FIELD EVALUATION OF GRAFTED GRAPE LINES EXPRESSING POLYGALACTURONASE-INHIBITING PROTEINS

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ABSTRACT

The CDFA Pierce's Disease (PD) and Glassy-winged Sharpshooter Board's Research Scientific Advisory Panel review in 2007 and subsequent RFPs have given top priority to delivery of PD control candidates, including polygalacturonase-inhibiting proteins (PGIPs), from grafted rootstocks. Previously transformed 'Thompson Seedless' and 'Chardonnay' grapevines expressing a PGIP from pear fruit show reduced PD incidence when inoculated with *Xylella fastidiosa (Xf).* These grapevines were propagated vegetatively for PD assessment in field trial locations in Solano and Riverside Counties. Fifty-one transgenic and null-transformed control, own-rooted, grapevines were planted in Solano County on 7/6/2010. Grafted plants, utilizing the pPGIP-expressing vines as rootstocks, are being generated to be planted in 2011. PD resistance and agronomic viability will be assessed in future seasons.

LAYPERSON SUMMARY

Xylella fastidiosa (Xf) utilizes a key enzyme, polygalacturonase (PG), to spread from one xylem vessel to the next, eventually leading to the development of Pierce's disease (PD) symptoms in infected vines. Plant proteins called PGIPs selectively inhibit PGs from bacteria, fungi, and insets. Our collective work has identified a PGIP from pear fruits as partially inhibiting PD symptom development in grapevines expressing the pear PGIP. These vines are being analyzed in two field trials to measure their resistance to both mechanical *Xf* inoculations and to natural PD pressure in Solano and Riverside Counties, respectively. PGIPs have been shown to be graft transmissible so the transgenic grapevines will also be used as rootstocks to measure how much resistance will be provided to scion tissues taken from grapevines without the transgenic PGIP.

INTRODUCTION

Pierce's disease (PD) symptom development has been extensively linked to the spread of the causal bacterium, *Xylella fastidiosa* (*Xf*), throughout the xylem network of infected grapevines. Previous research has highlighted a plant cell wall modifying enzyme of *Xf*, polygalacturonase (*Xf*PG), as a PD virulence factor and therefore, a target of potential PD resistance strategies (Roper et al., 2007). *Xf* uses cell wall modifying enzymes, such as *Xf*PG, to degrade the pectin-rich pit membranes separating adjoining xylem vessels, permitting the spread of the bacterium throughout the xylem tissue. Plant PG-inhibiting proteins (PGIPs) are selective inhibitors of bacterial, fungal, and insect PGs and have provided protection from pests producing such PGs when upregulated in transgenic crop plants. Agüero et al. (2005) generated 'Chardonnay' and 'Thompson Seedless' grapevines expressing a PGIP (pPGIP) from pear fruit. These plants had reduced PD susceptibility and it was shown that the pPGIP was active across a graft junction when the transgenic lines were used as rootstocks grafted to wild-type scions.

The grapevines transformed with the pPGIP protein are part of a funded project to optimize the activity, expression, and export of PGIP proteins from transgenic rootstocks to provide PD protection in wild-type scions: "Optimizing grape rootstock production and export of inhibitors of *Xf* PG activity" (PI Labavitch). These plants have previously only been observed in greenhouse settings. The goal of this project is to verify that the transgenic grapevines expressing pPGIP (1) have increased resistance to PD and (2) maintain the appropriate agronomic traits necessary for commercial release. This will be examined in own-rooted plants and in grafted plants where the pPGIP grapevine is the rootstock.

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 by G. Paulino 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 *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) have been maintained in the greenhouse. Vegetative cuttings of non-lignified stem sections from transgenic and null-transformed plants of both cultivars were rooted in an aeroponic cloning manifold (EZ-Clone Inc., Sacramento, CA). Stem cuttings, three nodes in length, were basally dipped in 1000 ppm IBA solution for five minutes and transferred to individual sites in the misting chamber. 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. Grafting was attempted with both green and semi-lignified stem segments for all possible graft combinations. A modified wedge grafting technique was used whereby scion sections of 1 to 2 nodes were stripped of foliage and cut into wedge. These sections were fit into notched rootstock stems regions of equal maturity. 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 green grafting techniques, such as saddle grafting and chip budding, were attempted with the rooted cuttings. So far, no technique has proven effective enough to generate the required number of grafted plants for either field trial location. More cuttings are being vegetatively propagated for grafting during the winter.

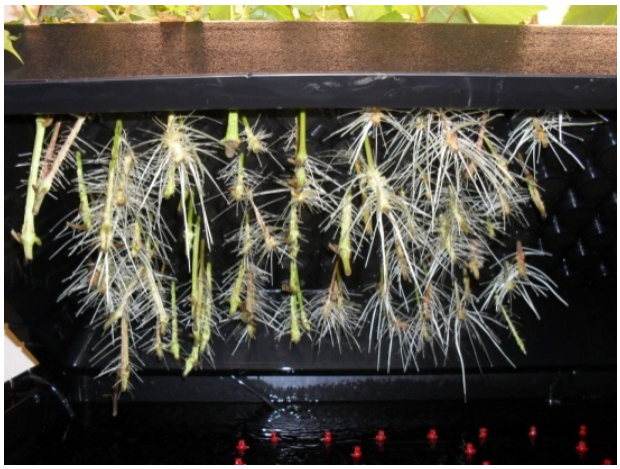

Figure 1. Grapevine cuttings rooting in the EZ-Clone aeroponic manifold.

Objective 2: Establish field trial sites

Two field trial sites were chosen to assess the PD resistance and general agronomic viability of own-rooted and grafted pPGIP expressing grapevines. The primary site in Solano County, CA has no natural PD pressure while the secondary site in Riverside County, CA has high natural PD pressure. The two locations will provide natural variation in climate and PD pressure so that trait assessments will be relevant to much of California's viticultural land.

Rooted cuttings of transgenic 'Chardonnay' and 'Thompson Seedless' grapevines were genotyped by PCR analysis for the presence of the pPGIP transgene. PCR primers were used to amplify the pPGIP gene from 0.5 mm leaf punches taken from young leaves near the growing tip (**Figure 2**). For this purpose, the Phire Plant Direct PCR Kit (Finnzymes Oy.) was used without the need for manual DNA extraction prior to PCR analysis. Grapevines with the proper gene expression were moved to lathe house facilities five weeks before field planting for hardening off. During this time, the vines were trained to one major shoot and pruned biweekly to encourage vigorous growth.

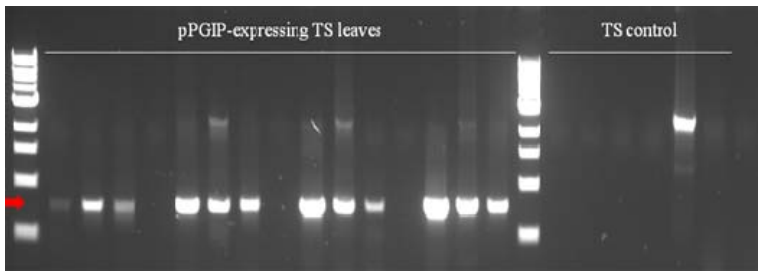

Figure 2. Representative DNA analysis. The pPGIP coding sequence was amplified from 12 cuttings taken from transgenic 'Thompson Seedless' (TS) grapevines. This band was absent in null-transformed control plants.

The field sites are shared by projects testing other transgenic PD control grapevines from PIs: D. Gilchrist, A. Dandekar, and S. Lindow. The 1.66 acre field in Solano County, CA was planted July 6, 2010. At this time, all own-rooted vines satisfying PCR analysis were hand-planted in a randomized block design with blocks consisting of two or three individuals in the same treatment (**Table 1**). The grapevines were planted approximately 8 ft. apart and tied to wooden stakes. After several weeks, trellising wires were added at 40 in. and 52 in. The vines grew vigorously throughout the summer, with most reaching at least the lower trellising wire (**Figure 3**). Flowers and fruit were not observed during year one. PD incidence recordings will begin in year two after mechanical inoculations with *Xf*. Newly generated grafted vines will be moved into the Solano County field early in year two.

Table 1. Total number of grapevines planted in Solano County. Dashed shapes represent pPGIP expressing grapevine rootstocks and/or scions; solid shapes are null-transformant controls (no pPGIP). Vines will be mechanically inoculated with *Xf* during year 2.

		Own-Rooted Plants (#)		Grafted Plants[a](#)	
Cultivar	Grafting Strategy	To Be Inoculated (year 2)	Non-Inoculated	To Be Inoculated (year 2)	Non-Inoculated
Chardonnay		8	4	9	4
		-	-	9	4
		9	4	9	4
Thompson Seedless		8	4	9	4
		-	-	9	4
		9	5	9	4

[a]Grafted plants are currently being generated and will be planted in year 2

Figure 3. The Solano County field location 15 weeks after planting. **A**, Panoramic view of the field, including PD control grapevines from other researchers. **B-C**, pPGIP-expressing Thompson Seedless and Chardonnay grapevines showing vigorous vegetative growth.

CONCLUSIONS

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, CA. Planned grafted vines are being generated to be planted next year. Mechanical *Xf* inoculations in Solano County and field plantings in Riverside County will take place next year.

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.

FUNDING AGENCIES

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