1. Project Title:

CHIMERIC ANTIMICROBIAL PROTEIN AND POLYGALACTURONASE-INHIBITING PROTEIN TRANSGENIC GRAPEVINES FIELD EVALUATION

II. Principal investigators and cooperators

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Reporting Period: The results reported here are from work conducted March 2010 to February 2011.

III. List of objectives and description of activities conducted to accomplish each objective

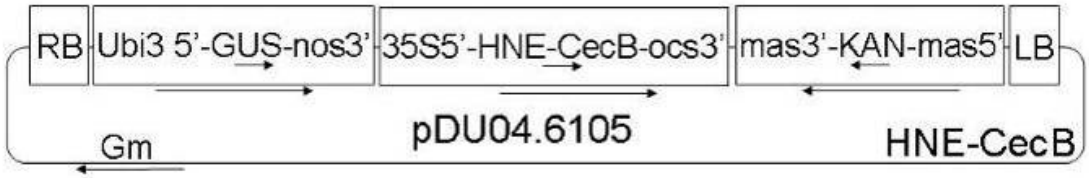
OBJECTIVES

The goals of this project are to field test four chimeric antimicrobial protein (CAP) and four polygalacturonase-inhibiting protein (PGIP) expressing transgenic Thompson Seedless (TS) grapevine lines to evaluate their horticultural characteristics and their resistance to Pierce's Disease (PD). Transgenic grapevines are being evaluated at two field locations as non-grafted plants and as transgenic rootstocks grafted with wild type scions. One field location has PD pressure and plants will be naturally infected with *Xylella fastidiosa* (*Xf*). In the location with no PD pressure, grapevines will be mechanically inoculated with *Xf*.

Objective 1. Validate the efficacy of *in planta* expressed CAP and PGIP, containing different signal peptides to inhibit and clear *Xf* infection in xylem tissue and their expression through the graft union in grapevines under field conditions.

We are field testing four independent transgenic lines (40-41, 40-89, 40-92, and 41-151) resulting from transforming grapevine with the vector pDU04.6105 expressing the CAP protein (**Figure 1**).

Fig. 1: Vector construct used for expressing disease resistance in grapevine plants



We have also planted vines representing four different constructs of PGIP (**Figure 2**). The four different modifications allow us to better understand how to control/restrict disease spread and virulence of *Xf*. Two versions have different signal peptide sequences to identify which most efficiently localizes PGIP to xylem tissues and which provides the best distribution through the graft union into untransformed scion tissues. In vector pDU05.1910 (event 52-08), the pear PGIP signal peptide was replaced with a signal peptide from a grapevine xylem-secreted protein that is similar to the PRp27-like protein from *Nicotiana tobacum*. In vector pDU06.0201 (event 45-77), the pear PGIP protein was linked to a signal peptide from the Ch1b chitinase protein found in the xylem of grapevine (*Vitis vinifera*). The remaining two vectors, serve as controls where pDU94.0928 (event TS50) contains the native pear PGIP signal peptide and pDU05.1002 (event 31-25) eliminates the endogenous signal peptide so the expressed PGIP should not be secreted and should not be able to limit the spread of *Xf*. These lines showed resistance to PD under greenhouse conditions with mechanical *Xf* stem inoculations.

Fig. 2: Vector constructs used for expressing disease resistance in grapevine plants

Activity 1: **Propagation, field planting, and grafting of CAP and PGIP transgenic grapevines.**

Four selected transgenic grapevine lines expressing CAP and the four expressing different PGIP constructs were propagated from cuttings in the greenhouse to obtain 48 clones of each line. After the root system developed, the cuttings were transferred to 5.5-inch pots to develop plants. Twenty-four clones were grafted with wild type TS scions **(Figure 3**). Once the growth phase in the greenhouse was completed, they were transferred to the lath house to acclimatize and then planted in two experimental fields. Two hundred and ten transgenic and wild type (controls), own rooted or grafted with wild type TS scions, were planted in Riverside County on 5/8/10 and the remaining 10 were planted on 3/6/11 completing the planting at this location **(Figure 4, Table 1)**. We also planted 112 transgenic and wild type controls on their own roots in Solano County on 8/2/2010 (**Figure 4, Table 2**) Additional 56 CAP, 56 PGIP and 12 controls grafted plants for Solano County field are being generated for completing this planting later this year (2011).

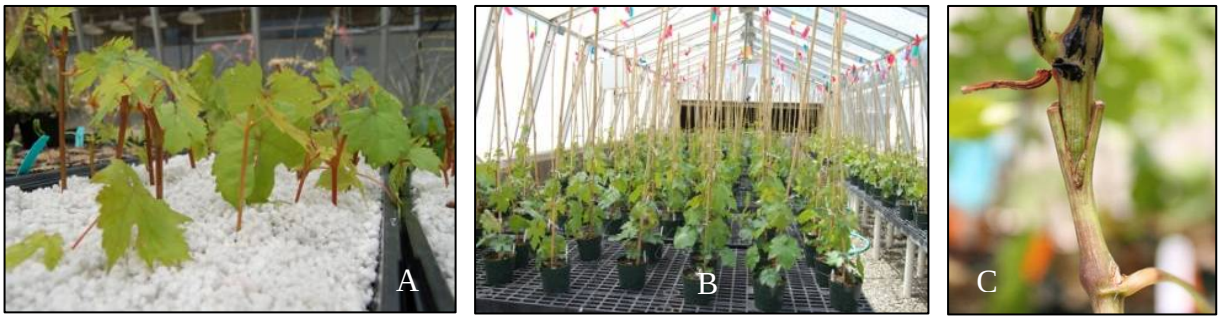


Figure 3. A) Propagated plants from vegetative tissue; B) Rooted plants and C) Grafted plant

Table 1. Riverside Field Evaluation planted on May 18, 2010 and March 6th 2011			
Non-grafted		**Grafted**	
Event ID	**# Planted**	**Event ID**	**# Planted**
CAP lines			
40-41	12	40-41G	12
40-89	12	40-89G	12
40-92	12	40-92G	12
41-151	12	41-151G	12
PGIP Lines			
31-25	12	31-25G	12
45-77	12	45-77G	12
52-08	12	52-08G	12
TS50	12	TS50G	12
Control lines			
TS	16	TG-G	12

Table 2. Solano County Field Evaluation planted on July 6th 2010			
Non-grafted		**Grafted**	
Event ID	**# Planted**	**Event ID**	**# Planted**
CAP lines			
40-41	12	40-41G	0
40-89	12	40-89G	0
40-92	12	40-92G	0
41-151	12	41-151G	0
PGIP Lines			
31-25	12	31-25G	0
45-77	12	45-77G	0
52-08	12	52-08G	0
TS50	12	TS50G	0
Control lines			
TS	16	TG-G	0

Figure 4. Riverside (left) and Solano County (right) transgenic grapevine palntings.

Activity 2. Evaluate preservation of varietal characteristics in transgenic grapevines grown on their own roots or as rootstocks.

CAP and PGIP-expressing lines will be evaluated in the Riverside and Solano County vineyards in 2011. Grafted and non-grafted transgenic grapevine lines will be evaluated phenotypically for shoot growth and leaf shape to see if they are normal and have maintained the horticultural and varietal characteristics of the parental genotype, TS. This examination will use the variables proposed by the International Organization of Vine and Wine (OIV, 1983).

Activity 3. Evaluate PD resistance of CAP and PGIP grapevines after inoculation with *Xf*.

Fifteen pooled leaf tissue samples from grafted and non-grafted transgenic and control grapevines planted in Riverside County were evaluated using Quantitative Real-Time PCR (qRT-PCR) for infection with *Xf* in season 1; the result showed no *Xf* infection. Grafted and non-grafted grapevines planted in Solano County will be mechanically inoculated with *Xf* (Almeida and Purcell, 2003) in 2011. PD symptoms will be scored on each infected plant using a standardized score based on percentage of leaf area scorching, a characteristic of PD (Krivanek et al., 2005a, 2005b). Xylem sap will be collected from grafted and non-grafted grapevines to evaluate the expression of neutrophil elastase (*ne*). PGIP movement in xylem from transformed rootstocks into wild type scions will be monitored using a radial diffusion assay that evaluates PGIP activity.

IV. Summary of major research accomplishments and results for each objective

We have successfully initiated two field trials to validate two greenhouse-tested strategies to control the movement and clearance of *Xylella fastidiosa* (*Xf*), a xylem-limited, Gram-negative bacterium that is the causative agent of Pierce's Disease (PD). A key virulence feature of *Xf* resides in its ability to digest pectin-rich pit pore membranes inside the host plant's xylem elements, permitting long distance movement and potentially enhancing vector transmission. The first strategy being evaluated tests the ability of a xylem-targeted polygalacturonase-inhibiting protein (PGIP) from pear as an effector protein to counter virulence associated with Xf PG activity. Our second strategy enhances clearance of bacteria from *Xf*-infected xylem tissues via the expression of a chimeric antimicrobial protein, CAP. The expectation is that expressing these proteins will prevent *Xf* movement and reduce its inoculum, curbing the spread of PD in California vineyards. Transgenic grapevine plants expressing either PGIP or the CAP proteins along with controls have been successfully planted in two locations. In Riverside County where the planting is now complete with100% (220 plants) of the plants are in the ground, 210 were planted on 05/08/2010 with the remaining 10 planted on 03/06/2011. In the Solano County location we have planted 112 plants on 08/02/2010 which corresponds to about 50% of the proposed planting now in the ground. These transgenic grapevines will be evaluated as plants on their own roots and as rootstocks grafted with wild type Thompson Seedless (TS) scions. At the Riverside County site, the plants will be naturally infected by wild GWSS presumably infected with wild strains of Xf while at the Solano County site, plants will be mechanically infected with *Xf* type strain (Temecula 1) to validate resistance to PD under field conditions.

V. Publications or reports resulting from the project

Dandekar, A.M., A.M. Ibáñez, H. Gouran, S.L. Uratsu, D. Gilchrist and T. Miller. 2010. Chimeric antimicrobial protein and polygalacturonase-inghibiting protein transgenic grapevines field evaluation. Proceedings of the Pierce's Disease Research Symposium. Dec 15-17. San Diego, CA. pp. 161-164.

V1. Presentations on research

Dandekar, A.M., A.M. Ibáñez, H. Gouran, S.L. Uratsu, D. Gilchrist and T. Miller. 2010. Chimeric antimicrobial protein and polygalacturonase-inghibiting protein transgenic grapevines field evaluation. Proceedings of the Pierce's Disease Research Symposium. Dec 15-17. San Diego, CA. pp. 161-164.

VII. Research relevance statement

The objective described in this report directly address the number 1 RSAP priority outlined in the "Top 5 to 10 Project Objectives to Accelerate Research to Practice" handout released at the December 2009 Pierce's Disease Research Symposium: "Accelerate regulatory process: Establish and facilitate field trials of current PD control candidate vines / endophytes / compounds in multiple locations". This document updates the priority research recommendations provided in the report "PD/GWSS Research Scientific Review: Final Report" released in August 2007 by the CDFA's Pierce's Disease Research Scientific Advisory Panel.

VIII Lay summary of current year's results

Transgenic grapevines are being evaluated as rootstocks to demonstrate the field efficacy of two strategies to control Pierce's Disease in California grapevines. The first strategy uses transgenic rootstocks to control the movement of the bacterium *Xylella fastidiosa (Xf)* in the water-conducting xylem of the vine through the expression of polygalacturonase-inhibiting protein (PGIP). The second strategy tests whether transgenic rootstocks can clear *Xf* infections in xylem tissues through the expression of a chimeric antimicrobial protein (CAP). Two vineyards have been planted with these transgenic vines to validate the in-field effectiveness of this strategy

IX. Status of funds

First years funding has been spent (100%).

X. Summary and status of intellectual property producing during this research project

No intellectual property was generated during this time period.

XI. References cited

Almeida, R.P.P. and A.H. Purcell. 2003. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. App. Env. Microbiol. 68:7447-7452.

International Organization of Vine and Wine (OIV). 1983. Code of descriptive characteristics of *Vitis* varieties and species. Ed. Dedon, Paris.

Krivanek, A.F., J.F Stevenson and M.A. Walker. 2005a. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's Disease. Phytopathology 95:36-43.

Krivanek, A.F., J.F Stevenson and M.A. Walker. 2005b. *Vitis* resistance to Pierce's Disease is characterized by differential *Xylella fastidiosa* population in stems and leaves. Phytopathology 95:44-52.