"Final Report for CDFA Agreement Number 09-0729"

Project Title: HNE-CecB and PGIP transgenic grapevines field trial.

Principal investigators (PI):

Abhaya M. Dandekar, Department of Plant Sciences, University of California, Davis, CA 95616
Phone 530-752-7784; fax: 530-752-0382; Email: amdandekar@ucdavis.edu

Cooperators
Ana M. Ibáñez. Department of Plant Sciences, University of California, Davis, CA 95616
Phone 530-752-5325; fax: 530-752-0382; Email: amibanez@ucdavis.edu

David Dolan, Department of Plant Sciences, University of California, Davis, CA 95616
Phone 530-752-5325; fax: 530-752-0382; Email: dcdolan@ucdavis.edu

Hossein Gouran, Department of Plant Sciences, University of California, Davis, CA 95616
Phone 530-752-5325; fax: 530-752-0382; Email: hgouran@ucdavis.edu

Field Coordinators:
David Gilchrist. Department of Plant Pathology, University of California, Davis, CA 95616
Phone : 530-752-6614 ; Email : dggilchrist@ucdavis.edu

Thomas Miller, Department of Entomology, University of California, Riverside, CA 92521
Phone: 951-827-2278; Email : thomas.miller@ucr.edu

Time period covered by the report: March 2010 to February 2013

Introduction.
Xylella fastidiosa (Xf), a xylem-limited, Gram-negative bacterium, is the causative agent of Pierce's disease (PD). A key feature of *Xf* virulence is its ability to digest pectin-rich pit pore membranes that connect individual xylem elements (Roper et al., 2007), enhancing long distance movement and vector transmission. In this project, we are examining the ability of xylem-targeted polygalacturonase inhibiting protein (PGIP, Aguero et al., 2005, 2006) and a chimeric antimicrobial protein (HNE-CecB, Kunkel et al., 2007) to restrict bacterial movement and clear *Xf* under field conditions (Dandekar et al., 2009, 2012). The expectation is that expression of these proteins will prevent *Xf* movement and reduce its inoculum, decreasing spread of PD.

We are field-testing four independent transgenic lines (40-41, 40-89, 40-92, and 41-151) resulting from transforming grapevine plants with the vector pDU04.6105 expressing the chimeric antimicrobial protein (**Figure 1**). In each location, 24 plants of each line are being field-tested: 12 replicates as non-grafted plants and 12 as transgenic rootstocks grafted with untransformed Thompson Seedless scions.

We have also planted vines carrying four different constructs of PGIP (**Figure 2**). The four different modifications allow us to better understand how to control/restrict *Xf* spread and thus disease incidence. 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, with and without the endogenous signal peptide, will serve as controls. The construct pDU94.0928 (event TS50), which uses the pear PGIP's own endogenous peptide, serves as a control to evaluate the efficiency

of exogenous signal peptides in targeting PGIP to the xylem tissue. Vector pDU05.1002 (event 31-25) eliminates the endogenous signal peptide; the expressed PGIP cannot be secreted and should not limit *Xf* spread.

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

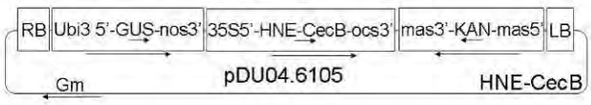
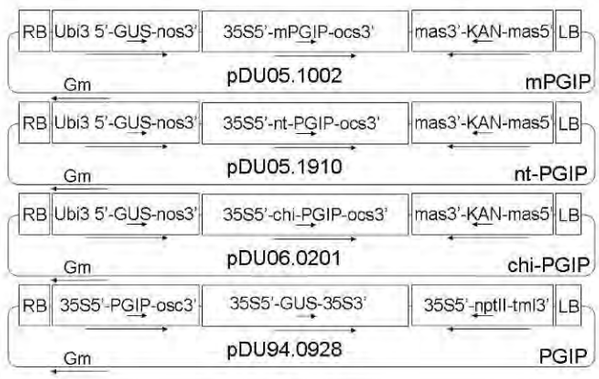


Fig. 2: Vector constructs used for expressing disease resistance in grapevine plants



List of objectives.

The goals of this project are to field-test four HNE-CecB- and four PGIP-expressing transgenic TS grapevine lines to evaluate their horticultural characteristics and resistance to Pierce's Disease (PD). Transgenic grapevines are being evaluated at two field locations as own-rooted plants and as transgenic rootstocks grafted with untransformed TS scions. One field location has endemic PD pressure and plants have been naturally infected with *Xf*. In the location with no PD pressure, grapevines have been mechanically inoculated with *Xf*.

Objective 1. Validate the efficacy of *in planta*-expressed chimeric HNE-CecB and PGIP with different signal peptides to inhibit and clear *Xf* infection in xylem tissue and to pass through the graft union under field conditions.

Activity 1. Propagation, field planting, and grafting of HNE-CecB and PGIP transgenic grapevines.

Activity 2. Evaluate preservation of varietal characteristics in transgenic grapevines grown as whole plants or used as rootstocks.

Activity 3. Evaluate PD resistance of HNE-CecB and PGIP transgenic grapevines after inoculation with *Xf*.

Description of activities conducted to accomplish each objective

Activity 1. Propagation, field planting, and grafting of HNE-CecB and PGIP transgenic grapevines.

Four selected transgenic grapevine lines expressing HNE-CecB and four expressing different PGIP constructs were propagated from cuttings in the greenhouse to obtain 48 clones of each line. After the root system developed, cuttings were transferred to 5.5-inch pots to develop into plants. Twenty-four clones were grafted with untransformed TS scions. Well-established plants were transferred to the lath house to acclimatize and then planted in two experimental fields. Two hundred and ten transgenic or untransformed vines, own-rooted or grafted with untransformed TS scions, were planted in Riverside County on 5/18/10 and the remaining 10 were planted on 3/6/11, completing the planting at this location **(Fig 3, Table 1)**. We also planted 110 transgenic and untransformed vines on their own roots on 8/2/2010 and 110 vines grafted with untransformed TS scions on 6/27/11 in Solano County, completing the planting at this location (**Fig 3, Table 1**).

Figure 3. Riverside (left) and Solano County (right) transgenic grapevine plantings (Summer 2012).

Table 1. Transgenic and control grapevines planted at Riverside and Solano fields

Non-grafted		Grafted	
Event ID	**# Planted**	**Event ID**	**# Planted**
HNE-CecB 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	TS-G	12

HNE-CecB- and PGIP-expressing transgenic and untransformed grapevine lines in Solano County were randomly sampled and tested for the transgenes by PCR (**Table 2**). DNA was isolated from young leaves collected from the field using the Qiagen DNeasy Plant Mini kit according to manufacturer's instructions. DNA was PCR amplified using ActinF (TACAATGAGCTTCGGGTTGC) and ActinR (GCTCTTTGCAGTTTCCAGCT) to determine DNA quality. Elastase primers were HNE5' (GCAGTTCAGAGGATCTTCGAGGATGG) and HNE3' (TTACTAGAGTGCTTTTGCTTCTCCCAG). Primers for PGIP determination were CaMV 35S-2 (GACGTAAGGGATGACGCACAAT) and MPGIP-4 (CGGATCCTTACTTGCAGCTTGGGAGTGGAGC ACCG).

Table 2. PCR genotyping of Solano County transgenic grapevine lines

Event ID	Inserted Gene	ActinF/R	HNE3/5	CaMV35S/mPGIP4
HNE-CecB lines				
40-41	HNE	Positive	Positive	Negative
40-89	HNE	Positive	Positive	Negative
40-92	HNE	Positive	Positive	Negative
41-151	HNE	Positive	Positive	Negative

PGIP Lines				
31-25	PGIP	Positive	Negative	Positive
45-77	PGIP	Positive	Negative	Positive
52-08	PGIP	Positive	Negative	Positive
TS50	PGIP	Positive	Negative	Positive
Control				
TS	None	Positive	Negative	Negative

Activity 2. Evaluate preservation of varietal characteristics in transgenic grapevines grown as whole plants or used as rootstocks.

To verify that horticultural and varietal characteristics of the parental genotype TS were unchanged, HNE-CecB- and PGIP-expressing transgenic grapevine lines in Solano and Riverside Counties were evaluated phenotypically in September 2011 and November 2011, respectively. This examination was accomplished using the first 12 descriptors from the "Primary descriptor priority list" proposed by the International Organization of Vine and Wine (OIV, 1983). The descriptors used were 1) aperture of young shoot tip/opening of young shoot tip, 2) density of prostrate hairs between main veins on 4th leaf lower side of blade, 3) number of consecutive shoot tendrils, 4) color of upper side of blade on 4th young leaf (**Fig. 4**), 5) shape of mature leaf blades, 6) number of lobes on mature leaf (**Fig. 4**), 7) area of anthocyanin coloration on main veins on upper side of mature leaf blades, 8) shape of teeth on mature leaves, 9) degree of opening of mature leaves/overlapping of petiole sinuses, 10) mature leaf petiole sinus bases limited by veins, 11) density of prostrate hairs between main veins on lower side of mature leaf blades, and 12) density of erect hairs on main veins on lower sides of mature leaf blades. Riverside and Solano Counties, HNE-CecB- and PGIP-expressing transgenic grapevines lines were also phenotypically evaluated in the fall of 2012.

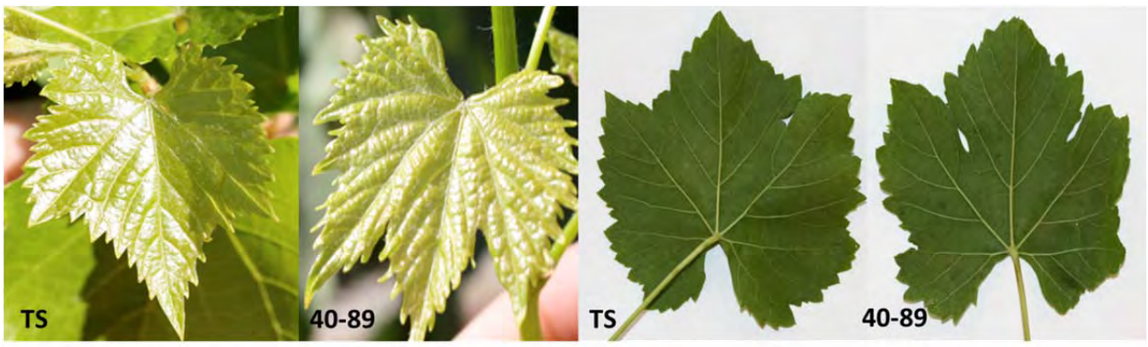


Figure 4. Color of upper side of blade on 4th young leaf (left) and number of lobes of mature leaf of TS and 40-89 transgenic line (right).

Activity 3. Evaluate PD resistance of HNE-CecB and PGIP transgenic grapevines after inoculation with *Xf*.

Two hundred and twenty petiole samples from grafted and ungrafted transgenic and control grapevines planted in Riverside County were evaluated for *Xf* using a commercial ELISA kit (Agdia, Elkhart, IN) in fall 2011. The ELISA assay is based on a mixture of *Xf* antibodies against eight grape *Xf* isolates. Sample extracts were also plated on PD3 medium and *Xf* growth was verified by PCR using EFTu and 16s primers. The ELISA cell count (**Fig. 5**), plate cell count (**Fig. 6**) and PCR assay (**Fig. 7**) results confirmed *Xf* infection in Riverside County.

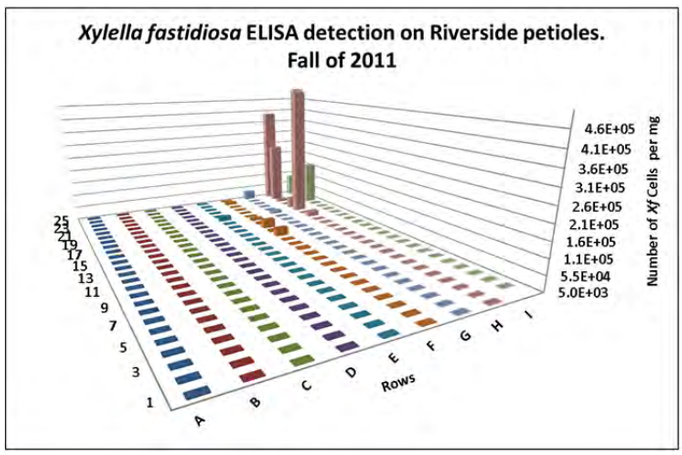


Figure 5. ELISA *Xylella fastidiosa* detection in 2011 Riverside County's petiole samples.

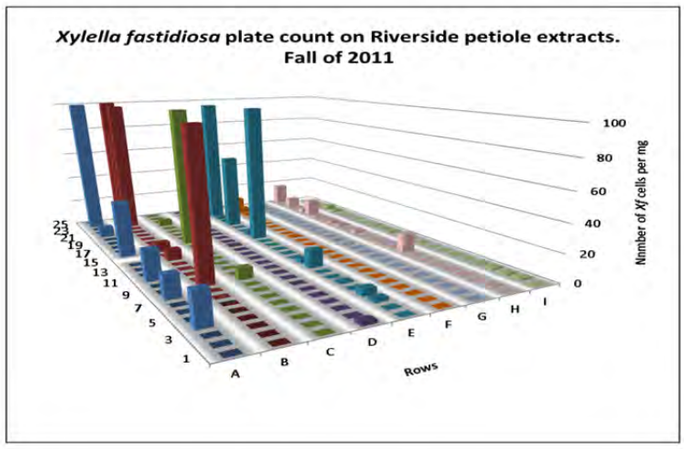


Figure 6. Plating *Xylella fastidiosa* cell counts from 2011 Riverside County's petiole samples

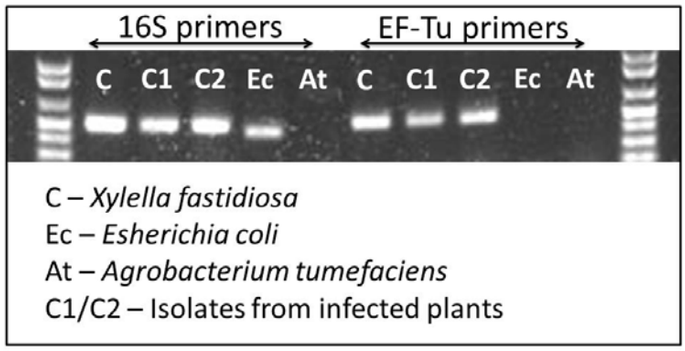


Figure 7. *Xylella fastidiosa* detection in Riverside County samples using PCR

PD symptoms in each single Riverside County HNE-CecB- and PGIP-expressing grapevine were scored using a standardized score based on percentage of leaf area scorching (**Fig. 8**), a characteristic of PD (Krivanek et al., 2005a, 2005b). The following scoring system was used: 0 = no infection, 1 = potential infection, 2 = definitive infection (1-5 leaves infected), 3 = 5-10 leaves infected, 4 = more than 10 leaves infected, 5 = systematic infection on 1 runner, 6 = systematic infection in more than one runner, 7=

systematic infection in all runners, 8 = completely systematic with less than 50% leaf loss, 9 = completely systematic with more than 50% leaf loss and 10 = dead plant. Riverside field average score in the fall of 2011 was 3.25 = 5-10 leaves infected.

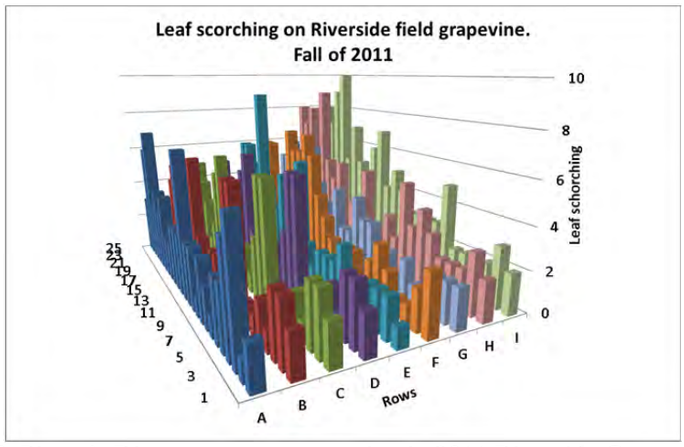


Figure 8. Pierce's Disease symptoms scoring in 2011 Riverside County's plants

One hundred and nineteen xylem sap samples from grafted and non-grafted transgenic and control grapevines planted in Riverside County were evaluated using ELISA for *Xf* detection in the spring of 2012. *Xf* was found in every single xylem sap sample collected: the average *Xf* cell number was 3.2 X 10^4 per 50 µL. The results confirmed once again the presence of Pierce's Disease at the Riverside County site (**Fig. 9)**.

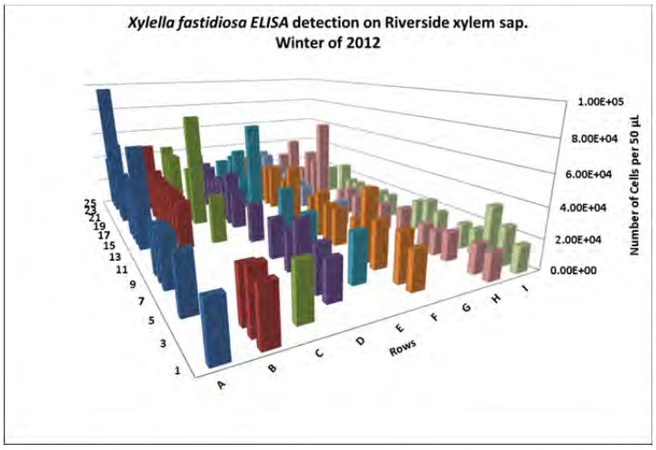


Figure 9. ELISA *Xylella fastidiosa* detection in Riverside County's 2012 xylem sap samples

In the fall of 2012, PD symptoms present in Riverside County HNE-CecB- and PGIP-expressing grapevine were scored using the standardized score described above based on percentage of leaf area scorching a characteristic of PD (**Table 3.**), Riverside field average leaf scorching in fall 2012 was 6 = systemic infection in more than one runner. Petiole samples from own-rooted and grafted transgenic and control grapevines planted in Riverside County were evaluated for *Xf* using ELISA. The ELISA data showed that *Xf* infection in Riverside County is higher in the fall 2012 than was in fall 2011 (**Fig. 10, Table 4**).

Table 3. Leaf Scorching detected on Riverside Field Transgenic and Control Grapevines. Fall of 2012						
Gene	**Own-rooted lines**	**Leaf scorching[a]**	**Leaf scorching[b]**	**Grafted Lines**	**Leaf scorching[a]**	**Leaf scorching[b]**
TS[c] Control	**TS**	**6 ± 1**	**6**	**TS**	**6 ± 1**	**6**
pPGIP	**TS-50**	**7 ± 1**	**6**	**TS-50-G**	**7 ± 1**	**6**
mPGIP	**31-25**	**7 ± 1**	**6**	**31-25-G**	**7 ± 1**	**5**
chiPGIP	**45-77**	**4 ± 1**	**4**	**45-77-G**	**4 ± 1**	**6**
ntPGIP	**52-08**	**5 ± 1**	**5**	**52-08-G**	**5 ± 1**	**5**
HNE-CecB	**40-41**	**6 ± 2**	**6**	**40-41-G**	**6 ± 2**	**5**
HNE-CecB	**40-89**	**8 ± 2**	**9**	**40-89-G**	**8 ± 2**	**7**
HNE-CecB	**40-92**	**6 ± 1**	**6**	**40-92-G**	**6 ± 1**	**5**
HNE-CecB	**41-151**	**6 ± 1**	**6**	**41-151-G**	**6 ± 1**	**6**

[a]Mean value from 12 biological replicates of each own-rooted or grafted transgenic lines and non-transgenic controls; [b]Median value from 12 biological replicates

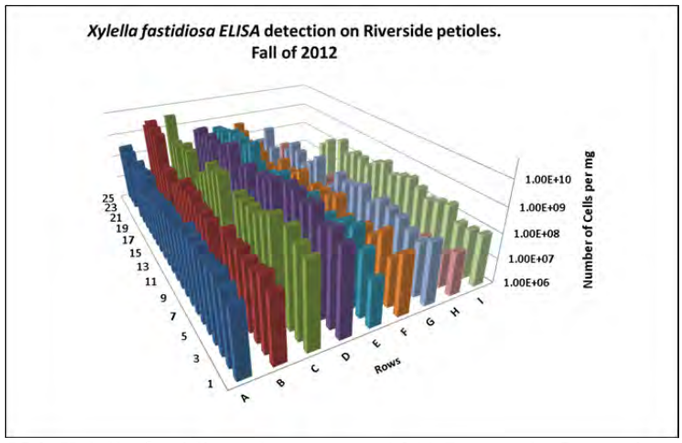


Figure 10. ELISA *Xylella fastidiosa* detection in 2012 Riverside County's petiole samples.

Table 4. *Xylella fastidiosa* ELISA detection on Riverside field petioles samples from transgenic and control grapevines. Fall of 2012						
Gene	**Own-rooted lines**	**Xf cells[a] (mg)**	**Xf cells[b] (mg)**	**Grafted Lines**	**Xf cells[a] (mg)**	**Xf cells[b] (mg)**
TS control	**TS**	**5.77E+08**	**2.76E+08**	**TS-G**	**3.08E+08**	**2.36E+08**
mPGIP	**31-25**	**1.29E+09**	**1.70E+08**	**31-25-G**	**2.08E+09**	**7.33E+08**
chiPGIP	**45-77**	**4.35E+08**	**4.63E+08**	**45-77-G**	**4.82E+08**	**2.32E+08**
ntPGIP	**52-08**	**7.85E+08**	**3.21E+08**	**52-08-G**	**8.88E+08**	**3.96E+08**
HNE-CecB	**40-41**	**7.59E+08**	**6.56E+08**	**40-41-G**	**1.71E+09**	**4.40E+08**
HNE-CecB	**40-89**	**2.15E+08**	**2.12E+08**	**40-89-G**	**1.75E+09**	**9.42E+08**
HNE-CecB	**40-92**	**4.76E+08**	**5.18E+08**	**40-92-G**	**2.13E+08**	**2.10E+08**
HNE-CecB	**41-151**	**3.74E+08**	**4.17E+08**	**41-151-G**	**8.56E+08**	**1.16E+09**
pearPGIP	**TS-50**	**5.15E+08**	**2.80E+08**	**TS-50-G**	**2.62E+08**	**2.76E+08**

[a]Mean value from 12 biological replicates; [b]Median value from 12 biological replicates.

At the Solano County site, petioles from transgenic and non-transgenic plants that were mechanically inoculated with *Xf* (Almeida and Purcell, 2003) in July 2011 and from TS and TS50 non-inoculated plants were evaluated for *Xf* infection using the ELISA in fall 2011. Solano County sample extracts were also plated on PD3 medium. *Xf* was detected in petiole extracts by ELISA (**Fig. 11, Table 5**), but no growth was observed when petiole extracts were plated.

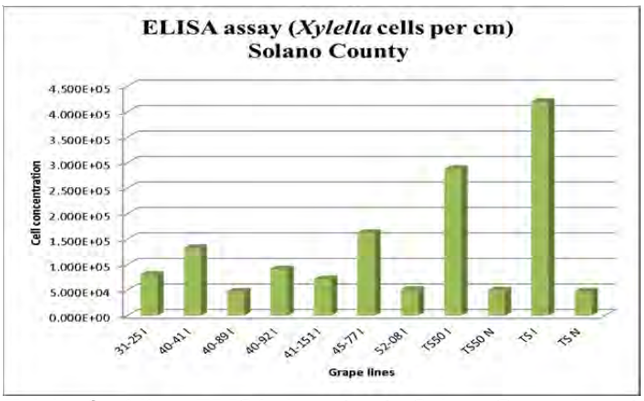


Figure 11. *Xylella fastidiosa* ELISA detection in Solano County's petiole samples

Table 5. ELISA cell count on Solano County petiole extracts		
Line	**Gene**	**Cell/cm**
31-25 inoculated	mPGIP	8.026E+04
40-41 inoculated	HNE	1.329E+05
40-89 inoculated	HNE	4.728E+04
40-92 inoculated	HNE	9.104E+04
41-155 inoculated	HNE	7.136E+04
45-77 inoculated	chiPGIP	1.625E+05
52-08 inoculated	ntPGIP	5.099E+04
TS50 inoculated	Control	2.877E+05
TS50 non-inoculated	Control	4.931E+04
TS inoculated	Wild type	4.199E+05
TS non-inoculated	Wild type	4.768E+04
TS non-inoculated + *Xf*	Positive control	3.675E+12

Solano County grafted plants were mechanically inoculated for the first time with *Xf* and own-rooted plants were re-inoculated on May 29, 2012. Two runners per plant were inoculated with an inoculum size of 2.5 x 10^5 cells/20 µL. In fall 2012 Solano County's petioles from own-rooted and grafted TS and TS50 non-inoculated controls, as well as *Xf* mechanically inoculated own-rooted and grafted transgenic and non-transgenic plants were evaluated for *Xf* infection using ELISA. *Xf* was detected in petiole extracts (**Fig. 12, Table 6**). Percentage of leaf area scorching a characteristic of PD was scored (**Table 7**) as described above.

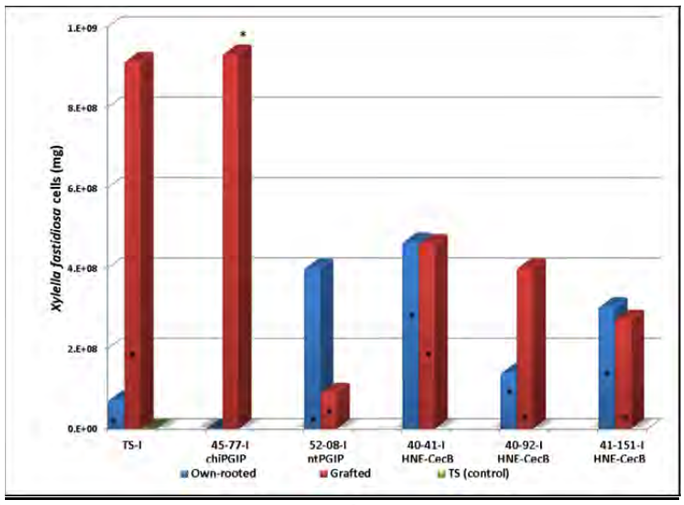


Figure 12. *Xylella fastidiosa* ELISA detection in fall 2012 Solano County's petiole samples. Bars represents the mean value from 3 biological replicates of each control, own-rooted or grafted inoculated transgenic line; * = Median value from 3 biological replicates.

Table 6. *Xylella fastidiosa* ELISA detection on petiole samples of Solano County field control and transgenic inoculated grapevines. Fall of 2012.

Gene	Own-rooted lines	*Xf* cells[a] (mg)	Xf cells[b] (mg)	Grafted Lines	Xf cells[a] (mg)	Xf cells[b] (mg)
TS[c] Control	TS	UD[d]	UD			
TS	TS-I	7.28E+07	6.18E+07	TS-G-I	9.15E+08	2.58E+08
pearPGIP	TS-50-I	ND[e]	ND	TS-50-G-I	ND	ND
mPGIP	31-25-I	ND	ND	31-25-G-I	ND	ND
chiPGIP	45-77-I	N/D	N/D	45-77-G-I	9.34E+08	1.33E+09
ntPGIP	52-08-I	4.01E+08	1.99E+07	52-08-G-I	9.17E+07	3.86E+07
HNE-CecB	40-41-I	4.66E+08	3.85E+08	40-41-G-I	4.62E+08	2.79E+08
HNE-CecB	40-92-I	1.40E+08	1.15E+08	40-92-G-I	4.02E+08	3.14E+07
HNE-CecB	41-151-I	3.05E+08	1.90E+08	41-151-G	2.74E+08	1.93E+07
HNE-CecB	40-89-I	ND	ND	40-89-G-I	ND	ND

[a]Mean ± STD from 3 biological replicates of each control, own-rooted or grafted inoculated transgenic line; [b]Median value from 3 biological replicates; [c]TS control non inoculated; [d]UD = undetectable; [e]ND = Not-done.

Table 7. Leaf Scorching observed on control and inoculated transgenic lines at Solano County field. Fall of 2012

Gene	Own-rooted lines	Leaf scorching[a]	Leaf scorching[b]	Grafted Lines	Leaf scorching[a]	Leaf scorching[b]
TS[c] Control	TS	UD	UD			
TS	TS-I	4 ± 1	4	TS-G-I	5 ± 2	6
pPGIP	TS-50-I	4 ± 1	5	TS-50-G-I	5 ± 2	5
mPGIP	31-25-I	3 ± 1	3	31-25-G-I	5 ± 3	6

chiPGIP	**45-77-I**	**5 ± 1**	**5**	**45-77-G-I**	**4 ± 2**	**4**
ntPGIP	**52-08-I**	**3 ± 1**	**3**	**52-08-G-I**	**4 ± 3**	**4**
HNE-CecB	**40-41-I**	**3 ± 1**	**4**	**40-41-G-I**	**4 ± 1**	**4**
HNE-CecB	**40-89-I**	**4 ± 1**	**3**	**40-89-G-I**	**6 ± 2**	**6**
HNE-CecB	**40-92-I**	**4 ± 2**	**4**	**40-92-G-I**	**6 ± 1**	**6**
HNE-CecB	**41-151-I**	**4 ± 1**	**5**	**41-151-G**	**6 ± 2**	**8**

[a]Mean ± STD from 6 biological replicates of each control, own-rooted or grafted inoculated transgenic line; [b]Median value from 6 biological replicates; [c]TS control non inoculated; [d]UD = undetectable.

Summary of accomplishments and results for each objective.

We have successfully established 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 that interconnect the host plant's xylem elements, enhancing long distance movement and vector transmission. The first strategy evaluated the ability of a xylem-targeted polygalacturonase-inhibiting protein (PGIP) from pear to counter virulence associated with *Xf* PG activity. Our second strategy enhances clearance of bacteria from *Xf*-infected xylem tissues using a chimeric antimicrobial protein, HNE-CecB. The expectation is that expressing these proteins will prevent *Xf* movement and reduce its inoculum size, curbing the spread of PD in California vineyards.

Transgenic grapevine plants expressing either PGIP or HNE-CecB along with untransformed controls have been successfully planted in two locations. In Riverside County, planting was completed with 220 vines in the ground: 210 planted on 05/18/2010 with the remaining 10 planted on 03/06/2011. In Solano County, where planting was also completed with all 220 vines in the ground, 110 were planted on 08/02/2010 and the remaining 110 on 6/27/2011. These transgenic grapevines have been evaluated as plants on their own roots and as rootstocks grafted with untransformed Thompson Seedless (TS) scions. HNE-CecB- and PGIP-expressing transgenic grapevine lines in Riverside and Solano County have been evaluated phenotypically using the first 12 descriptors from the "Primary descriptor priority list" proposed by the International Organization of Vine and Wine (OIV). No phenotypical/horticultural differences were observed between transgenic and untransformed TS vines. HNE-CecB- and PGIP-expressing transgenic grapevine lines in Solano County have also been genotyped, confirming the presence of the inserted transgene in all lines. At the Riverside County site, the plants have been naturally infected by wild populations of GWSS and *Xf* presence in petioles extracts was confirmed by ELISA, PCR, and plate cell count in fall 2011. *Xf* presence was also confirmed in Riverside xylem sap samples collected in spring 2012 and in petiole's extracts collected in fall 2012. PD symptoms were assessed using a standardized score based on percentage of leaf area scorching to validate resistance to PD under field conditions. At the Solano County site, non-grafted vines were mechanically inoculated with the *Xf* type strain (Temecula 1) in 2011 to validate resistance to PD under field conditions, *Xf* presence was confirmed by ELISA in fall 2011, but no *Xf* growth in plate or PD symptoms were detected. Solano County grafted plants were for the first time mechanically inoculated with *Xf* and non-grafted plants were re-inoculated on spring 2012. Leaf scorching the characteristic symptom of PD was observed in Solano Country for the first time in fall 2012 and *Xf* presence was confirmed by ELISA in petiole extracts collected in the same season.

For field trials, please include information on the status of the field trial, including planting and sampling activities, the condition of the plants, and any factors impacting the progress of the field trial. Also, please include photos of the field planting.

Riverside and Solano Fields grapevines were infected at the end of fall 2012, but we will take observations on how the grapevines come back in the spring 2013. Riverside and Solano Fields planting were completed on 03/06/2011 and 6/27/11, respectively. After each of the fields wereplanted completely no additional planting activities have been made. At Riverside field 220

petioles and leaves were sampled on 9/26/2011 and 10/17/2012, xylem sap was sampled on 4/2/2012. At Solano Field 220 petioles and leaves were sampled on 9,14/2011, 10/4-2011 and 10/22/2012, xylem sap was sampled on 4/30/2012.

Publications produced and pending, and presentations made that relate to the funded project.

Dandekar, A.M., H. Gouran, A.M. Ibáñez, S.L. Uratsu, C.B. Aguero, S. McFarland, Y. Borhani, P.A. Feldstein, G. Bruening, R. Nascimento, L.R. Goulart, P.E. Pardington, A. Chaudhary, M. Norvell, E. Civerelo and G. Gupta. 2012. An Engineered innate defense protects grapevines from Pierce's disease. Proc. Nat. Acad. Sci. USA 109 (10): 3721-3725.

Dandekar, A.M., A. M. Ibáñez, D. Dolan, H. Gouran, D. Gilchrist and T. Miller. 2012. Chimeric antimicrobial protein and polygalacturonase-inhibiting protein transgenic grapevines field trial. Pierce's Disease Research Program Report. Dec 2012. pp. 94-103.

Dandekar, A.M., A. M. Ibáñez, H. Gouran, S. Uratsu, D. Gilchrist and T. Miller. 2011. Chimeric antimicrobial protein and polygalacturonase-inhibiting protein transgenic grapevines field trial. Proceedings of the Pierce's Disease Research Symposium. Dec 2011. pp. 101-106.

Dandekar, A.M., A. M. Ibáñez, H. Gouran, S. Uratsu. D. Gilchrist and T. Miller. 2010. Chimeric antimicrobial protein and polygalacturonase-inhibiting protein transgenic grapevines field trial. Proceedings of the Pierce's Disease Research Symposium. Dec 2010. pp. 161-164.

Research relevance statement, indicating how this research will contribute towards finding solutions to Pierce's disease in California.

The objective described here directly addresses the first 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.

Layperson summary of project accomplishments.

Transgenic grapevine plants expressing either polygalacturonase-inhibiting protein (PGIP) or a chimeric antimicrobial protein (HNE-CecB) have been planted in two locations, one in Riverside County and the other in Solano County. These transgenic grapevines are being evaluated both as plants on their own roots and as rootstocks grafted with untransformed Thompson Seedless (TS) scions to demonstrate the field efficacy of two strategies to control Pierce's Disease (PD) 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 expression of PGIP. The second strategy tests whether transgenic rootstocks can clear *Xf* infections in xylem tissue by expressing HNE-CecB. PGIP- and HNE-CecB-expressing transgenic grapevine lines in Riverside and Solano Counties have been evaluated phenotypically; no visible differences were seen between transgenic and untransformed vines. At the Riverside County site, natural *Xf* infection has been confirmed in petioles and xylem sap by ELISA. PD symptoms were scored using a standardized score based on percentage of leaf area scorching to validate resistance to PD under field conditions. At the Solano County site, non-grafted plants were mechanically inoculated with *Xf* type strain Temecula 1 in 2011. The presence of *Xf* was confirmed in petiole extracts but not in xylem sap from mechanically inoculated grapevines using the ELISA assay. *Xf* growth was not observed when petiole extracts were plated and no PD symptoms were detected. HNE-CecB- and PGIP-expressing transgenic grapevine lines in Solano County have also been tested to confirm the presence of the transgene. At the Solano County site, grafted plants were mechanically inoculated with *Xf* and non-

grafted plants were re-inoculated in spring 2012. Leaf scorching the characteristic symptom of PD was observed in Solano Country for the first time in fall 2012 and *Xf* presence was confirmed by ELISA in petiole extracts collected in the same season.

Status of funds. The funds for this project have been spent (100%).

Summary and status of intellectual property associated with the project.

No additional intellectual property was generated during this project period

Literature cited

Aguero, C.B., S.L. Uratsu, C. Greve, A.L.T. Powell, J.M. Labavitch, and A.M. Dandekar. 2005. Evaluation of tolerance to Pierce's disease and *Botrytis* in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Mol. Plant Pat. 6:43-51.

Aguero, C.B., C.P. Meredith, and A.M. Dandekar. 2006. Genetic transformation of *Vitis vinifera* L. cvs. 'Thompson Seedless' and 'Chardonnay' with the pear PGIP and GFP encoding genes. Vitis 45:1-8.

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.

Dandekar, A.M., J.M.. Labavitch, R. Almeida, A.M. Ibáñez, S.L. Uratsu, H. Gouran and C. Aguero. 2009. In planta testing of signal peptides and anti-microbial proteins for rapid clearance of *Xylella*. Proceedings of the Pierces Disease Research Symposium. Dec 9-11. Sacramento CA. pp. 117-122.

Dandekar, A.M., H. Gouran, A.M. Ibáñez, S.L. Uratsu, C.B. Aguero, S. McFarland, Y. Borhani, P.A. Feldstein, G. Bruening, R. Nascimento, L.R. Goulart, P.E. Pardington, A. Chaudhary, M. Norvell, E. Civerelo and G. Gupta. 2012. An Engineered innate defense protects grapevines from Pierce's disease. Proc. Nat. Acad, Sci. USA 109 (10): 3721-3725.

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.

Kunkel, M., M. Vuyisich, G. Gnanakaran, G.E. Bruening, A.M. Dandekar, E. Civerolo, J.J. Marchalonis and Gupta, G. 2007. Rapid clearance of bacteria and their toxins: Development of therapeutic proteins. Critical Reviews in Immunology 27: 233-245.

Roper, M.C., L.C. Greve, J.G.Warren, J.M. Labavitch and B.C. Kirkpatrick. 2007. *Xylella fastidiosa* Requires Polygalacturonase for Colonization and Pathogenicity in *Vitis vinifera* Grapevines. MPMI 20: 411-419.