored for visual signs of scorching, death, photos and samples for PCR (AP UCD)
7 October 2015	Solano	Scored for visual signs of scorching, death, photos and samples for PCR (AP, JMc, JA UCD)
14 March 2016	Solano	Observation of field to project when pruning and assessments can be done (AP)

Table 2. Activities at the Solano and Riverside sites for this project through 15 March 2016.

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

Activities: Other than differences due to the variety ('Chardonnay' or 'Thompson seedless'), no difference in over-all growth, time to flower, fruit set or yield was noticed between the vines expressing pPGIP and the controls. All produced buds in mid-March and flower buds broke by the end of March in 2014 and 2015. In 2016, little sign of growth was evident on the vines on 14 March, probably due to heavy rain and cool weather. The fields were saturated due to heavy rains and no weeding had been done between rows so the PI was unable to walk the field. The vines had not been pruned as of mid-March 2016. Depending on the weather, assessments and pruning will be attempted by the PI by the end of March, 2016.

Non-grafted vines were inoculated for three years by March, 2014. Numbers of bud producing, no-bud producing and scorched leaves along canes inoculated in 2011, 2012 and 2013 were recorded in 2014 and 2015 and will be analyzed for further details. The data has not yet been analyzed for statistical significance or for effects due to grafting. Photos of each vine were taken throughout the 2015 growing season. Vine death was noted at the Solano site and was monitored for each infected vine during the 2015 growing season (Table 3) and will be repeated in late March 2016.

The PI visited the Riverside site on 2 June 2015 and scored the vines for apparently PD damage and for herbicide damage as reported in 2015. Herbicide damage was independently assessed by Peggy Mauk and Philippe Rolshausen at the Riverside site on 17 June 2017 and the results were provided in 2015 reports.

Table 3. Vine death during 2014-2015 at Solano site.

			late 2014		25-Mar-15		27-May-15		7-Aug-15		7-Oct-15	
	Total infected plants	Total uninfected plants	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected	Infected	Not infected
CC	17	8	6	0	6	0	7	0	10	0	10	1
CC/CC	9	4	2	0	2	0	2	0	3	0	7	0
329	0	2	0	0	0	0	0	0	0	0	0	0
329/329	9	4	3	0	3	0	3	0	3	0	3	0
CC/329	11	4	1	1	2	1	2	1	3	1	3	1
TSC	8	4	2	0	4	0	4	0	7	0	8	0
TSC/TSC	9	4	0	0	2	0	2	0	5	0	8	0
79	9	5	3	0	3	0	3	0	3	0	4	0
79/79	7	4	2	0	2	0	2	0	3	0	6	0
TSC/79	10	3	1	0	2	0	2	0	5	0	8	0

Table 3. Observations of vine death at the Solano plot from late 2014 through the 2015 growing season. wtch= Chardonnay wildtype, CC= 'Chardonnay' control, wtTS = 'Thompson seedless' wildtype and TSC = 'Thompson

seedless' control. / denotes grafted plants with the genotypes expressed as scion/rootstock. 329 and 79 genotypes express pPGIP in 'Chardonnay' or 'Thompson seedless' backgrounds, respectively.

Results: By the end of the 2015 season, it is clear that some vines had died in the Solano plot. Table 3 shows the number of dead vines of each genotypes as determined by assessments in 2014 and four times in 2015. It is clear that the number of dead vines increased during the 2015 season, possibly due to stress caused by the severe drought conditions but it is also clear that the plants that did not express pPGIP either in the rootstock or in the scion were far more susceptible to death under these stress conditions. The data clearly indicate that vines that had been infected at least once were far more susceptible to death; only 2 uninoculated vines appeared to be dead.

Previous reports showed the damage assessments made on 2 June 2015 at the Riverside site. Since up to 25% of the plantings in the Riverside plot were compromised by the herbicide drift, it was decided in late June 2015 to terminate the site with no further observations because it was going to be impossible to distinguish between damage caused by Pierces Disease and effects caused by the potential exposure to herbicides.

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 Solano plot, the leaves/petioles with evidence of PD were counted twice during the 2015 season, including on canes which had been infected in 2011, 2012 and 2013. Infected cane material was twice collected during the summer of 2015, approximately when other groups collect their samples. Tissue collected in the summer of 2014 had hand ground and frozen at -80^{o}C. The material has been kept frozen. The Powell group received separate funds for a GenoGrinder and worked on protocols to effectively grind the infected stem tissue until the machine sustained damage. Approximately 6 weeks were needed for repairs to be made. The group plans to refine protocols for macerating the tissue and proceed to PCR analysis.

The data analyzing the genotypes of the dead vines (shown in Table 3) was preliminarily analyzed by plotting (Figure 2). The grafted and transgrafted vines at the Solano site were reinoculated along with the vines in the plots of the other PIs on 28 May 2014 and 27 May 2015. Up to 4 canes per vine were inoculated as previously with inoculum provided by D. Gilchrist. In our plot, only vines that were grafted or transgrafted were inoculated in 2015, like in 2014. Previous inoculations in 2011-2013 had included vines that were own-rooted. The extent of disease along the canes inoculated in 2014 and 2015 was measured twice during the 2015 season. Examples of the photo evidence of vine phenotypes on 7 October 2015 was provided in reports in 2015.

At the Riverside site, vine vigor was analyzed on early June 2015. Since it was difficult to unequivocally distinguish between damage caused by natural PD infections or by herbicide drift, the observations have not been further analyzed. To obtain the data for the visual assessments of disease throughout the vines , in October 2014 evaluators, PR and AP, used the same general assessment scale going from 0 (no disease) to 5 (dead) to assess the vines. Additionally, AP counted the total number of canes per vine and the number of canes with scorched leaves or no growth (diseased canes). The initial analyses of the results are given in Figure 3. In general expression of pPGIP throughout the vine or via grafting to pPGIP expressing rootstocks, reduced slightly the disease score and reduced the number of infected canes. The data has not yet been analyzed for statistical significance or for effects due to grafting. Examples of the photo evidence of the vine phenotypes on 2 June 2015 in Riverside was provided in 2015 reports.

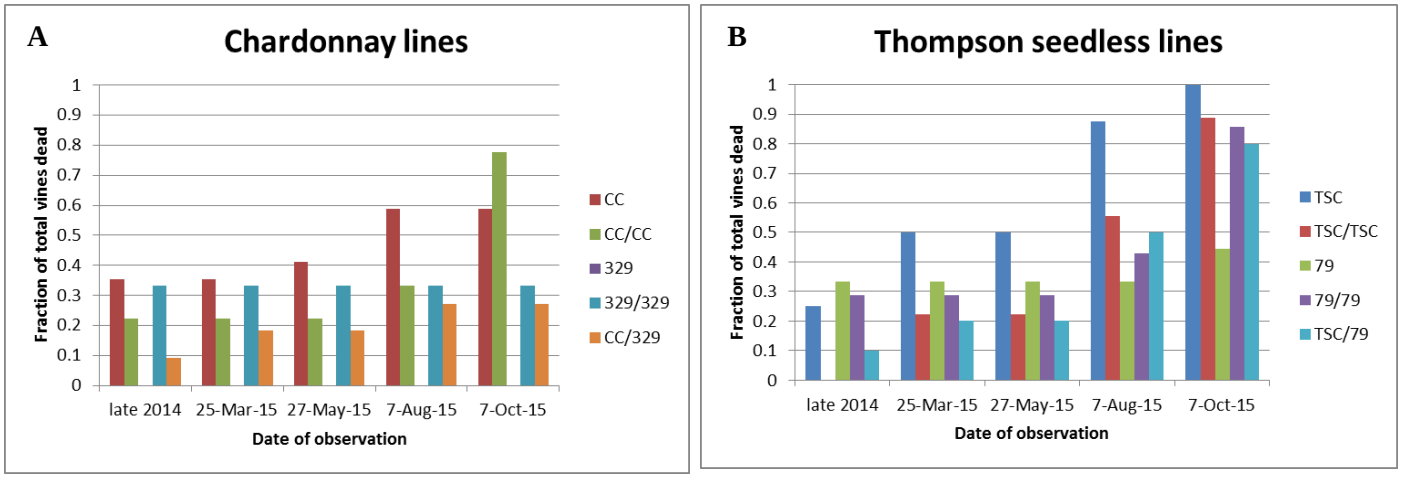


Figure 2. Vine death incidence in Solano plot of 'Chardonnay' and 'Thompson seedless' vines measured in 2014 and throughout the 2015 season. **A. '**Chardonnay' lines. **B.** 'Thompson Seedless' lines. / denotes grafted plants with the genotypes expressed as scion/rootstock. 329 and 79 genotypes express pPGIP in 'Chardonnay' (CC) or 'Thompson seedless' (TSC) backgrounds, respectively.

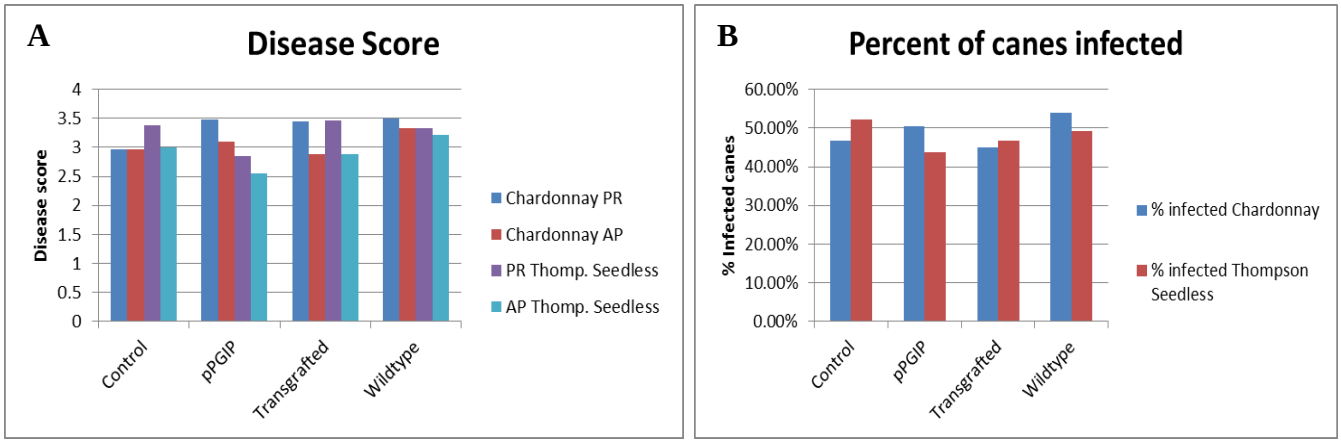


Figure 3. Disease incidence in Riverside plot of 'Chardonnay' and 'Thompson seedless' vines measured in October 2014. **A.** Disease score based on 0-5 scale. **B.** Percent of vine canes with symptoms or evidence of PD disease. PR= data collected by P. Rollhausen, AP= data collected by A. Powell/

Results: In general, the expression of pPGIP either in the scion or the rootstock or both does not impact the over-all phenotype of the plant but infected plants without pPGIP were more likely to die, especially in the Solano county site, during the 2015 season than those plants with pPGIP.

In Solano County, analysis of the infected vines demonstrated disease progression over the summer of 2015 and suggested that pPGIP expression provided reduced disease development and ultimately vine death. The effect was clearly due to infection with *Xf* as only 2 uninfected plants had died by the end of 2015. Furthermore, as was shown in the previous annual report, the number of leaves or petioles along canes infected in 2014 was greater when assessed on 9 October 2014 than on the 29 July 2014 observations. These results indicated that disease was developing in these canes. However, vines with pPGIP in the scion portion had a slower increase in disease symptoms, especially in the 'Chardonnay' variety. Notably vines with pPGIP in the rootstocks also showed fewer numbers of diseased leaves or petioles along the infected canes although the increase during the 2014 season was about what was observed in the controls, vines that had been grafted using material that had been transformed with the empty vector construct. In all genotypes, the number of dead infected plants increased over the course of the 2015 summer but the percentage of the vines that died was clearly reduced if the infected plants were expressing pPGIP. It is possible that the severe drought heightened the effect of disease. The effects of *Xf* infections were much more pronounced on the 'Thompson seedless' genotype; by the end of the 2015 season, nearly 100% of the infected 'Thompson seedless' vines at the Solano site were dead. In both varieties, vines with rootstocks expressing pPGIP early in the season were the least likely to die but by the end of the season plants

expressing pPGIP in the scion and the rootstock or only in the rootstock were about equally likely to die. Examples of infected vines were shown previous results. Data from the own rooted 'Thompson seedless' line (79) should probably not be considered since an equivalent 'Chardonnay' line (329) was not infected. The conclusion is tentatively made that pPGIP expression even in the rootstocks alone was sufficient to delay PD symptoms and vine death but in 'Thompson seedless' lines, ultimately the plants may succumb to PD even when pPGIP is expressed. pPGIP expression seems to offer more protection to the 'Chardonnay' variety. The plants will be reanalyzed during the 2016 growing season once the fields are accessible since it is possible that parts of the plants can recover from the disease and regrow.

The disease scoring analyses done by PR and AP at the Riverside site in 2014 produced approximately equivalent scores. Analysis of the actual number of infected canes generally supported the over-all disease score analyses. The results even with natural infections suggested that some beneficial effects of pPGIP expression in rootstocks as well as in the scion portions of the vines could be seen although the 'Thompson seedless' variety grown at the Riverside site and infected naturally showed a more pronounced positive effect than the 'Chardonnay' variety.

CONCLUSIONS:

All of the grafted plants necessary for the studies at both locations were generated, planted and inoculated with protocols similar to the other groups' at the sites. 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 as shown in previous reports. Symptoms of PD infection were visible on the mechanically 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 unless the vines have been inoculated with *Xf*. The evaluations of the leaf and cane phenotypes of infected plants suggest that pPGIP expression improves resistance of vines to PD, probably more in the ' Chardonnay' vines with pPGIP which 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.

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 of the vines in Solano County produced no visible symptoms for over a year. Successive sets of inoculations, beginning in Year 2, resulted in detectable *Xf* DNA in infected vines. Symptoms of PD infection were visible on subsequently inoculated vines and were evident on newly infected canes in the Spring of the year following the inoculations *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 uninfected 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. Only 2 uninoculated vines died at the Solano site. The evaluations of vine death suggest that pPGIP expression improves resistance to PD in inoculated vines, probably more in the 'Chardonnay' transgrafted lines than in the control or pPGIP expressing self-grafted lines and the 'Thompson seedless' variety vines were generally more prone to die at both sites. Based on counting leaves with evidence of scorching, the 'Chardonnay' vines with pPGIP had fewer PD symptoms than the 'Thompson Seedless' variety when mechanically inoculated. 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:** None in this reporting period.
- **RESEARCH RELEVANCE STATEMENT:**

Work in this project evaluates the performance of grafted grapevine lines whose rootstocks produce a protein that is a candidate for control of Pierce's Disease (PD). The experiments were designed to evaluate whether, when the rootstock-produced protein is transported to naïve fruit-bearing scion, the symptoms of PD can be reduced and the

vines can recover from infections. The vines were 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 a polygalacturonase-inhibiting protein (PGIP) normally expressed in pear fruit (pPGIP) from grafted rootstocks to control PD in the scion, fruit-bearing portions of grapevines. Established transformed 'Thompson seedless' and 'Chardonnay' grapevines expressing pPGIP had shown 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, for the experiments in this project, 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 producing 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. Mechanical inoculations with *X. fastidiosa* bacteria were done annually from 2011-2015 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	1 February, 2013 – 29 February 2016	1 March 2016- 30 June 2016
Personnel			
Professional, 8% Ann Powell, Feb 13 – Oct 13, 16% Nov 13 – June 14 (Monthly base $7,741.67)	**41,547**	34,383.03	2,734.63
Lab Asst. I, 1 month (monthly base $2,368)	**2,368**	4355.20	
Student Asst., 150 hrs at $10/hr	**5,100**	14,402.10	
Employee Benefits (30.3%, 33.3%, 1.3%, 3.1%)	**14,024**	9,116.45	35.55
SUBTOTAL **(**Personnel + Benefits)	**63,039**	62,256.78	2,770.18
Supplies and Expenses	**16,907**	13,644.63	500
Equipment/ Equipment/Computer time/ Other			
Travel	**3,000**	3,774.41	
SUBTOTAL (Supplies, Expenses, Equipment, etc.)	**19,907**	17,419.04	500
TOTAL	**82,946**	79,675.82	3,270.18

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

- **LITERATURE CITED:**

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