A. Title of Report: INTERIM PROGRESS REPORT FOR CDFA CONTRACT 09-0746

B. Title of Project: FIELD EVALUATION OF GRAFTED GRAPE LINES EXPRESSING PGIPs

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D. **Time Period covered by the Report:** 7 March 2012 to 28 August 2012.

E. Introduction:

Work in this project evaluates 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*)*. Therefore, cuttings from these grapevines have been grafted with non-pPGIP producing scions to make comparisons between the efficacy of pPGIP produced in grafted rootstocks vs throughout the plant for PD control. Grafted and non-grafted grapevines have been propagated vegetatively for PD assessments in fields in Solano and Riverside Counties. Fifty-one transgenic and control, own-rooted, grapevines were planted in Solano County on 7/6/2010. The field plantings in the plot in Solano County were severely winter pruned in 2011 and were pruned to establish primary canes and cordon positions in early spring 2012. Thirty-one grafted plants, utilizing the pPGIP-expressing vines as rootstocks or the appropriate control combinations, were planted in Solano County on 5/18/12 and closely monitored to ensure survival throughout the dry summer season. The established, own-rooted vines were inoculated with *X. fastidiosa* on 5/29/2012 and PD resistance and plant growth characteristics are currently being assessed.

The grapevines transformed with the pPGIP protein are also being analyzed in a separate 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.

F. 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.

G. 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 (Figure 1)), as described in previous reports. These plants are referred to as "own-rooted plants." Rooted cuttings were transferred to soil and maintained in the greenhouse.

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

Since reusing the EZ-Clone tanks during the second season was problematic as the foam plugs were a source of contamination, a new system using different media combinations has been utilized beginning in mid-2011.

Grafted plants were made by green grafting rootstock stem sections with budded scion material as described in previous reports. As of 28 August, 2012, 46 grafted plants and an additional 8 grafts (by grafting budded scions onto rooted plants) are currently in 1 gal. pots in greenhouses. The current inventory of potted grafted plants are: 11 Chardonnay trans-grafts (Chardonnay pPGIP expressing rootstock grafted with Chardonnay scion not expressing pPGIP), 4 Thompson Seedless transgrafted plants (Thompson seedless pPGIP expressing rootstock grafted with Thompson seedless scion not expressing pPGIP), 16 Chardonnay null-transformant control grafted plants (Chardonnay rootstock not expressing pPGIP grafted onto Chardonnay scion not expressing pPGIP), 5 Thompson seedless null control grafted plants (non-transformed Thompson seedless rootstock grafted onto Thompson seedless scion not expressing pPGIP) , and 10 Thompson seedless pPGIP expressing control grafted plants (Thompson seedless expressing pPGIP rootstock grafted onto Thompson seedless expressing pPGIP scion). DNA was prepared from the vines used as source tissue for grafting and tested genotype confirmed by PCR (Figure 2). In addition to the grafts listed previously, 31 grafted plants were generated, confirmed, and transferred to the Solano

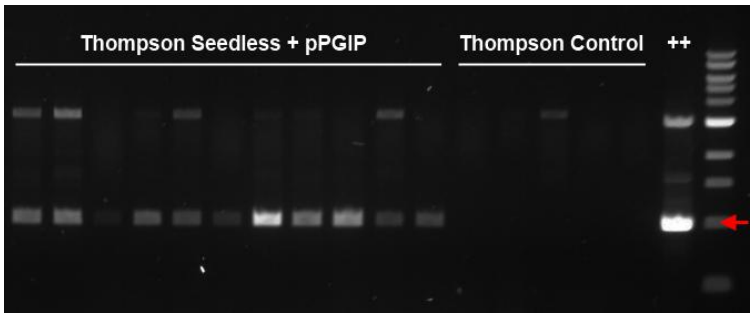

Figure 2. Sample genotyping PCR of grape leaf tissue from Thompson seedless vines expressing pPGIP and null-transformed (no pPGIP) controls used to generate the transgrafted vines planted in May 2012. A 1 kb band (arrow) corresponding to pPGIP is expected only in samples used as rootstocks for transgrafts and pPGIP self-grafted controls. Each sample's quality was verified by amplifying a control fragment (not shown).

field site in May 2012. Grafting efforts, which in the last six months have been hindered by mealy bug and podery mildew infestations in the green houses will continue to complete the population for the Riverside site.

Objective 2: Establish field trial sites

Two field trial sites in Solano and Riverside Counties are being used to assess the PD resistance and general agronomic viability of own-rooted and grafted pPGIP expressing grapevines. The field sites are shared by projects testing PD resistance of other transgenic grapevines from PIs, D. Gilchrist, A. Dandekar, and S. Lindow. The vines satisfying our initial PCR analysis in 2010 for 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). Thirty-one grafted plants, either utilizing the pPGIP expressing material as rootstocks or the appropriate control graft combinations, were prepared as described above and hardened in a lathe house for two months prior to planting in Solano County in May 2012. These younger, grafted plants were surrounded by protective grow tubes and hand-watered every two weeks or as needed. The grapevines are planted approximately 8 ft. apart and tied to wooden stakes with trellising wires at 40 in. and 52 in. Their growth during the 2012 growing season has been vigorous (Figure 3).

The vines have been pruned both to maximize potential cane number for inoculations and to establish vigorous positions for future growth. With the permit amendment granted to M. Szczerba by the BRS-USDA in March 2012, we have been able to allow flowers and fruiting clusters to persist. All own-rooted Chardonnay vines were cordon trained and spur pruned whereas the majority of the Thompson Seedless vines were cane pruned in an attempt to maintain proper vine balance and ensure fruit development. The Solano field site has been under weekly observation for the duration of the growing season.

Figure 3. Examples of vines in the field in March (left) and August (right). *X. fastidiosa* mechanical inoculation sites are marked with tags.

		Chardonnay			Thompson Seedless		
	Grafting Strategy						
Own-Rooted Plants (#)	Inoculated	17	-	(9)	8	-	9
	Non-Inoculated	8	-	(4)	4	-	5
Grafted Plants (#)	Inoculated	9	(9)	(9)	3 (6)	8 (1)	2 (7)
	Non-Inoculated	4	(4)	(4)	1 (3)	4	(4)
Grafting In Progress (#)	Mist Beds	0	22	0	9	0	3
	EZ-Clone	0	11	5	0	0	2
	Potted	16	11	0	5	4	10

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). Own-rooted vines were inoculated on 7/21/2011 and 5/29/2012; grafted vines have not been mechanically inoculated. Grafting in progress numbers include all grafted cuttings at each checkpoint. Parentheses indicate vines that have not yet been planted in the field.

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. With the permit amendment mentioned above, agronomic trait analyses are being discussed. Any measurements of fruit number or quality will be made in the fall.

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

Two-thirds of the own-rooted vines at the Solano County site were mechanically inoculated with *X. fastidiosa* Temecula on 21 July 2011, to monitor PD incidence during the late summer 2011. No visual evidence of PD infection was observed throughout the 2011 growing season or in the early 2012 months following bud break. The same 34 own-rooted vines were resubmitted to mechanical inoculations on 29 May 2012 with a mixture of *X. fastidiosa* Temecula and Stags Leap strains (3:2, v:v). Young, green tissue was chosen for inoculation with 3-4 canes chosen per plant. Mechanical inoculations were performed as in 2011 except that approximately 1.5×10^7 cells were used per inoculation, an increase of 750-fold over the previous year. The inoculations were performed simultaneously with the other field site collaborators. The bacterial suspension was provided by D. Gilchrist. At this time, no visual PD symptoms are apparent. Molecular assays of infection will be performed in the coming weeks.

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.

H. Publications produced:

1. Haroldsen VM, Szczerba MW, Aktas H, Lopez-Baltazar J, Odias MJ, Chi-Ham CL, Labavitch JM, Bennett AB and Powell ALT (2012) Mobility of transgenic nucleic acids and proteins within grafted rootstocks for agricultural improvement. *Frontiers in Plant Science* **3**:39, Published 2 March 2012.

I. 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 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.

J. Layperson summary of project accomplishments:

Fifty-one own-rooted Chardonnay and Thompson Seedless grapevines, including those expressing pPGIP, were generated by vegetative propagation, genotyped by PCR, and planted as part of a field trial in Solano County in 2010. Transgrafted combinations of these same plants have been generated and planted as part of the Solano site in 2012. Grafted vines are being generated to add to the existing site in Riverside. Mechanical inoculations with *X. fastidiosa* bacteria were done in 2011 and 2012 in Solano County but no evidence of infections has been observed, to date.

K. Summary and status of intellectual property associated with the project:

Evaluation of the intellectual property status of the strategy to control PD from PGIPs produced in rootstocks has been evaluated by PIPRA and reported previously. No change has occurred.