A. Title of Report: RENEWAL 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:** 22 July 2011 to 6 March 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. Forty-six grafted plants, utilizing the pPGIP-expressing vines as rootstocks, have been prepared, rooted and they will be planted early in 2012 when field conditions are appropriate. The field plantings in the plot in Solano County were winter pruned in 2011 and will be pruned in the second year during the second week of March, 2012, in anticipation of bud break shortly thereafter. PD resistance and plant growth characteristics are 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. Grapevine 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 rooted scions with budded scion material as described in previous reports. As of 5 March, 2012, 49 grafted plants and an additional 8 grafts (by grafting budded scions onto rooted plants) are currently in 1 gal. pots in greenhouses. We expect to transfer them to the lathe house within a week. The current inventory of grafted plants are: 27 Chardonnay trans-grafts (Chardonnay pPGIP expressing rootstock grafted with Chardonnay scion not expressing pPGIP), 19 Thompson Seedless trans-grafted plants (Thompson seedless pPGIP expressing rootstock grafted with Thompson seedless scion not expressing pPGIP), 3 Chardonnay null-transformant control grafted plants (Chardonnay rootstock not expressing pPGIP grafted onto Chardonnay scion not expressing pPGIP), 1 Chardonnay pPGIP expressing control grafted plants (Chardonnay expressing pPGIP rootstock grafted onto Chardonnay expressing pPGIP scion) , 6 Thompson seedless null control grafted plants (non-transformed Thompson seedless rootstock grafted onto Thompson seedless scion not expressing pPGIP) , and 1 Thompson seedless pPGIP expressing control grafted plants (Thompson seedless expressing pPGIP rootstock grafted onto Thompson seedless expressing pPGIP scion). DNA has been prepared from the scion portion of most of these plants and is being analyzed by PCR using pPGIP and 35S promoter specific primers to confirm the genotypes of the scion portion of the plants (Figure 2). These grafted plants will be sufficient to complete the planting of our portion of the field trial in Solano County. Grafting will continue to complete the population for the Riverside site. Confirmation of the genotypes of the plants used for the rootstock portions of the grafts is

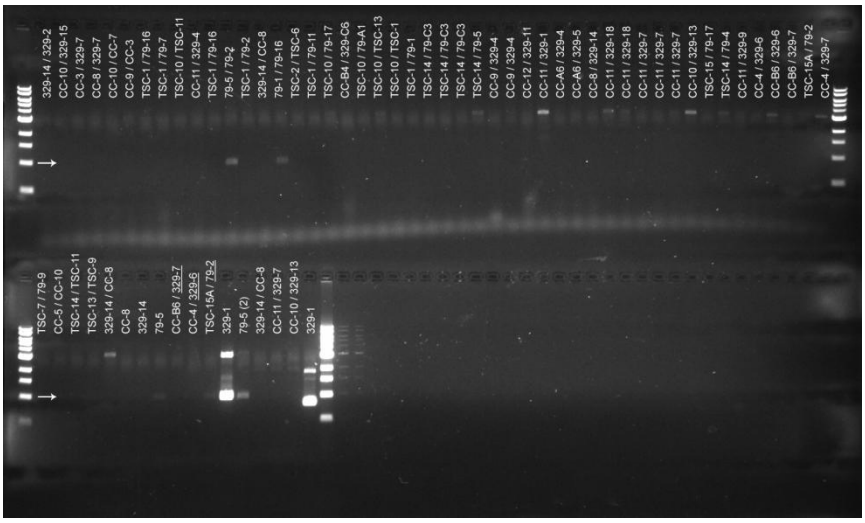

Figure 2. Genotyping PCR of scion leaf tissue from grafted vines. A 1 kb band (arrow) corresponding to pPGIP is expected only in control grafts with pPGIP-expressing scion genotypes (329 and 79). Trans-grafts have scions (Chardonnay (CC) and Thompson seedless (TSC)), lacking pPGIP and therefore, not expressing pPGIP. Graft genotypes are labeled as "scion / rootstock" except where own-rooted vines were used as controls. Each sample's quality was verified by amplifying a control fragment (not shown).

underway. In all but 2 cases, DNA from the scion portions of the transgrafted plants did not produce PCR products with two combinations of primers, indicating that their genotypes were correct, ie. they did not contain the introduced pPGIP genes. The two exceptions are being retested and will be relabeled appropriately if it is confirmed that they contain pPGIP DNA sequences.

For the Solano County site, as of 5 March 2012, there are approximately 180 green grafted rooting plants in the mist beds for the trial at this site. 90% of these are control grafts. Transgrafted plants will be developed once the plants in the greenhouse recover from a mealy bug infestation.

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) and the remainder of the grafted plants currently in pots expressing pPGIP in the rootstocks will be planted in Solano County in March, 2012. The grapevines are 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 will be pruned again in March, 2012. Their growth during the 2011 growing season was vigorous (Figure 3).

The vines are being pruned to maximize potential cane number. All vines are being cordon trained and spur pruned. If flowers are allowed to persist pending a favorable response to the modification request made to the BRS-USDA by M. Szczerba in February, 2012, the remaining unpruned Thompson seedless vines will be cane pruned to ensure flower and fruit development from the later bud positions. The vines in the Solano site were pruned, weeded and monitored weekly throughout the 2011 growing season and observed monthly during the dormant 2011/2012 winter 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.

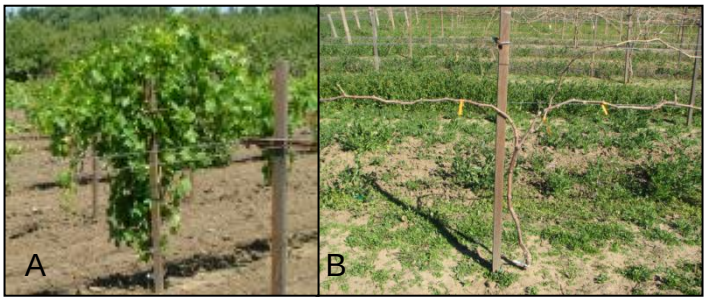

Figure 3. Examples of vines in the field in July, 2011 (A) and March, 2012 (B). Vines have been trained to the central post and trellising wires. *X. fastidiosa* mechanical inoculation sites are marked with orange tags.

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 as described in previous reports. Graft translocation is not seen in control transgenic grapes expressing a cellular-localized protein.

		Own-Rooted Plants (#)		Grafted plants (#)			
Cultivar	Grafting Strategy	Inoculated	Non-Inoculated	Mist Beds	EZ-Clone	Potted in Greenhouse	Originally Planned
Chardonnay		8	4	39	0	3 (1)	13
		-	-	14	1	27 (1)	13
		9	4	41	0	1	13
Thompson Seedless		8	4	45	0	6	13
		-	-	3	0	19 (2)	13
		9	5	38	0	1	13
Subtotals		34	17	180	1	57	
Aggregate Totals		51		238			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 thus far 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 have worked with PIPRA to explore possible exceptions to this policy for future seasons and therefore in February Mark Szczerba filed a request for a variance to retain flowers and evaluate fruit.

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. Mechanical stem inoculations were chosen so that an even introduction of the bacteria in this site with no natural PD pressure would be achieved. 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 simultaneously with the other field site collaborators. The bacterial suspension was provided by D. Gilchrist. No evidence of PD infections was observed for the remainder of the growing season. The trial will be repeated in the summer of 2012.

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 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 prject 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 plants to complete the field site for this project in Solano County have been rooted and their genotypes are being confirmed before they are transplanted by late March, 2012, to the field.. Grafted vines are being generated to be planted later this season in Riverside. Mechanical inoculations with *X. fastidiosa* bacteria were done in Summer 2011 in Solano County but no evidence of infections was observed.

K. Status of funds:

The current balance in the account as of 5 March 2012 is approximately $30,000. During this reporting period, approximately $9100 was spent on salaries plus $1600 in required benefits. Approximately $4600 was charged for supplies, primarily for the PCR genotyping. $218 was charged to the project for the PI to register to attend the Annual PD-GWSS meeting in Sacramento.

L. 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.