FIELD EVALUATIONS OF GRAFTED GRAPE LINES EXPRESSING POLYGALACTURONASE INHIBITING PROTEINS (PGIPS)

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- **REPORTING PERIOD:** The results reported here are from work conducted from 1 February 2013 - 30 June 2016.

- **ABSTRACT:**

The aim of the project was to determine whether introduction of a plant protein that is naturally produced in edible fruit can restrict the spread of *Xylella fastidiosa* (*Xf*) and symptoms of Pierce's Disease (PD) in grapevines without altering the agricultural attributes of the plants. The PD and Glassy Winged Sharpshooter Scientific Advisory Panel had identified, based on previous work, the plant protein, polygalacturonase (PG) inhibiting protein (PGIP) naturally expressed in pear fruit (pPGIP), as a promising candidate to consider for advancement towards commercialization. Prior to this project, it was known that *Xf* produces a PG that is inhibited by pPGIP (Agüero et al., 2005) and previous work had also shown that PD incidence and symptoms decreased in 'Thompson Seedless' and 'Chardonnay' grapevines if the pPGIP was expressed throughout the vine. The aim of this project was to determine whether pPGIP, when delivered from grafted rootstocks, can control PD in the scion, fruit bearing parts of the grapevines. Work in this project evaluated the performance of vines grown in two commercial-type vineyards and determined whether their susceptibility to PD depended on the pPGIP protein, especially when delivered from pPGIP expressing rootstocks. Cuttings from the two varieties of grapevines that had been transformed to express pPGIP were grafted as rootstocks with non-pPGIP producing 'Chardonnay' or 'Thompson Seedless' scions to make comparisons between vines producing pPGIP in their grafted rootstocks (transgrafted), those producing pPGIP throughout the vine, and vines with no pPGIP. Once the two vineyards were established with the grafted, transgrafted and ungrafted vines, an objective of the project was to determine whether sufficient pPGIP that reduces PD symptoms is delivered from rootstocks expressing pPGIP to scions, which themselves did not produce pPGIP. Active pPGIP protein that had been produced in transgrafted rootstocks was detected in the xylem exudates that were collected from scions (Agüero et al., 2005; Haroldsen et al., 2012). Vineyards approximating commercial settings were established with own-rooted and transgrafted vines in locations in Solano and Riverside counties with naturally low and high PD disease pressure, respectively; vines in Solano County were mechanically inoculated and disease progress was monitored on known infected vines and at known times after inoculation. Evaluations of performance and susceptibility were made for comparisons of scion susceptibility to PD based on the mode of infection (introduced vs natural), varietal background ('Thompson Seedless' and 'Chardonnay') and origin of pPGIP (rootstock only vs entire vine).

- **LAYPERSON SUMMARY:**

In order to determine whether polygalacturonase (PG) inhibiting proteins (PGIPs) have potential for the commercial development and deployment to reduce Pierces Disease (PD), two test vineyards were established in California. The model PGIP evaluated in this project is produced naturally in pear fruit and inhibits the PG that *X. fastidiosa* produces as it spreads and causes damage in infected grapevines. Each vineyard contained 'Chardonnay' and 'Thompson Seedless' grapevines that were growing on their own roots (own-rooted) and others that were "transgrafted" (with rootstocks of the same variety expressing pPGIP grafted to fruit producing non-modified scions that, thus, do not themselves produce pPGIP). The vineyards were designed to enable comparisons of plant performance and susceptibility to PD based on mode of infection (deliberate vs natural introductions of *X. fastidiosa*), varietal background ('Thompson Seedless' vs 'Chardonnay') and origin of the pPGIP (delivered from transgrafted rootstock to grafted non-PGIP producing scions vs plants expressing pPGIP

in all parts). Mechanical inoculations with *X. fastidiosa* bacteria were done yearly from 2011-2015 in Solano County and, beginning with the establishment of the vineyard in Riverside County in June 2013, natural infections were permitted. Data describing the total vine and disease characteristics of the own-rooted or transgrafted vines were collected during growing seasons in both locations. Since this project evaluated grape varieties grown for fresh fruit or wine production in California, we tested varieties important to most California grape growers; the 'Thompson Seedless' and 'Chardonnay' varieties have different growth habits and products and the project provided information for the wine and fresh product sectors of the grape industry. The initial evaluations of the symptoms, 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. Eventually however, when the accumulations of inoculations were repeated and allowed to develop, 'Chardonnay' vines benefited more from the introduction of the pPGIP than 'Thompson seedless' vines.

- **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 and/or delivery of the 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-glucanases (EGases) could participate in the digestion of pectin and xyloglucan polymers in pit membranes and, thereby, facilitate PD development as *Xf* moves within the vine xylem elements. 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, presumably because it permits *Xf* movement.

The aim of this project is to use plant PGIPs to limit *Xf* spread in grapevines. PGIPs are produced in flowers and edible fruit and are induced by contact with pathogens and are selective inhibitors of pathogen and pest PGs (Powell et al., 2000; Shackel et al., 2005; Stotz et al., 1993, 1994). Grapevines transformed to express the pPGIP-encoding gene from pear fruit have reduced susceptibility to *Xf* and pPGIP is transported from rootstocks across the graft junction into wild-type scions (Agüero et al., 2005, Haroldsen et al., 2012).

Because the scions do not contain an introduced pPGIP gene, grafting pPGIP-producing rootstocks to non-pPGIP expressing scions is an opportunity to deliver a beneficial plant fruit protein (i.e., pPGIP) without introducing a pPGIP gene into the part of the plant producing the berries used for produce and wine. This project was designed to generate sufficient numbers of grafted and own-rooted pPGIP expressing grapevines, plant them in field settings comparable to commercial fields, and evaluate their agronomic performance and their resistance to PD due to intentional inoculation or natural modes of transmission.

- **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.

- **RESULTS AND DISCUSSION:**

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

Activities: This objective were completed in June 2013. DNA was prepared from the vines used as source tissue for grafting and the genotypes were confirmed by PCR (Figure 1). Results (see Objectives 3 and 4 below) were that some of the vines over the past 3 years died due to PD and a few died because of other causes.

After the first year, none of the dead vines were replaced. Table 1 shows the number of grafted and non-grafted vines of each genotype that were planted at the sites by June 2013.

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

Table 1. Plant 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 no 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.

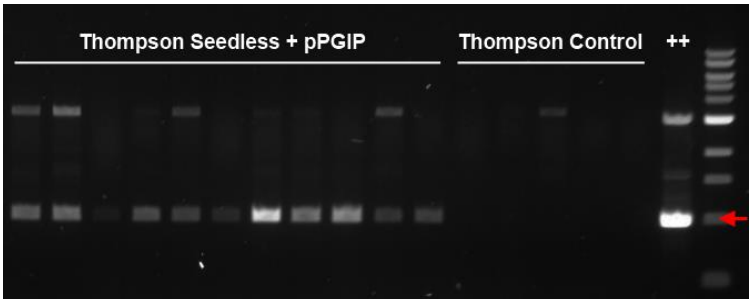

Figure 1. A gel used to genotype by PCR with genomic DNA from grape leaf tissue from Thompson Seedless vines expressing pPGIP and null-transformed (no pPGIP) controls used to generate transgrafted vines. A 1 kb band (arrow) indicating the *pPGIP* DNA sequence is expected only in samples used as rootstocks for transgrafts and pPGIP self-grafted controls. Each sample's quality was verified by amplifying a control fragment (not shown).

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 characteristics 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 2. 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; natural rainfall accounted for most of the watering. In Riverside County, the

plants were watered by drip irrigation. In Riverside, the plot was at the bottom of a small hill and the soil was very sandy and porous; irrigation water accumulated 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 2-3 times per year 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. After 2012, pruning has 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 was observed approximately monthly in the 2014 and 2015 growing seasons and twice in 2016. 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: The Riverside and Solano County sites were planted by 3 June 2013 with all the vine combinations planned for this project. A consistent pruning regime was a goal for this plot so comparisons can be made with other evaluators but pruning was variable. 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. Two evaluations of the Solano field were made in 2016. The vines at the Riverside site were removed in late 2015 because evaluations at that site had been completed and presumed herbicide drift caused unrelated vine symptoms and death.

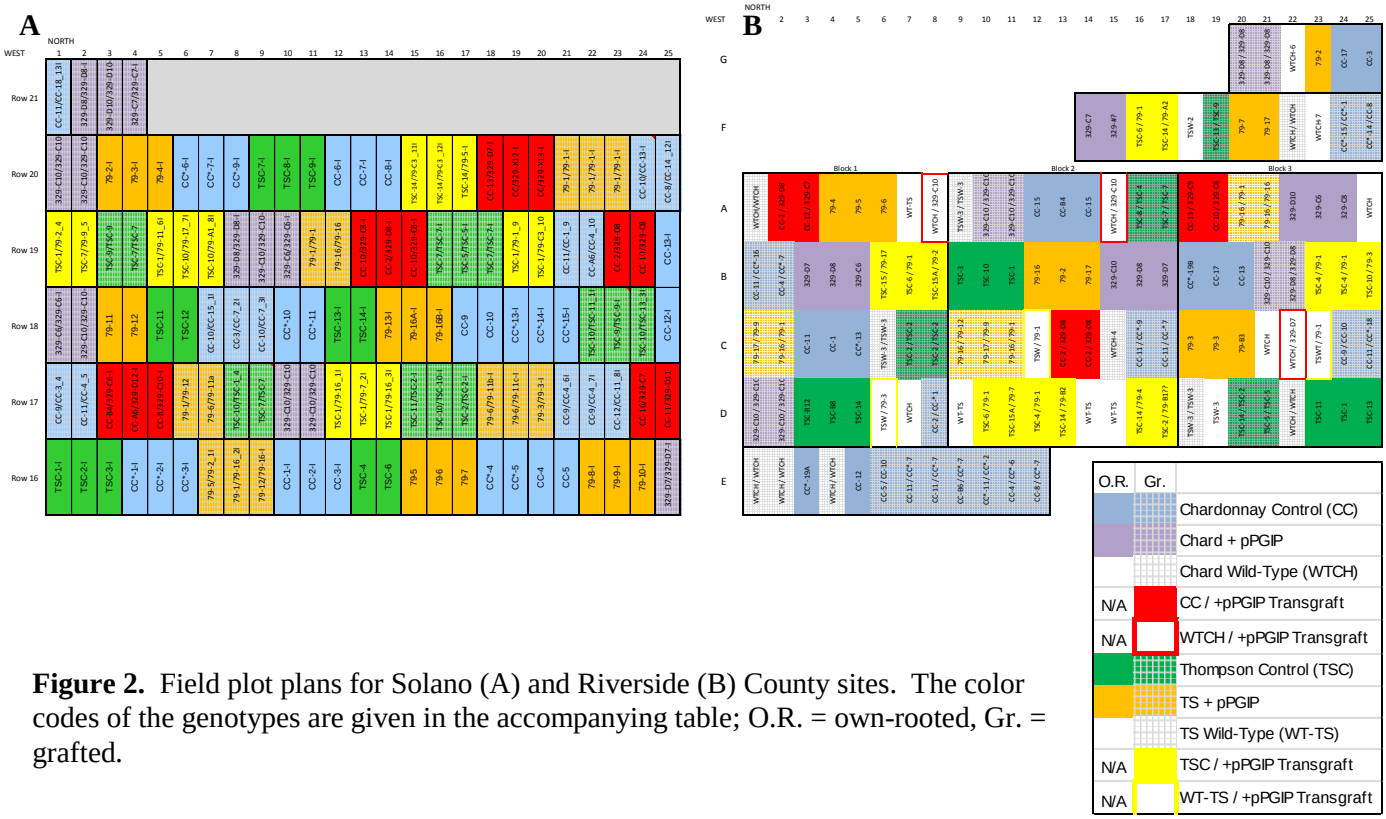

Figure 2. Field plot plans for Solano (A) and Riverside (B) County sites. The color codes of the genotypes are given in the accompanying table; O.R. = own-rooted, Gr. = grafted.

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 UCD)
21 April 2016	Solano	Observation of plants in the field, record dead plants (AP UCD)
27 April 2016	Solano	Confirm observations of plants in the field and record dead plants (AP UCD), field crew prunes plants.

Table 2. Activities at the Solano and Riverside sites for this project through 30 July 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. Observations of the vines were made 21 and 27 April 2016 (Table 3).

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. 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 and were repeated in April 2016 (Table 3).

Agronomic traits such as grape cluster size, berry size, berry and seed phenotypes were measured at the Solano site in the Summer of 2013 but were not repeated. No consistent changes were observed; observations were made only for 1 year and are therefore are not significant. 29 August 2013, 25 berries total were collected from 3 plants of each own-rooted genotype and inoculation state at the Solano site; grafted plants were too juvenile to bear fruit in 2013 and were not sampled. Sample collection was randomized by choosing 5 berries spread across 1-2 clusters per plant. Clusters were chosen from inside the fruiting zone on each plant. Berries were crushed by hand and the free-run juice was combined with juice pressed from the solids, strained through cheesecloth. Sediments were precipitated overnight at 4°C and clarified juice was sampled for pH and °Brix. Soluble solids ranged from 21.7-24.4 °BRIX and pH values were 3.56-4.00. A smaller subsample was repeated on 4 September 2013 with similar results. After one week, total cluster numbers were counted and one cluster was harvested per plant. Some inoculated own-rooted vines did not bear fruit; grafted plants, with one exception, were fruitless in 2013. Cluster weight, length, and peduncle length were measured upon returning to the lab.

Twenty-five berries were removed from each cluster for further analysis after counting the total number of healthy and raisined berries per cluster. Assessments of the subsamples include weight of 25 berries, retention of pedicels, number and class of seeds (trace, rudimentary, or mature), dimensions of 5 berries, soluble solids, titratable acidity, and pH of juice. Each cluster and 5 individual berries were photographed for assessment of cluster density and berry color and shape.

The Riverside site was visited in the late summer of 2014 and plants phenotypes recorded and photographs taken. PI visited the Riverside site on 2 June 2015 and rescored the vines for phenotypes, PD damage and for herbicide damage. Herbicide damage was independently assessed by Peggy Mauk and Philippe Rolshausen at the Riverside site on 17 June 2015 (Table 4).

Table 3. Vine death 2014-2016 at Solano site.

			late 2014		25-Mar-15		27-May-15		7-Aug-15		7-Oct-15		21 April 16	
	Total infected plants	Total uninfected plants	Infected	Not infected	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	11	0
CC/CC	9	4	2	0	2	0	2	0	3	0	7	0	7	0
329	0	2	0	0	0	0	0	0	0	0	0	0	0	0
329/329	9	4	3	0	3	0	3	0	3	0	3	0	3	0
CC/329	11	4	1	1	2	1	2	1	3	1	3	1	3	2
TSC	8	4	2	0	4	0	4	0	7	0	8	0	8	0
TSC/TSC	9	4	0	0	2	0	2	0	5	0	8	0	8	0
79	9	5	3	0	3	0	3	0	3	0	4	0	4	0
79/79	7	4	2	0	2	0	2	0	3	0	6	0	6	0
TSC/79	10	3	1	0	2	0	2	0	5	0	8	0	8	0

Table 3. Observations of vine death at the Solano plot from late 2014 through the 2016 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.

Table 4. Vine symptoms and death at Riverside site in 2015

Genotype	Total number of vines	Severely compromised growth due to Round-up	Moderate growth due to Round-up	Minimal or slight impact on growth due to Round-up	Probably Dead	Dead
CC	13	0	1	0	0	0
CC/CC	16	4	3	3	1	0
wtch	6	0	1	0	1	0
wtch/wtch	6	3	1	0	0	0
329	11	0	2	0	0	1
329/329	7	0	1	3	1	0
cc/329	6	0	1	1	0	0

wtch/329	3	1	0	0	0	0
Total Chard.	68	8	10	7	3	1
TSC	9	1	1	0	0	4
TSC/TSC	7	2	1	0	1	2
wtTS	6	0	1	0	0	2
wtTS/wtTS	3	1	0	1	0	1
79	11	3	1	3	0	1
79/79	7	0	2	2	0	2
TSC/79	14	5	2	3	2	0
wtTS/79	3	0	0	1	0	0
Total TS	60	12	8	10	3	12
Total	128	20	18	17	6	13

Table 4. Observations of herbicide damage and vine death at the Riverside plot 2 June 2015. wtch= Chardonnay wildtype, CC= 'Chardonnay' control, wtTS = 'Thompson seedless' wildtype and TSC and '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 in 2014, four times in 2015 and once in 2016. It is clear that the number of dead vines increased from the 2015 season through the late spring of 2016, 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 caused by infections with *Xf* 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 or were missing.

Table 4 shows 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 not going to be possible to distinguish between damage caused by Pierces Disease and by the herbicide exposure.

Images of the vines at the Solano and Riverside sites is provided in Figure 3.

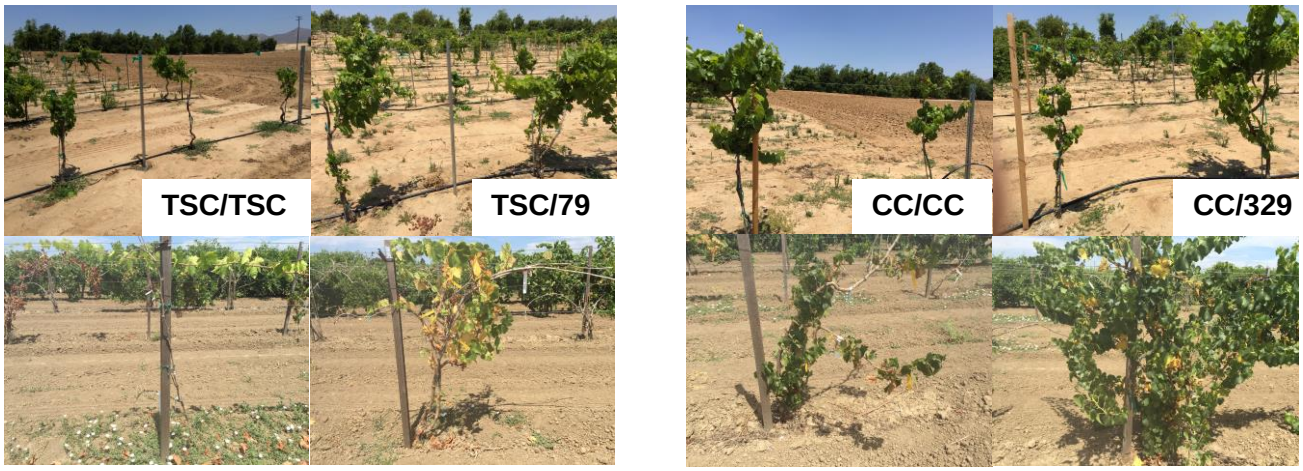

Figure 3. Examples of vines in the Riverside (top row, 2 June 2015) and Solano (bottom row, 7 October 2015) plots of 'Chardonnay' and 'Thompson seedless'. The genotypes of the grafted or transgrafted vines are indicated.

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, after a few test inoculations in 2011, 34 own-rooted vines were resubmitted to mechanical inoculations on 29 May 2012 with a mixture of *X. fastidiosa* Temecula and Stag's Leap strains (3:2, v:v). Young, green tissue was chosen for inoculation with 3-4 canes selected per plant. In

2013-2015, mechanical inoculations were performed as in 2011 except that approximately 1.5 x 10^7 cells were used per inoculation. The inoculations in 2013, 2104 and 2015 were done only on grafted and transgrafted vines although phenotype observations were made on all inoculated vines. Inoculated vines were identified by colored tags denoting the times of inoculations. Inoculations in this PI's plot were performed simultaneously with the other field site collaborators.

The leaves/petioles with evidence of PD symptoms were counted twice during the 2013 and assessments were made again in the 2015 (data not shown) season, including on canes which had been infected in 2011, 2012 and 2013 (Table 5) and increasing symptoms of disease. 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. 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.

Table 5. Vine symptoms during Spring and Summer 2013

Genotype		Plants (#)	% Plants with Excessive Base Growth		% Plants with Marginal Leaf Necrosis on Inoculated Canes		% Plants with Marginal Leaf Necrosis on Un-inoculated Canes		% Plants with Atypical Berry Clusters (partial, aborted, or absent)	
			Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
Inoculated Thompson+pPGIP	79-I	9	77.8 (7/9)	66.7 (6/9)	0 (0/9)	33.3 (3/9)	0 (0/9)	11.1 (1/9)	-	44.4 (4/9)
Thompson+pPGIP	79	5	0 (0/5)	0 (0/5)	-	-	0 (0/5)	0 (0/5)	-	20 (1/5)
Inoculated Thompson	TSC-I	8	25 (2/8)	100 (8/8)	0 (0/8)	12.5 (1/8)	0 (0/8)	0 (0/8)	-	75 (6/8)
Thompson Control	TSC	4	0 (0/4)	50 (2/4)	-	-	0 (0/4)	0 (0/4)	-	0 (0/4)
Inoculated Chardonnay	CC-I	17	17.7 (3/17)	82.4 (14/17)	0 (0/17)	11.8 (2/17)	0 (0/17)	0 (0/17)	-	58.8 (10/17)
Chardonnay Control	CC	8	0 (0/8)	37.5 (3/8)	-	-	0	0	-	25 (2/8)

Table 5. Observations of PD damage and vine responses at the Solano site in late April (Spring) and late August (Summer) 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 was hand ground and frozen at -80ºC. The Powell group received separate funds to purchase a GenoGrinder, similar to equipment used by the Galino group. The Powell group worked on protocols to effectively grind the frozen infected stem tissue until the machine sustained damage. Approximately 6 weeks were needed for repairs to be made. The group tried subsequently for several weeks to refine protocols for macerating the tissue using the machine for PCR analysis but protocols were unsuccessful.

The data analyzing the relationship between the genotypes and the appearance of dead vines were preliminarily analyzed by plotting (Figure 4). Examples of the photo evidence of vine phenotypes are shown in Figure 3. The data demonstrate that vine death increased in late 2015 and continued in Spring 2016 and fewer 'Chardonnay' lines expressing pPGIP either throughout the plant or in grafted rootstocks were dead.

At the Riverside site, vine vigor was analyzed for evidence of PD in 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 analyses of the results are given in

Figure 5. 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 is shown in Figure 3.

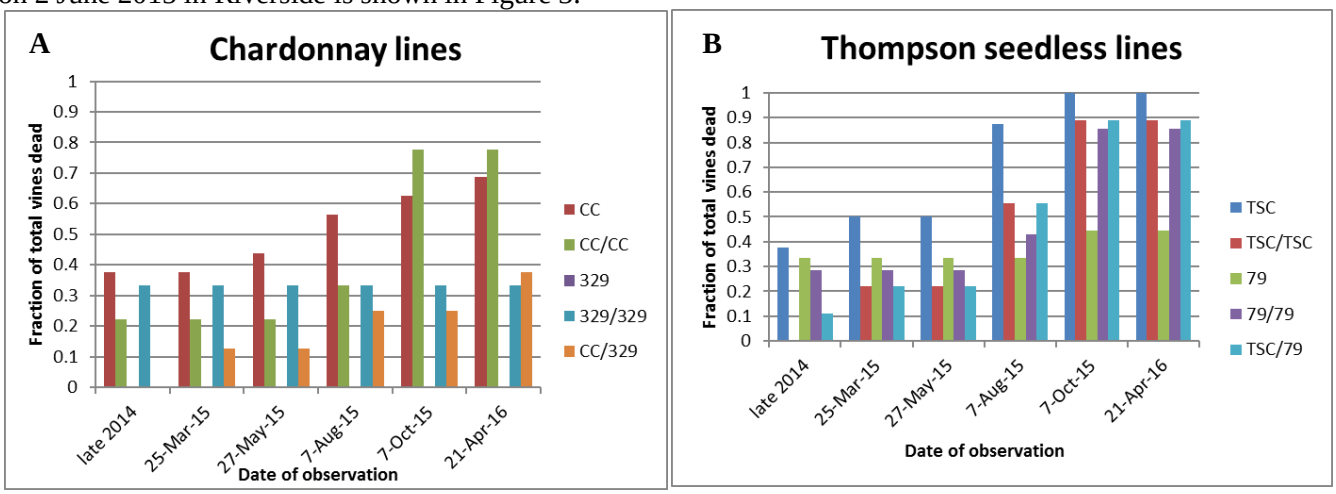

Figure 4. Vine death incidence in Solano plot of 'Chardonnay' and 'Thompson seedless' vines measured in 2014, throughout the 2015 season and initially in Spring 2016. **A. '**Chardonnay' lines. **B.** 'Thompson Seedless' lines. / denotes grafted plants with the genotypes expressed as scion/rootstock. 329 and 79 genotypes express pPGIP in 'Chardonnay' (CC) or 'Thompson seedless' (TSC) backgrounds, respectively.

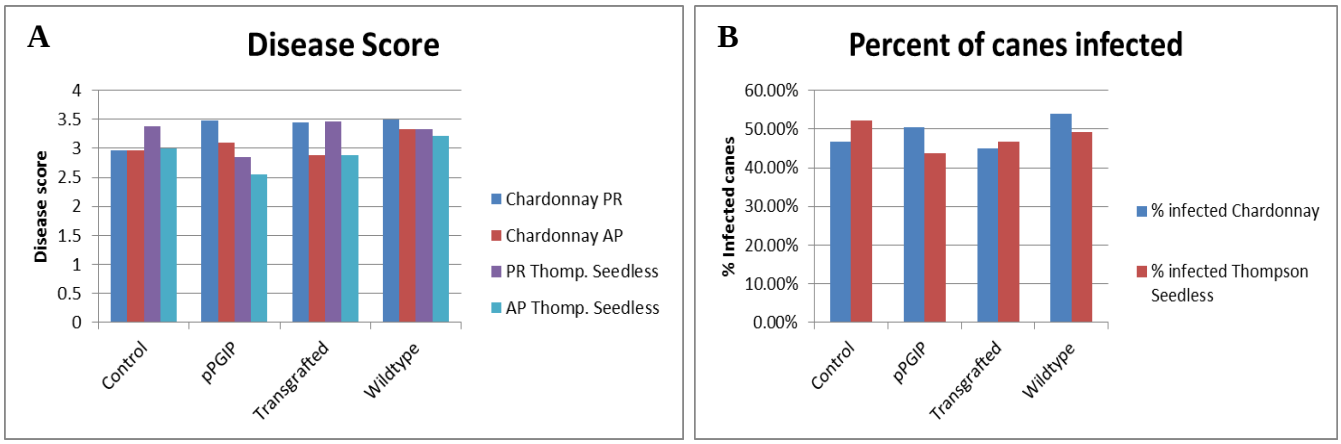

Figure 5. Evidence of disease in Riverside plot of 'Chardonnay' and 'Thompson seedless' vines measured in October 2014. **A.** Disease score based on 0-5 scale. **B.** Percent of vine canes with symptoms or evidence of PD disease. PR= data collected by P. Rolshausen, AP= data collected by A. Powell/

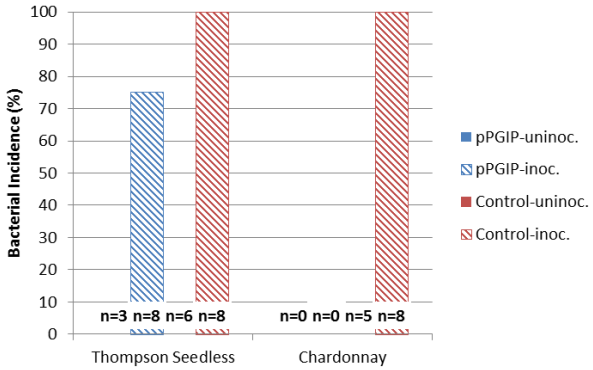

Figure 6. Results of PCR detection of *Xf* DNA sequences in inoculated vines from the Solano County site.

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 'Chardonnay' variety vines in the Solano county site, by the 2015 season than those plants with pPGIP.

In Solano County, initial analyses by PCR showed *Xf* DNA only in inoculated plants and less *Xf* DNA was detected in plants expressing pPGIP (Figure 6). In order to monitor earlier stages of disease development, the number of leaves or petioles along canes infected in 2014 was measured and found to be greater when assessed on in the Spring than in the Summer of 2013 (Table 5). The observations of disease development along leaves and petioles was repeated in 2014. These results indicated that disease was developing in these canes. However, leaf and petiole disease symptoms developed more slowly invines with pPGIP in the scion portion, especially in the 'Chardonnay' variety. Notably vines with pPGIP in the rootstocks 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. Subsequent analysis of the infected vines demonstrated disease progression leading to vine death especially over the summer of 2015 leading to the conclusion that pPGIP expression provided reduced disease development and ultimately less vine death. The effect was clearly due to infection with *Xf* as only 2 uninfected plants had died by April 2016. It is possible that the severe drought heightened the vine-killing effects of disease. The deleterious effects of *Xf* infections were much more pronounced on the 'Thompson seedless' variety than the 'Chardonnay' variety; 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 compared to varieties expressing pPGIP throughout the vine, but by the end of the projects plants expressing pPGIP in the scion and the rootstock or only in the rootstock were about equally likely to die. 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 succumb to PD due to repeated *Xf* inoculations even when pPGIP is expressed. pPGIP expression seems to offer more protection to the 'Chardonnay' than to the 'Thompson seedless' variety. The plants were reanalyzed during the 2016 growing season once the fields were accessible to see if any parts of the plants could recover from the disease and regrow but no growth was observed.

The disease scoring analyses done by PR and AP at the Riverside site in 2014 produced approximately equivalent scores. Analysis of the counted 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 with pPGIP grown at the Riverside site and infected naturally showed a slightly more 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' procedures 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 for 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 confirming the identity and history of the inoculations. 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 was challenging, However, the over-all performance of the '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. Only 2 uninoculated vines died at the Solano site. Based on counting leaves with evidence of scorching, the 'Chardonnay' vines with pPGIP had initially also had fewer PD symptoms than the 'Thompson Seedless' variety when mechanically inoculated. By evaluating varieties grown for fresh fruit and for wine production in California, we provided information about the impacts of pPGIP and its delivery using varieties which grow with different habits and which are important to different segments of the community of California grape growers.

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