PROGRESS REPORT FOR CDFA CONTRACT 09-0746

Title: FIELD EVALUATION OF GRAFTED GRAPE LINES EXPRESSING PGIPS

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Objectives, activities, progress:

This project is to evaluate the performance in the field of grafted grapevine lines that produce in the rootstock, a protein that is a candidate for control of Pierce's Disease (PD). The CDFA PD and Glassy-winged Sharpshooter Board's 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 *X. fastidiosa (*Agüero *et al*., 2005*)*. 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 7/6/2010. 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 and are currently in the midst of their main growing season. PD resistance and plant growth characteristics are being assessed.

The grapevines transformed with the pPGIP protein are also being analyzed in a funded project to optimize the activity, expression, and export of PGIP proteins from transgenic rootstocks to provide PD protection in the scion portions of the vines by inhibiting the enzyme, polygalacturonase (PG) that *X. fastidiosa* uses to spread infections (Roper *et al.*, 2007): "Optimizing grape rootstock production and export of inhibitors of *X. fastidiosa* PG activity" (PI Labavitch). These plants were previously only observed in greenhouse settings. The goal of this project is to verify that the transgenic grapevines expressing pPGIP as grafted rootstocks (1) have increased resistance to PD and (2) maintain the appropriate agronomic traits necessary for commercial release.

This field trial proposal was funded jointly with proposals from D. Gilchrist, A. Dandekar and S. Lindow. The plants from these trials have been planted at the same locations and the APHIS-USDA authorizations have been handled through PIPRA.

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.

Activities and Progress:

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. Vegetative cuttings of non-lignified stem sections from transgenic and control plants of both cultivars have been rooted in an aeroponic cloning manifold (EZ-Clone Inc., Sacramento, CA), as described in previous reports. Apical regions received continuous light while basal nodes received constant misting in darkness until roots began to form (Figure 1). Rooted cuttings were transferred to soil and maintained in the greenhouse. Since reusing the EZ-Clone tanks for a second season was problematic as the foam plugs were a source of contamination, a new system using polyfill and peat plugs has been utilized and 23 own-rooted plants have been successfully rooted with these changes and transferred to pots during the reporting period.

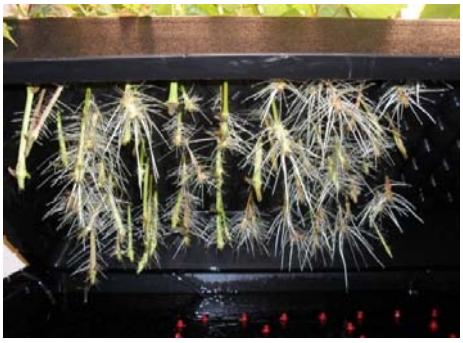

Figure 1. Grapevine cuttings rooting in the EZ-Clone aeroponic manifold.

Grafting has been done with green and semi-lignified stem segments for all possible graft combinations. A modified wedge grafting technique is used whereby scion sections of 1 to 2 nodes were stripped of foliage and cut into a wedge. These sections were fit into notched rootstock line stems of equal maturity, alternating 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 were basally dipped in 5.7 µM IAA and 2.7 µM NAA for 5 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 so that the grafted plants develop more robust roots before they are transferred to soil prior to transplantation to the field. 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 18 potted, grafted plants with another 87 grafted cuttings currently callusing in the mist beds. We expect to complete the generation of sufficient grafted plants by the end of August, 2011 and transplant the vines to the field in the fall.

Objective 2: Establish field trial sites

Two field trial sites are being established to assess the PD resistance and general agronomic viability 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 satisfying PCR analysis 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 in July 2010 (Table 1) and the remainder of the grafted lines expressing pPGIP in the rootstocks will be planted in the early fall 2011. The grapevines were planted approximately 8 ft. apart and tied to wooden stakes with trellising wires at 40 in. and 52 in. All plants were winter pruned in February, 2011 and have grown vigorously so far in 2011 (Figure 2).

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

Two-thirds of the own-rooted vines have been mechanically inoculated with *X. fastidiosa* to monitor PD incidence throughout the summer. The vines in the Solano site have been pruned, weeded and monitored weekly throughout the 2011 growing 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 prepared by training to one major shoot and pruning biweekly to encourage vigorous growth.

The rooted cuttings of transgenic 'Chardonnay' and 'Thompson Seedless' grapevines were genotyped by PCR analysis to confirm the presence of the pPGIP transgene. The pPGIP protein, cross-reacting with a pPGIP-specific polyclonal antibody, was observed only in samples from scions grafted to pPGIP expressing rootstocks or where otherwise expected (Figure 3). Graft translocation is not seen in control transgenic grapes expressing a cellular-localized protein.

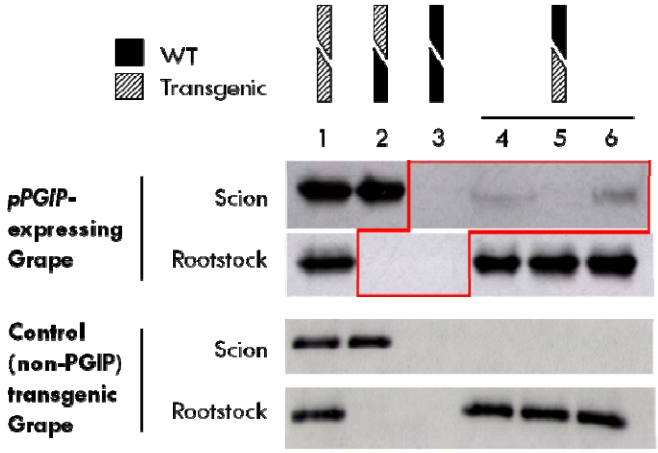

Figure 3. Western blot of leaf extracts taken from rootstock and scion portions of grafted 'Thompson Seedless' grapevines. Transgenic vines are expressing either pPGIP or NPTII (control). pPGIP is visualized crossing from transgenic rootstocks into wild-type (WT) scion tissue (lanes 4-6). This movement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

		Own-Rooted Plants (#)		Grafting Progress (#)			
Cultivar	Grafting Strategy	Inoculated	Non-Inoculated	Mist Beds	EZ-Clone	Potted in Greenhouse	Originally Planned
Chardonnay		8	4	0	0	2	13
		-	-	28	1 (3)	3 (1)	13
		9	4	0	0	2	13
Thompson Seedless		8	4	0	0	1	13
		-	-	37	6 (1)	3 (1)	13
		9	5	0	0	0	13
Subtotals		34	17	65	7	11	
Aggregate Totals		51		83			78

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 *X. fastidiosa* on 7/21/2011. Grafting progress numbers include all grafted cuttings at each checkpoint; parentheses indicate own-rooted cuttings from failed grafts.

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. As such, we have not been able to perform any of the agronomic measurements necessary for the commercial viability assessment. We are working with PIPRA to explore possible exceptions to this policy for future seasons.

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

The grapevines at the Solano field site were inoculated with *X. fastidiosa* Temecula on 7/21/2011. Mechanical stem inoculations will ensure an even introduction of the bacteria in this site with no natural PD pressure. Each of the 34 own-rooted vines were inoculated 3-4 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.

References cited:

Agüero CB, Uratsu SL, Greve LC, Powell ALT, Labavitch JM, Meredith CP, Dandekar AM. 2005. Evaluation of Tolerance to Pierce's Disease and *Botrytis* in Transgenic Plants of *Vitis vinifera* L. expressing the pear PGIP gene. Mol. Plant Pathol. 6: 43-51.

Roper MC, Greve LC, Warren JG, Labavitch JM, Kirkpatrick BC. 2007. *Xylella fastidiosa* Requires Polygalacturonase for Colonization and Pathogenicity in Vitis vinifera Grapevines. Mol. Plant Microbe Interact. 20: 411-419.

How the work will contribute to solving the PD problem in California:

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