FIELD EVALUATION OF GRAFTED GRAPEVINE LINES EXPRESSING POLYGALACTURONASE-INHIBITING PROTEINS

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ABSTRACT

The aim of this project is to evaluate in two California field vineyards, the performance of grafted grapevine lines that produce in the rootstock, a protein that is a candidate for control of Pierce's disease (PD). The PD and Glassy-winged Sharpshooter Board's (GWSS-PD) Research Scientific Advisory Panel gave priority to the delivery of polygalacturonase-inhibiting proteins (PGIPs), from grafted rootstocks to control PD. Previously transformed 'Thompson Seedless' and 'Chardonnay' grapevines expressing a PGIP from pear fruit (pPGIP) show reduced PD incidence when inoculated with *Xylella fastidiosa (*Agüero *et al*., 2005*).* In this field evaluation, these grapevines were propagated vegetatively for PD assessment in field trial locations in Solano and Riverside Counties. Fifty-one transgenic and control, own-rooted, grapevines were planted in Solano County on 07/06/2010. Additional grafted plants, utilizing the pPGIP-expressing vines as rootstocks, have been prepared, rooted and they will be planted later in 2011 when field conditions are appropriate. The field plantings in the plot in Solano County were winter pruned in 2011 and currently are in the midst of their main growing season. PD resistance and plant growth characteristics are being assessed on the plants in the northern California location. The test field also contains plots from the three collaborating groups of D. Gilchrist, A. Dandekar and S. Lindow who were funded jointly. Inoculations and disease assessments of the vines developed in the four projects are being evaluated simultaneously. The plants from these trials have been planted at the same locations and the APHIS-USDA authorizations for all projects have been handled through PIPRA.

LAYPERSON SUMMARY

The goal of this project is to verify that the Pierce's disease (PD) resistance provided by expression of polygalacturonase inhibiting protein (PGIP) in grapevines is evident when the plants are grown in field vineyard settings in California. The overall health and robustness of the plants expressing the pear fruit (p) PGIP will be compared to the plants from other jointly funded groups evaluating other strategies to limit PD development. The resistance of plants expressing PGIPs in grafted rootstocks will be compared following manual infections with *Xylella fastidiosa* through stem inoculations in the Northern California location and as a result of natural infections in the Southern California site. Funding for this project was needed to develop sufficient plants for both locations, manage the vineyard plantings, confirm the genetic identity of rootstocks as well as the scion and do the resistance testing. The performance and resistance of the grapevines in the field are being evaluated as the vines become established in the vineyards.

INTRODUCTION

Grapevines transformed to express the pear fruit polygalacturonase inhibiting protein (pPGIP) were grrown in greenhouses prior to the work in this proposal. These vines displayed fewer symptoms of Pierce's disease (PD) infections when inoculated with *Xylella fastidiosa (Xf) (*Agüero *et al*., 2005*).* The additional PGIP in the grape plants inhibits the enzyme, polygalacturonase (PG), that *Xf* employs to spread infections throughout the vine (Roper *et al.,* 2007). In a separate glassy-winged sharpshooter (GWSS)-PD funded project aiming to optimize the activity, expression, and export of PGIP proteins expressed in transgenic grape rootstocks to provide optimal PD protection in the scion portions of the vines ("Optimizing grape rootstock production and export of inhibitors of *Xf* PG activity" (PI Labavitch)), PGIPs from various plants are being evaluated for their efficacy. While these evaluations strongly suggested that expression of additional PGIPs could be an appropriate strategy for improving grapevine resistance to PD, the vines had only been grown and evaluated in greenhouses and to be acceptable to the California grape industry, their growth performance and susceptibility to PD in vineyard settings comparable to commercial production locations was necessary. The goal of this project is to verify that the transgenic grapevines expressing pPGIP in grafted rootstocks (1) have increased resistance to PD and (2) maintain the appropriate performance traits necessary for commercial release when grown in field vineyard settings in California. The project was funded jointly with other groups evaluating anti-*Xf* strategies so that uniform field conditions could be achieved for all of the trials. Comparisons of protection and performance outcomes from the groups should be achieved.

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 performance traits of vines in two locations.

4. Determine PD incidence in pPGIP expressing grafted and own-rooted lines. Test for *Xf* presence and, if present, determine the extent of infection.

RESULTS AND DISCUSSION

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

The pPGIP expressing 'Chardonnay' and 'Thompson Seedless' grapevines generated by Agüero et al. (2005) continue to be maintained at the UC Davis Core Greenhouses. Grafting has been done with green and semi-lignified stem segments for the possible graft combinations. A modified wedge grafting technique is used whereby scion sections of one to two nodes were stripped of foliage and cut into a wedge. These sections were fit into notched rootstock line stems of equal maturity, alternating the bud position. The graft union was covered with Parafilm M, secured by a clothespin, and the entire scion piece was covered loosely by a translucent bag to prevent desiccation. Other similarly grafted vegetative cuttings - one node scions grafted onto three node, disbudded rootstock cuttings - were basally dipped in 5.7 µM IAA and 2.7 µM NAA for five min. before transferring to a loose perlite:vermiculite medium (1:1). We have utilized mist beds to increase the success of callusing these green grafted cuttings and have modified the EZ-Clone aeroponic system discussed in previous reports so that the grafted plants develop more robust roots before they are transferred to soil prior to transplantation to the field (**Figure 1**). We have made significant progress toward generating the grafted plants needed to complete the project design in the field trial. Our grafting techniques are continuously evolving to yield higher success rates. For this season, we have generated 21 potted, grafted plants with another 142 grafted cuttings currently callusing and hardening off.

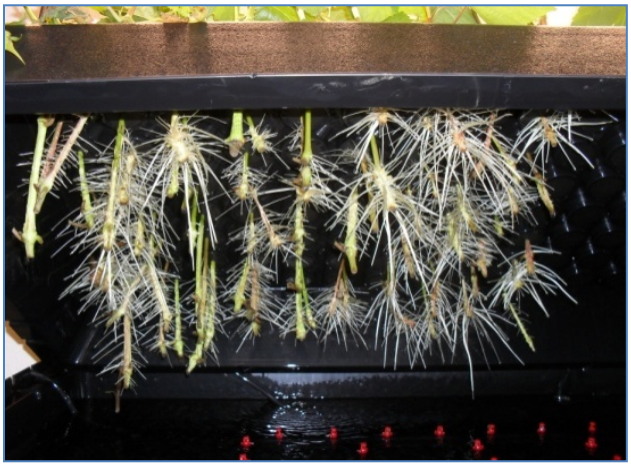

Figure 1. Grapevine cuttings rooting in the EZ-Clone aeroponic manifold.

Objective 2: Establish field trial sites.

Two field trial sites are being established to assess the PD resistance and general agronomic viability ("performance") of own-rooted and grafted pPGIP expressing grapevines. We have focused our efforts on generating sufficient high quality vines for the primary site in Solano County, CA. The Solano site has no natural PD pressure and the secondary site in Riverside County, CA has high natural PD pressure. The field sites are shared by projects testing other transgenic PD control grapevines from PIs, D. Gilchrist, A. Dandekar, and S. Lindow. All vines, both grafted and ungrafted, that by PCR analysis have the correct genotypes, from our portion of the field trial were hand-planted in a randomized block design with blocks consisting of two or three individuals in the same treatment beginning in July 2010. In **Table 1** the number of each combination of each genotype of grafted and ungrafted plants is shown. Not all of the plants that are needed for the experimental design have been transplanted to the field vineyards as of October, 2011 but most of the remaining plants have been grafted and are developing roots and hardening off in lathe houses. The remaining grafted lines expressing pPGIP in the rootstocks will be planted in the early fall 2011.

The grapevines are planted approximately eight ft. apart and tied to wooden stakes with trellising wires at 40 in. and 52 in. All plants in the field vineyard were winter pruned in February 2011 and have grown vigorously so far in 2011 (**Figure 2**). The vines in the Solano site have been weeded and monitored weekly throughout the 2011growing season. Grow tubes were initially placed around the vines to minimize damage by rabbits, mechanical weeding, and herbicides. We have lost none of the vines that we placed in the field in 2010, a result we attribute to planting robust vines that were sufficiently hardened off, combined with tending the vines in the field with appropriate care. The propagated vines were trained to one major shoot and pruning biweekly to encourage growth. The vines were topped and major cordons were extended bidirectionally, keeping additional positions to account for the vigorous nature of the vines at the site.

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-transformant controls (no pPGIP). Vines were mechanically inoculated with *Xf* on 7/21/2011. Grafting progress numbers include all grafted cuttings at each checkpoint.

		Own-Rooted Plants (#)		Grafting Progress (#)			
Cultivar	Grafting Strategy	Inoculated	Non-Inoculated	Mist Beds	EZ-Clone	Potted in Greenhouse	Originally Planned
Chardonnay		8	4	0	2	2	13
		-	-	70	2	5	13
		9	4	0	1	1	13
Thompson Seedless		8	4	0	3	4	13
		-	-	58	4	8	13
		9	5	0	2	1	13
Subtotals		34	17	128	14	21	
Aggregate Totals		51		163			78

Figure 2. Examples of vines in the field in July, 2011. Vines have been trained to the central post and trellising wires. *Xf* mechanical inoculation sites are marked with orange tags (C).

The rooted cuttings of 'Chardonnay' and 'Thompson Seedless' grapevines engineered to express pPGIP were genotyped by PCR analysis prior to transplantation to the field vineyard to confirm the presence of the pPGIP sequences in the appropriate rootstock or scion portions of the plants. To confirm that the pPGIP sequences produced the expected pPGIP protein, total extracted proteins were cross-reacted with a pPGIP-specific polyclonal antibody on Western blots. The pPGIP protein was observed only in samples from scions grafted to pPGIP expressing rootstocks or where otherwise expected in scions expressing the pPGIP sequences (**Figure 3**). Graft translocation is not seen in control transgenic grapes expressing a cellular-localized protein.

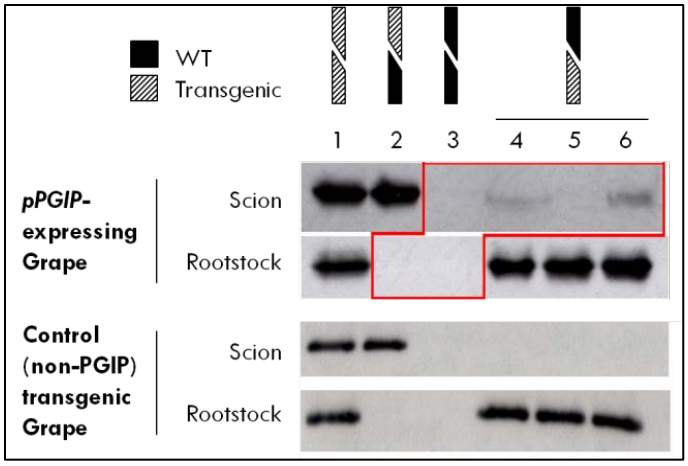

Figure 3. Western blot of leaf extracts taken from rootstock and scion portions of grafted 'Thompson Seedless' grapevines. Transgenic vines are expressing either pPGIP or NPTII (control). pPGIP is visualized crossing from transgenic rootstocks into wild-type (WT) scion tissue (lanes 4-6). This movement is not seen in the reciprocal graft (lane 2).

Objective 3. Evaluate relevant agronomic traits of vines in two locations.
The grapevines planted in Solano County have been monitored for general health and maintained on a weekly basis. Our regulatory permits require that all flowers be removed to prevent the potential for pollen escape. Because of this restriction, we have not been able to perform the agronomic fruit production measurements necessary for the commercial viability assessment. We are working with PIPRA to explore possible exceptions to this policy for future seasons. We however, monitor health and vigor of individual plants.

Objective 4. Determine PD incidence in pPGIP expressing grafted and own-rooted lines.
Two-thirds of the own-rooted vines at the Solano site have been mechanically inoculated with *Xf* to monitor PD incidence throughout the summer. Mechanical stem inoculations were utilized to ensure an even introduction of the bacteria in this site with no natural PD pressure. Each of the 34 own-rooted vines were inoculated three-four times per plant using a pin-prick technique by which a 20 µl (20,000 cells) drop was placed on a 21 gauge needle piercing the cane, and the needle was then withdrawn. The bacterial suspension was taken up into the xylem by the natural negative turgor pressure associated with evapotranspiration. The inoculations were performed in conjunction with the other field site collaborators. The bacterial suspension was provided by D. Gilchrist. A subset of the inoculated plants will be screened for *Xf* presence and movement by culturing xylem sap extracts on PD3 media.

CONCLUSIONS
Field vineyards are being established that contain grapevines that express pPGIP protein in rootstocks and these rootstock export the pPGIP protein to the scion portion of the plant. The efficacy of the exported pPGIP to reduce the PD damage caused by *Xf* is being evaluated in the field plantings to verify that the resistance observed in greenhouse settings can be replicated by plants grown in typical field settings in California. We are completing the generation of the remaining plants needed for the sites and confirming the genotype of the lines transplanted to the field. The general health and performance of the plants in the field vineyard continue to be monitored.
The results of the field evaluation will confirm that delivery of the pPGIP from rootstocks provides a means of controlling PD and *Xf* infection in a typical vineyard setting in California, an outcome that is important for the acceptance of this strategy to control PD in California. The evaluations of the performance and productivity of the plants will confirm that that expression and presence of pPGIP does not unintentionally adversely affect 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.

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