**CONTROL OF PIERCE'S DISEASE BY ALTERED EXPRESSION OF ANTI-APOPTOTIC GENES OR THEIR RNA-BASED REGULATORY ELEMENTS**

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**Reporting Period:** The results herein are from work conducted July 1, 2009 to March 1, 2010.

**Objectives; 2008-2010:**
This project has two related thrusts. The first and major thrust builds on our previous successful demonstration that P14 and UT456 transgenes effectively suppress PD symptoms and restrict bacterial titer in two grape genotypes highly susceptible to PD (Figures 2&5). The goals were to complete the greenhouse evaluation of all six genes recovered originally from the cDNA screen for anti-PCD (anti-apoptotic) genes and to extend the data set on quantitative evaluation of the effect of P14 and UT456 on suppression of PD symptoms and Xf titer. Studies were initiated to quantitatively measure the potential for graft transmissibility of the PD protection from transgenic rootstocks of P14 and UT456. The second thrust was focused on the mode of action for the P14 gene and the UT456 in suppression of PD symptoms.

1. Complete evaluation of Thompson Seedless transgenic grape plants expressing four additional candidate anti-apoptotic genes CB390, WG23, WG71 and CB376 for ability to block PD symptoms (Table 1).
2. Determine the effect, over a time course, of blocking PD symptoms with anti-apoptotic transgenes P14 and UT456 on *Xf* bacterial population levels and movement in the xylem by quantitative PCR (qPCR) and confocal laser scanning fluorescence microscopy of GFP-tagged *X. fastidiosa*. Simultaneously measure the level of the messages in the transformed plants of P14 and UT 456.
3. Graft untransformed winegrape scions to each of two transgenic rootstocks, Freedom and Thompson Seedless expressing P14 and UT456 sequences to 9 commercial winegrape varieties (Chardonnay, Pinot Gris, Sauvignon Blanc, Cabernet Sauvignon, Pinot Noir, Zinfandel, Syrah and Merlot). These experiments will assess the potential for protection of untransformed winegrape varieties scions grafted to rootstocks expressing these anti-PCD transgenes. Initial greenhouse analysis will include: visual monitoring of symptoms, qPCR monitoring of mRNA transcripts across the graft and of bacterial titer. This will be followed by field evaluations of equivalent later in 2010 or 2011.
4. Prepare cuttings of the resistant representatives of full plant P14 and UT456 transgenics for field planting in the spring of 2010.
5. Continue mode of action studies on P14 and UT456 transgenes leading to the suppression of PD symptoms and reduction in bacterial titer. This objective is not currently funded from PD sources but is integral to the funded project but partial future funding is being requested.

**Summary of major research accomplishments and results for each objective.**
We established previously that the cell death leading to leaf scorch and cane death symptoms in Pierce's Disease (PD) of grape, caused by *Xylella fastidiosa* (Xf) is due to the activation of a programmed cell death (PCD) process, also known as apoptosis. A functional screen identified several grape genes that, when expressed transgenically, could block the symptoms of PD by suppressing Xf-triggered PCD. In the past year, we addressed the combined objectives of two

grants (now converged into one) to examine the biological behavior of *Xf* in transgenic grape plants expressing the P14 and UT456 sequences (Figure 1&2), measure the level of message expression in relation to relative protection (Figure 6), conduct experiments on the mode of action of P14 and UT456 (Figures 7, 8&9), and began to assess the potential for cross graft movement of the protective factors (Figures 10&11). In 2009, inoculation experiments involving more than 50 P14 and UT456 transgenic plants established that suppression of PD symptoms was associated with a dramatic reduction in bacterial titer of up to four to six orders of magnitude compared to untransformed control vines that were killed within two to four months after inoculation (8). Quantitative PCR (qPCR) of *Xf* measured over distance and time indicated that the amount and movement of the bacteria in the asymptomatic transgenic grapes was similar in distance even though the titer was dramatically reduced several orders of magnitude compared to controls (Figure 5A). Functionally, the pathogenic bacteria introduced into Thompson Seedless-derived P14 and UT456 transgenics now appears as a benign endophyte in both distribution and relative titer within the plants in a manner equivalent to that found in asymptomatic *Vitis californica*. We further observed that the protective sequences are active only when secreted outside the cells suggesting they may function across a graft union (Table 1).

**By Objective:**

1. The results of *Xf* inoculation of CB390, WG23, WG71 and CB376 are summarized in Table 1**.** In each case the genes also suppressed PD symptoms and limited bacterial titer as seen previously with P14 and UT456 but to varying degrees. A brief description of BLAST search comparison follows, which indicates the relationship to orthologous genes in the database and what is known from the literature about the possible biochemical function of the putative gene products.

   **WG23 member of the cupin superfamily:** The coding sequence of the gene designated as WG23 is a member of a family of proteins with conserved barrel domains. Family members related to this gene isolated from the function screen of the grape cDNA library have been described as enzymes having a diversity of function including oxalate oxidase activity, which has been linked to resistance in rice to rice blast disease.

   **WG71 theta-class glutathione S-transferase homolog:** The enzymes glutathione-*S*-transferases (GSTs, E.C.2.5.1.18) have been associated with detoxification of xenobiotics, limiting oxidative damage and other stress responses in plants. This protein has long been associated with cell death in the hypersensitive response of plants to avirulent pathogens but its precise role is unclear.

   **CB390 member of the metallothionein family, an example from tomato was previously published for our lab (2):** The, metallothionein (MT) recovered in this screen is an ortholog of one we cloned earlier from tomato. The tomato MT suppressed accumulation of reactive oxygen species in tomato roots treated with the apoptosis-inducing mycotoxin FB1and suppressed symptoms of PCD (2). MT is reported to modulate both PCD and disease in animal cells

   **CB376 is a member of the Ribosomal S14 superfamily:** This gene encodes a ribosomal protein that is a component of the 40S subunit and a member of the S14P family of ribosomal proteins. Variable expression of this gene is reported in colorectal cancers compared to adjacent normal tissues although no correlation between the level of expression and the severity of the disease has been found. However, this gene appears to play a role in the regulation of cell stability or in situations where cell proliferation or cell death is part of the syndrome.

2. **Extensive evaluation of P14 and UT456:**

In 2008, we began testing the anti-PCD genes expressed in Thompson Seedless and reported results of two inoculations of the Thompson Seedless transgenics bearing the P14 and the UT456 genes (3). All transgenic Thompson Seedless plants bearing either P14, or the UT456 sequences exhibited normal morphology, growth rates, were capable of bearing fruit, and were indistinguishable from the non-transgenic wild type Thompson Seedless control plants (Figure 1). After creating clones of these transgenic lines, the plants were trained to grow as 2 canes and maintained by periodic pruning of side and top branches. Transformed plants were individually inoculated April and May of 2009. The inoculation method was by needle puncture of the stem to allow uptake of 10-20 µl of Xf at $2x10^6$ cfu of the GFP-tagged Xf /ml. The plants were monitored visually for symptoms and by quantitative PCR (qPCR) for bacterial movement and multiplication (Figures 2&5). In both cases, the cell death ultimately concluding in the loss of all leaves and death of the plant in the unprotected control Thompson Seedless plants was completely suppressed in the transgenic P14 and UT456 plants with the bacterial titer reduced by 3-4 orders of magnitude in each case. (Table 1 and Figures 2&5).

**Biological and genetic information related to P14 and UT456 and suppression of PCD**
**P14, a Pathogenesis-related protein**: The P14 gene is a specific member of a family of so-called pathogenesis-related proteins of the PR1 class. P14 is widely used as a marker for resistance, including the onset of systemic acquired resistance (SAR) but has no known function, although the gene was first identified more than 2 decades ago. Sequence comparison to genome databases of the VvP14 from grape using the Clustal W algorithm confirmed high conservation of several domains in orthologs of the gene analyzed from humans, dog hookworm, *Meloidogyne incognita*, tomato and alfalfa. P14 from tomato and grape are essentially identical, as is their function in transgenic assays with *Xf*. In each case where expression of P14 orthologs is induced, the presence of the transcript is associated with a situation in which PCD or apoptosis was blocked or suppressed. Attempts to isolate the P14 protein from expression of a histidine tagged P14 gene failed to recover any P14 protein, suggesting that the P14 transcript that was present in abundance is under translational control.

Localized expression of the protein at the point of PCD induction is consistent with the suppression of pathogen growth and confinement to a zone surrounding lesion, a situation that occurs in PD and other "necrotrophic" plant-bacterial interactions as pointed out by Richael and Gilchrist (5). Working with tobacco plants transgenic for a P14-GFP fusion, Ph.D. student Juan Sanchez (supported by a UC Mexus Fellowship) found abundant mRNA of the P14-GFP fusion but no P14-GFP fusion protein. However, when the P14-GFP plants were inoculated with the tobacco pathogen, *Pseudomonas tabaci*, expression of the P14-GFP fusion protein was readily seen by confocal microscopy at the margin of the elicited lesion (Figure 3). No GFP fluorescence was detected elsewhere in the leaf. In addition, *P. tabaci* could be isolated from the green lesion border but not from elsewhere in the leaf indicating that live bacteria were confined to the region of the cells expressing P14-GFP. We interpret these data to indicate that once the lesion margin was defined by blocking further cell death no further lesion expansion occurs and the bacteria remained alive at the lesion margin while apparently adopting an endophytic association with the tissue.

P14 has a leader sequence for extracellular targeting and appears to be quite resistant to proteolytic cleavage due to the fact that it is recoverable in expressed plant sap (13). We

inoculated five P14B (leader sequence removed) plants along with 16 P14 transgenics (with the signal peptide intact) with *Xf.* (Table 1, P14B and P14). All 5 P14B plants died within 3 months after inoculation with *Xf*, at rates equal to untransformed control plants, and showed a titer of $10^6$ bacterial cells per 0.1 gram of stem tissue. In contrast, 26 grape plants expressing the P14 gene with the leader sequence intact were asymptomatic after 6 months and had a bacterial titer of $10^2$ cells per 0.1 gram of tissue (Table 1, P14).

**UT456 and potential for activity as a systemic anti-apoptotic regulatory molecule**
UT456 is a 270 bp DNA sequence that does not contain a protein coding sequence but does contain a stem/loop structure derived from the 3'UTR of a gene designated as p23 in potato and known as TCTP in animals. The p23 gene is reported to be up-regulated in *Meloidgyne incognita*-induced giant cells in tomato and has a protective effect against apoptosis in yeast. We investigated the secondary structure of the sequence by folding analysis with the program mFOLD, which revealed a striking conservation of the both the stem and loop sequence between UT456 and the P14 3'UTR (Figure 6&7). 3' UTRs have been shown to encode translational regulators. The UT456 protection in our experiments (15/25 independent transgenics fully protected after 10 months) was due to RNA (presence confirmed by Northern analysis) and not a translated protein (lack of open reading frame). This suggested that the PD suppressive action is resident in the 100 bases comprising the UT456-hp4 stem and loop (Figure 8). As of February 2010, we now have evidence that a 21 mer derived from the UT456 and containing the conserved bases highlighted in Figure 6 is present in cells where UT456 is expressed. If this data is confirmed, it will suggest the mechanism of action involves a microRNA-dependent regulation of translation, a situation accepted in the animal world but essentially unknown in plants.

3. **Grafting experiments:** We have generated experimental material in the form of transformed rootstocks (Freedom and Thompson Seedless) expressing P14 or UT456 grafted to untransformed winegrape scions for greenhouse inoculation and quantitative assessment of message level, message movement, and bacterial titer in untransformed grafted and ungrafted winegrape varieties (Figures 10&11). It is critical to do controlled greenhouse testing to establish quantitative and qualitative base line data before any field evaluation. The first preliminary inoculation experiment with Thompson Seedless indicated that *Xf* failed to establish in untransformed scion grafted to a P14 transgenic rootstock. Extensive inoculation experiments are now in progress.
4. **Prepare cuttings of the resistant representatives for field planting in the spring of 2010.** This objective is currently in progress with no additional results to report beyond those detailed in #2 above.
5. **Continue mode of action studies** on P14 and UT456 transgenes leading to the suppression of PD symptoms and reduction in bacterial titer. This objective is not currently funded from PD sources but is integral to the funded project but partial future funding is being requested. Functional studies on the role of P14 and UT456 revealed some novel findings. The transgenic P14 coding sequence is translationally blocked in healthy cells but is readily translated when tobacco, tomato or grape cells expressing the gene are under chemical or pathogenic (death) stress (Figure 3). Secondly, the noncoding UT456 sequence contains a small RNA hairpin structure (UT456-hp4) with a high degree of sequence conservation with the P14 3'UTR (Figures 6 & 7). Transient expression of UT456-hp4 in transgenic tobacco leaves expressing P14-GFP activated the P14-GFP translation (Figure 4) and also activated P14 translation in wheat germ *in vitro* translation assays. Lastly, P14 antibodies, in a classical P14 pulldown assay, identified 3 proteins, HSP70, HSP90 and RACK1 (Figure 9) that bound to P14 from plant extracts. Interestingly, these three proteins have previously been reported to interact directly

with each other and occur in a membrane associated complex involved in innate immunity in rice plants (Figure 9). The goal is to extend the information on the association of between P14 and the membrane associated RACK1 complex (Figure 9) and to further understand the functional activity of the sequences within UT456 that activate P14 translation (Figure 4). In the past month we have cloned RACK1 and will translate the protein in *E. coli* (as was done with P14) in order to generate antibodies against RACK1; antibodies to be used in further pulldown assays to identify additional interactors from the complex and to confirm the interaction with the P14 protein. Future plans include: to establish a role for the potential small RNA hairpin loop in P14 translation from the *in vivo* active UT456 RNA using RNA protection assays, to identify the region within the UT456 RNA responsible for the activation of translation (release of 3'UTR block) of P14 (Figure 4), to determine if the putative UT456 hairpin stem loop can anneal to the identical stem sequence in the 3'UTR of P14 using $^{32}$P-labelled UT456-hp4 RNA. Functionally this would result in a stem to stem shuffle, as in riboswitches, thus culminating in the release of translational blocks (Figure 7&8).

**Publications or reports on research:**

Pierce's Disease Research Symposium Proceedings. Sacramento, CA. December 9-11.

Two manuscripts in preparation

**Presentation of research:**

2009 Pierce's Disease Symposium, Sacramento, CA. December 9-11

University of Florida, Department of Plant Pathology Seminar. November 17, 2009

**Research relevance**

The results of this research have experimentally determined that the symptoms of Pierce's Disease (PD) result from the activation of programmed cell death (apoptosis) in the susceptible grape plants. The experiments directly address a genetic-based strategy for of grape against PD. Anti-apoptotic genes expressed in transgenic grape plants suppress PD symptoms and reduce the titer of the otherwise pathogenic *Xylella fastidiosa* to a level consistent with the endophytic association it establishes in the asymptomatic host *Vitis californica.* These data are consistent with other reports on protection against plant and animal disease by blocking apoptosis (1, 6, 7) Two DNA sequences obtained from a function screen for anti-apoptotic factors, P14 and UT456, have been studied extensively in transgenic grape plants and shown to suppress PD without otherwise altering the morphology of the grape plant or directly killing the bacteria via an antibiotic mechanism. These studies established the proof of concept which will now be examined further under field conditions. Lastly, studies are in progress that will address the question of whether the PD suppressive factors can function across a graft union to protect untransformed scions from the disease. These materials and the experiments to be conducted directly address a biological mechanism to protect grape plants against Pierce's Disease caused by *X. fastidiosa.*

**Lay summary of current year's results**

In the past year we successfully demonstrated resistance against PD in the susceptible grape variety Thompson Seedless by one anti-apoptotic transgene (P14) and one 270 bp DNA sequence homologous to the 3'UTR of a nematode up-regulated gene designated p23. We further demonstrated that expression of these sequences not only protected the transgenic plants against PD symptoms and plant death but maintained the population of *Xf* at four to six orders of magnitude below the level observed in untransformed plants that died within 2 months ($10^8$ bacteria per gram of stem tissue) following controlled inoculations in the greenhouse. Our

current experiments indicate that the effect of the anti-PCD genes suppresses symptom expression but do not exert a direct inhibiting effect on the bacteria. The symptom suppressive genes do not act as antibiotics and do not affect the natural endophytic ecology of the bacteria in the xylem. Lastly and most importantly, viable plants with resistance to Pierce's Disease have been produced; the proof of concept validated, and plans are in place for field evaluation of the material. One key point is that altered expression of the anti-apoptotic transgenes does not kill the bacteria but does restrain the titer in the asymptomatic transgenic plants from a lethal level of $10^8$ to a level of $10^4$ to $10^2$ cells/0.1 gm stem tissue in the most resistant lines; the $10^4$ titer is equivalent to that which we measured in the asymptomatic host *V. californica* 12 months after inoculation. Interestingly, the $10^4$ cells/0.1 gm stem tissue titer level in the asymptomatic transgenic plants and *V. californica* is equivalent to that observed by Dr. Lindow in his *rpfF* transformed plants that also are asymptomatic suggesting that susceptible grape plants can tolerate a bacterial population of $10^4$ cells per 0.1 gm of tissue without showing PD symptoms.

**Status of funds:** All funds will be expended by the end of the current fiscal year.

**Status of intellectual property produced during this research period.**
The grape plants containing the anti-PCD genes and the grafted rootstocks will require the use of several patented enabling technologies that have been brought into the process. Record of invention disclosures have been submitted to the UC Office of Technology Transfer. The research proposed here will provide data on the activity and mechanism of action of the protective transgenes in grape relative to the presence, amount and movement of *X. fastidiosa* in the transformed and untransformed grape plants. All steps related to future patent protection and licensing will be reviewed by PIPRA.

**Citations:**


1. Richael, C., Lincoln, J., Bostock, R., and Gilchrist, D. G. 2001.Caspase inhibitors reduce symptom development in compatible plant-pathogen interactions and limit pathogen multiplication *in planta.* Physiol. and Mol. Plant Pathol. 59(4) 213-221.
2. Harvey, J. JW, J. E. Lincoln, and D. G. Gilchrist 2007 Programmed cell death suppression in transformed plant tissue by cDNAs identified from an *Agrobacterium rhizogenes*-based functional screen. Molecular Genetics and Genomics. 279, 509-521.
3. Gilchrist, D, J Lincoln, 2008. Systemic control of Pierce's Disease by altered expression of anti-apoptotic genes or their RNA-based regulatory elements. Pierce's Disease Research Symposium Proceedings. San Diego, CA December 15-17.
4. Achim E. Gau, Mostafa Koutb, Markus Piotrowski, and Klaus Kloppstech 2004. Accumulation of pathogenesis-related proteins in the apoplast of a susceptible cultivar of apple (*Malus domestica* cv. Elstar) after infection by *Venturia inaequalis* and constitutive expression of PR genes in the resistant cultivar, Remo. *European Journal of Plant Pathology* 110: 703–711.
5. Richael, C. and Gilchrist, D.G. 1999. The hypersensitive response: A case of hold or fold? Physiol. Mol. Plant Pathol. 55:5-12.
6. Dickman, M. B., Y. K. Park, et al. (2001). "Abrogation of disease development in plants expressing animal antiapoptotic genes." Proc Natl Acad Sci U S A 98(12): 6957-62.
7. Chen, S. and M. B. Dickman (2004). Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. J. Exp. Bot. 55: 2617-2623.
8. Gilchrist, D, J Lincoln, 2009. Systemic resistance to Pierce's Disease by transgenic expression of plant-derived anti-apoptotic gene. Pierce's Disease Research Symposium Proceedings. Sacramento, CA. December 9-11.


**Figure 1.** Representative transgenic plants of Freedom and Thompson Seedless were morphologically and developmentally indistinguishable from the untransformed control plants. The Thompson Seedless plants were capable of bearing fruit, which also appeared normal

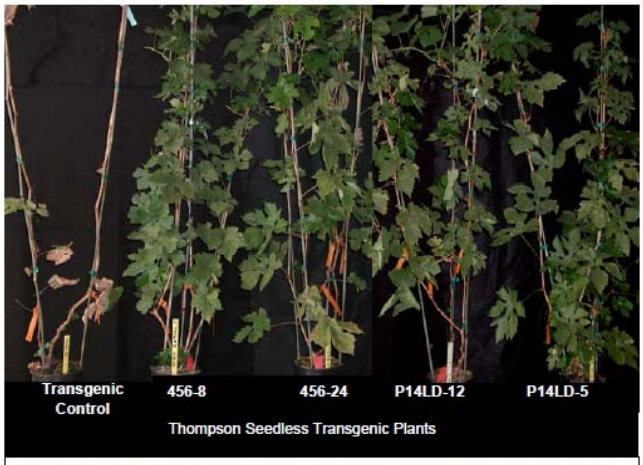


**Figure 2.** These plants had been infected for 6 months at the time the picture was taken; 80% of the transgenics in PR1 and 456 have a rating of 5 and very low bacterial counts $10^2$-$10^4$ cfu/0.1g/cane by qPCR. GFP transformed and untransformed control plants were dead at 4 months and had high titers ($10^8$) at the point of dying.

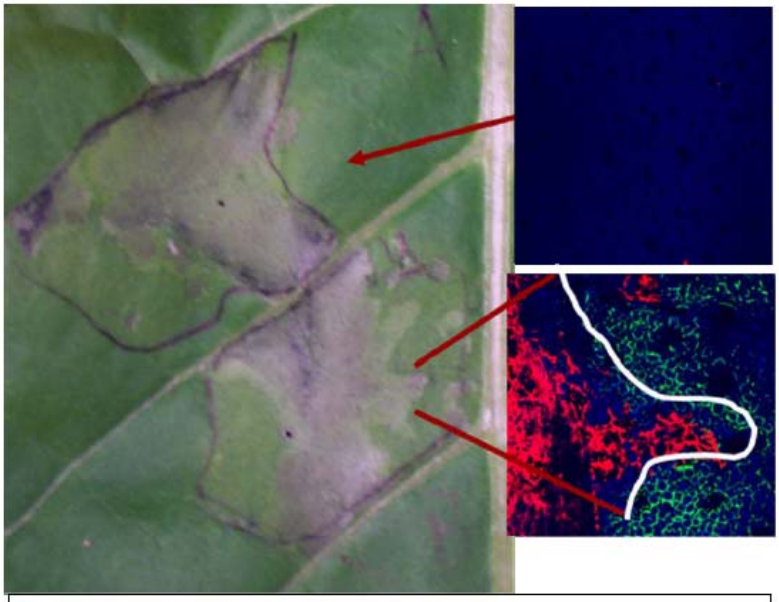

**Figure 3.** Lesion elicited by infiltration of transgenic tobacco expressing P14-GFP fusion message bearing both the 5' and 3' UTRs with *Pseudomonas tabaci*. Confocal image of the lesion margin and a healthy area distal to the lesion reveals expression of P14-GFP fusion protein is restricted to the area bordering the lesion (red cells=dead).. The lesion did not expand further and bacteria remained alive in the cells expressing P14-GFP protein for at least two weeks after infiltration.

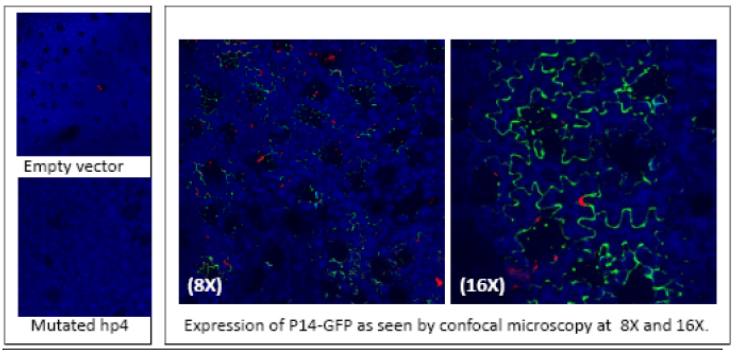


**Figure 4.** A 100bp region of UT456 called UT456-hp4 induces translation of the p14-GFP fusion protein in mesophyll and epidermal cells 3 days after infiltration of *Agrobacterium tumefaciens* expressing UT456-hp4 into tobacco constitutively expressing P14-GFP fusion mRNA with both 5' and 3' UTRs present. Leaf panels infiltrated with empty vector and a mutated form of UT456-hp4 were negative for P14-GFP.

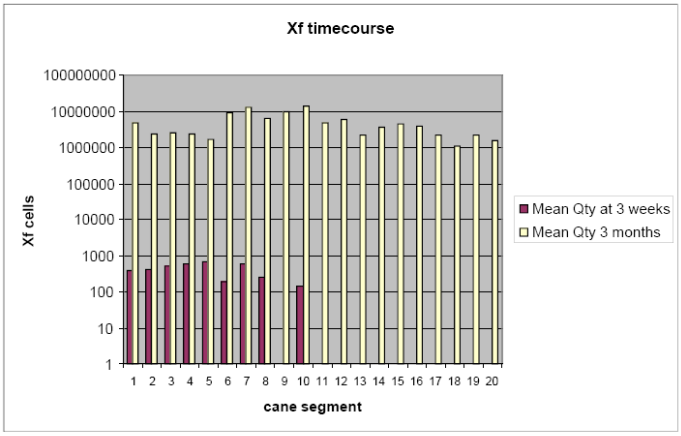


**Figure 5 A**. Distribution of *X. fastidiosa* cells in inoculated canes at 3 weeks and 3 months post inoculation Consecutive 1 cm sections of PD susceptible control plant (9353-2) were cut beginning at the site of inoculation (#1) to 20 cm up the stem. Bacteria titer at inoculation site 1 week after infiltration averaged 500 cells and gradually increased as seen in the figure.

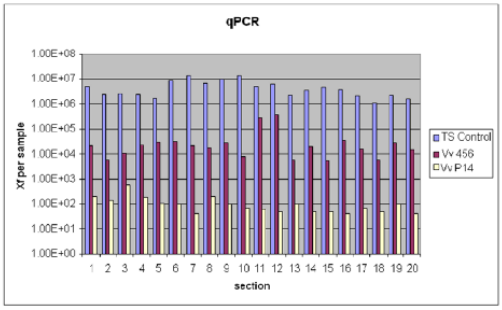


**Figure 5.** Comparison of *Xf* titer in inoculated Thompson Seedless susceptible control plants to Thompson Seedless plants expressing the UT456 and P14 sequences at 1.5 months post inoculation. Conclusion: bacterial cells are distributed evenly along the stem with the P14 and 456 lines showing 4-5 orders of magnitude less bacterial cells than the control.


**Figure 7. Structure of P14 genes.** The structure of the PR1 gene from tomato (top) and grape (bottom) are identical. Each has a leader (pink) for secretion of the mature protein (green) outside the plant cell. The position in the 3'UTR (blue) with homology to the UT456-hp4 sequence is marked as

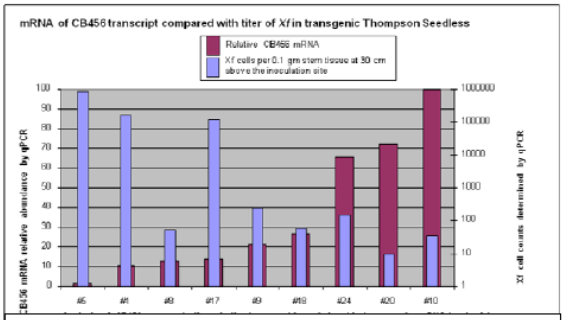


**Figure 6.** Relationship between the mRNA level of UT456 transcript compared to the titer of *Xf* in independently transformed Thompson Seedless lines Analysis of nine UT456 transgenic lines indicating a positive relationship between the mRNA level of the transcript and the suppression of Xf in the stems of individual transgenic lines. Data based on duplicate measurements with less than 1 % variation among duplicate samples.

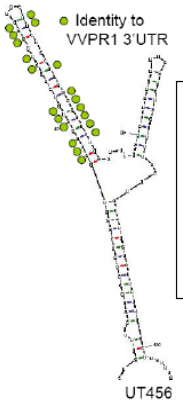


**Figure 8. UT456-hp4 has homology to 3'UTR of VvP14** Sequence of the hp4 stem-loop region of UT456 is shown folded into a hairpin structure with identical bases to the 3'UTR of VvPR1 marked with green ( ) circles.

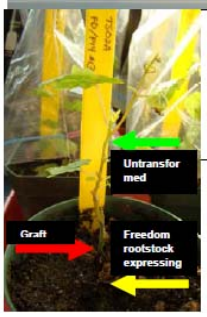


**Figure 10.** Micrografted untransformed scion to transformed rootstock bearing either P14 or UT456 (see inset). Examples of grafted plants shown on the bench above in preparation for movement to the greenhouse and inoculation with *Xf*.

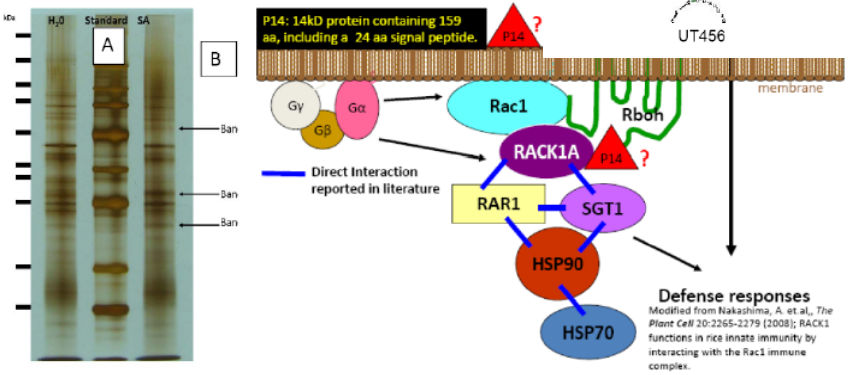


**Figure 9.** (A) PAGE image of P14 antibody pulldown assay of protein extracts from salicylic acid treated leaves expressing P14 protein. The 3 differentially expressed protein bands are marked with arrows. Each of the 3 bands were sequenced and the identity of each is based on 3 peptide sequence matches as Rack 1A, HSP 90 and HSP 70 . Each of these 3 putative P14 interactors have orthologs in plants and animals and have been reported to directly interact ( ) in the Rac1 membrane-anchored immune complex (B), which functions in conferring resistance in rice to a bacterial pathogen. Nakashima, A. et.al., *The **Plant Cell*** 20:2265-2279 (2008);


**Figure 11 .** Untransformed Thompson Seedless stems grafted to the Freedom P14 rootstock and inoculated with *Xf* @ 20,000 cells/site on each stem. Xylella cells not detected by PCR (< 100 cells) in the Thompson Seedless scion grafted to the PR1 rootstock at 5 months and plants appear healthy (6/6). Untransformed

**Table 1. Transgenes cloned from the cDNA functional screen and control plants used in the pathogenicity test with *Xylella fastidiosa* under controlled greenhouse conditions. Disease rating based on a five point scale; 5= no symptoms, 1= complete loss of leaves.**

| Construct | Gene | PD resistance rating average (n# plants) | Xf / 0.1g cane by qPCR (average) |
|---|---|---|---|
| CB390 | metallothionein | 3 (3) | $10^4$ |
| UT456 | 270bp sequence from 3'UTR of "nematode-inducible" p23 gene, orthologous to TCTP from humans | 5 (28) | $10^2$ |
| WG23 | Unknown but "cupin-like" | 4 (8) | $10^3$ |
| WG71 | cytokine-like gene (MIF) | 4 (6) | $10^4$ |
| O2A | Untransformed Thompson seedless | 1 (9) | $10^6$ |
| PR1A | VvPR1 | 5 (10) | $10^2$ |
| I35 | Intron p35 (anti-PCD control gene) | 3 (5) | $10^4$ |
| P14 | P14 (homolog of PR1) | 5 (26) | $10^2$ |
| P14B | Secretory leader deletion of P14 | 1 (5) | $10^6$ |
| CB376 | Mycorrhizal induced gene | 3 (12) | $10^4$ |
| 9353 | GFP (transformed control) | 1 (9) | $10^6$ |