OPTIMIZING GRAPE ROOTSTOCK PRODUCTION AND EXPORT OF INHIBITORS OF *XYLELLA FASTIDIOSA* POLYGALACTURONASE ACTIVITY

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ABSTRACT
In response to a recommendation by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Research Scientific Advisory Panel to express plant genes for particularly effective inhibitors of *Xylella fastidiosa* (*Xf*) polygalacturonase (PG) in transgenic grape, optimal plant polygalacturonase inhibiting proteins (PGIPs) are being selected and expressed in grape rootstocks to enhance grapevine Pierce's disease (PD) resistance. This project includes integrated approaches aimed at the eventual deployment of that strategy in commercial lines. To ease the path to commercialization, PIPRA investigators are examining the relevant Intellectual Property and regulatory issues associated with the use of selected PGIPs in transgenic grape rootstocks in combination with elite scion lines. The PGIPs that most effectively inhibit *Xf*PG are predicted to be the best candidates for providing significant PD resistance. Recombinant *Xf*PG is being developed to screen diverse PGIPs selected from a wide variety of plant sources for their ability to effectively inhibit the *Xf*PG enzyme. We are cloning the selected PGIPs so they can be expressed in plants for the tests of their efficacy in inhibiting *Xf*PG. Grape rootstock lines will be transformed with the most effective PGIPs and signal and target sequences will be used as needed to maximize PGIP expression in the rootstock and its export to the non-transgenic scions. At the conclusion of the project, the capacity of non-transgenic scions to resist PD and produce high quality grapes when grafted on transgenic rootstocks will be tested.

LAYPERSON SUMMARY
Plant proteins have been selected to inhibit a key enzyme called polygalacturonase (PG) that *Xylella fastidiosa (Xf)* uses to spread from the point of inoculation throughout the grapevine and cause Pierce's disease (PD). Proteins called PG-inhibiting proteins (PGIPs) are produced by many plants. PGIPs are selective in their ability to inhibit the PG enzymes of plant pathogens. We know that the pear fruit PGIP can inhibit *Xf*PG and that expression of the pear PGIP in transgenic grapevines slow PD development. We also know that pear PGIP produced in a rootstock can move into scions by crossing the graft union in the water-conducting tissues. The PGIPs from different plants are being tested for their ability to inhibit *Xf*PG and structural modeling is being used to characterize what parts of the PGIP are important for inhibition. The best inhibiting PGIPs will be expressed in grape and their ability to reduce PD development in grafted scions will be determined. Regulations regarding the release and use of transgenic rootstocks and intellectual property considerations associated with this approach are being addressed to maximize the commercial potential of this PD management strategy, an approach that has been advocated by the CDFA PD/GWSS Advisory Panel.

INTRODUCTION
Xylella fastidiosa (Xf), the causative agent of Pierce's disease (PD) in grapevines, has been detected in infected portions of vines. Several lines of evidence support the hypothesis that *Xf* uses cell wall-degrading enzymes to digest the polysaccharides of plant pit membranes separating the elements of the water-conducting vessel system, the xylem, of the vines (Thorne et al., 2006). *Xf* cell wall degrading enzymes break down these primary cell wall barriers between cells in the xylem, facilitating the systemic spread of the pathogen. The genome of *Xf* contains genes predicted to encode a polygalacturonase (PG) and several β-1,4-endo-glucanases (EGase), cell wall degrading enzymes that are known to digest cell wall pectin and xyloglucan polymers, respectively. To demonstrate that these wall degrading proteins facilitate *Xf* systemic movement and PD development, Roper et al. (2007) developed a PG-deficient strain of *Xf* and showed that the mutant bacterial strain was unable to cause PD symptoms; thus, the *Xf*PG is a virulence factor of the bacteria that contributes to the development and spread of PD. Labavitch et al. (2006) reported that introduction of PG and EGase enzymes into explanted stems of uninfected grapevines caused breakage of the pit membranes and demonstrated that substrates for these enzymes, pectins and xyloglucans, are present in grapevine pit membranes (Labavitch, 2007).

PG-inhibiting proteins (PGIPs) produced by plants limit damage caused by fungal pathogens (*B. cinerea*, or gray mold) as well as by insects (*Lygus hesperus*, the western tarnished plant bug) (Powell et al., 2000; Shackel et al., 2005) because PGIPs

are selective inhibitors of the PGs produced by fungal pathogens and insects (Cervone et al., 1990). Agüero et al. (2005) demonstrated that by introducing a pear fruit PGIP gene (Stotz et al., 1993; Powell et al., 2000) into transformed grapevines, the susceptibility to both fungal (*B. cinerea)* and bacterial (*Xf)* pathogens decreased. This result implied that the pear PGIP provided protection against PD by inhibiting the *Xf* PG, reducing its efficiency as a virulence factor. Using *in vitro* assays with *Xf*PG expressed in *E. coli*, Roper (2006) demonstrated that the recombinantly expressed *Xf*PG can be inhibited by the pear PGIP (Labavitch, 2006). In a key preliminary observation for the PD control approach investigated in this project, Agüero et al. (2005) demonstrated that transgenic pear PGIP protein could be transported across a graft junction of genetically engineered grapevines into the aerial portions of wild-type scions.

The overall goal of the project is to develop transgenic grape rootstock lines that optimally express PGIPs that most effectively reduce the virulence of *Xf*.. The project is designed to identify specific PGIPs that optimally inhibit the virulence factor, *Xf*PG, and to express efficiently the optimal PGIPs in grape rootstocks to provide PD protection in scions. The optimization of the expression of PGIPs includes the use of transformation components with defined intellectual property (IP) and regulatory characteristics, as well as expression regulating sequences that result in the maximal production of the PGIPs in rootstocks and efficient transport of the proteins through the graft junctions to the aerial portions of the vines so that *Xf*PG produced by the pathogen in scions is inhibited.

OBJECTIVES

1. Define a path to commercialization of a PD control strategy using PGIPs, focusing on IP and regulatory issues associated with the use of PGIPs in grape rootstocks.
2. Identify plant PGIPs that maximally inhibit *Xf*PG
3. Assemble transcription regulatory elements, *Xf*-inducible promoters, and signal sequences that maximize PGIP expression in and transport from roots.
4. Create PGIP-expressing rootstocks and evaluate their PD resistance.

RESULTS AND DISCUSSION

Objective 1: Regulatory issues associated with commercialization of transgenic rootstocks

A visit by PIPRA staff to federal agencies regulating the environmental release into the environment of genetically modified (GM) plants gave us insights on the regulatory issues related to *PGIP* expressing grape rootstocks. From a USDA/APHIS perspective, it will be recommended that field trials start with GM rootstocks and wild type scions will be grafted on later for deregulation of future commercial products. From an EPA standpoint, one potential issue for this project to address will be gene-flow from transgenic pollen. For regulatory approval only, it will be necessary to allow rootstocks to flower in commercial settings even though under normal practices, the rootstocks will not be allowed to flower. The EPA will have the final word on defining if grapes harvested from non-GM scions grafted on the *PGIP*-expressing rootstocks will be considered transgenic. This will determine if the FDA needs to be consulted before commercialization.

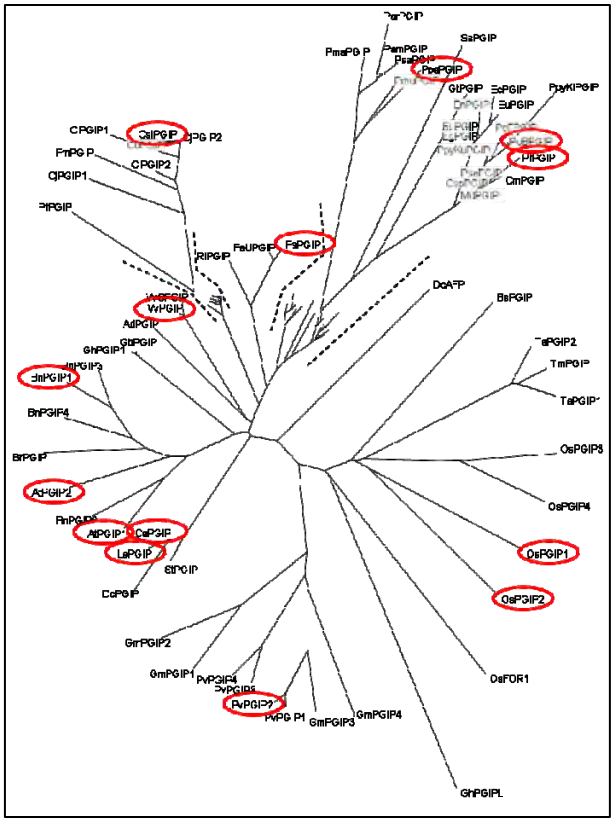

Figure 1. Unrooted phylogenetic tree of PGIP proteins. The 14 candidate PGIPs for *Xf*PG inhibition are circled in red. The protein names and organisms are given in Labavitch, 2008.

Objective 2: Propagation and grafting of existing grape lines expressing and exporting pear PGIP

Agüero et al. (2005) described the use of transgenic grapevine cultivars 'Thompson Seedless' and 'Chardonnay' expressing the 'Bartlett' pear fruit PGIP (*PcBPGIP*). These plants have been maintained in our greenhouse facilities with the intent to use them in grafting and *Xf* inoculation experiments. Vegetative propagation efforts to increase the total number of plants for these experiments yielded 66% efficiency last winter. PCR analysis has been used to verify the transgene identity in both grape cultivars containing either the *PcBPGIP* transgene or the empty vector.

Further work related to this objective has been delayed substantially due to quarantine measures implemented in response to a Panicle Rice Mite infestation in the greenhouse facilities. For more information regarding these matters, please refer to the August 2009 progress report for CDFA contract number 08-0171. The CDFA and UC Davis issued directives for treating the affected grape vines including drastic pruning and isolation, resulting in rootstocks with only one or two viable buds remaining prior to intensive chemical disinfestations treatments. Some of the

grape lines did not survive the mandated action and all remaining lines have shown slower than normal regeneration. Due to the considerable amount of time and effort to follow these directives and regenerate the plants, the grafting experiments to be conducted in years 1 and 2 have been delayed until the affected grapevines can be moved back to the appropriate facilities. As of October 2009, the grapevines remain in isolation.

Objective 2: Selection of PGIPs

The previously reported phylogenetic tree of PGIP sequences (Labavitch 2008) has been expanded upon to include a total of 68 PGIP-like amino acid sequences. These PGIPs represent a diverse array of plant families and expression patterns. The PG inhibition activities of some of them are known. The full-length protein sequences from GenBank were aligned using ClustalX 2.0.9. An unrooted, neighbor-joining tree (**Figure 1**) was constructed in ClustalX and visualized with TreeView 1.6. PGIPs are typically characterized by 10 leucine rich repeats (LRR) in the region thought to influence inhibition of PGs. The PGIP sequence diversity in this phylogenetic tree mirrors the diversity among plant families, crediting the use of PGIP sequence data in plant family classification studies.

Fourteen candidate PGIPs (**Figure 1, Table 1**) were selected from the phylogeny, representing the major clades of the tree and the inherent sequence variation dividing them. The candidates were also chosen by their predicted total protein charge at a given pH. The predicted charges were calculated for all 68 PGIP sequences but the lower total charges predicted for the candidates should prevent interference or repulsion between each PGIP and the highly charged *Xf*PG. The large positive charge on AtPGIP2 and the minimal charge on OsPGIP2 will be particularly informative as we correlate *Xf*PG inhibition with total PGIP charge.

Table 1. Predicted total protein charge analysis for the 14 candidate PGIPs and *Xf*PG in different pH environments.

Common name	Organism	Protein	Charge of Protein (at certain pH)					
			3.5	*4.0*	***4.5***	*5.0*	*5.5*	*6.0*
Thale cress	*Arabidopsis thaliana* (Col.)	AtPGIP1	27.5	20.9	**14.2**	10.0	7.4	5.2
Thale cress	*Arabidopsis thaliana* (Col.)	AtPGIP2	35.4	28.5	**21.6**	17.0	14.2	11.8
Rapeseed	*Brassica napus* cv. DH12075	BnPGIP1	30.5	22.2	**14.2**	9.4	6.8	4.8
Pepper	*Capsicum annum* cv. arka abhir	CaPGIP	20.7	15.2	**9.5**	5.9	3.8	2.2
Sweet orange	*Citrus sinensis* cv. Hamlin	CsiPGIP	28.0	21.7	**15.2**	11.1	8.7	6.7
Strawberry	*Fragaria x ananassa*	FaPGIP	25.4	18.7	**12.1**	8.0	5.6	3.7
Rice	*Oryza sativa* cv. Roma	OsPGIP1	18.4	12.9	**7.6**	4.3	2.2	0.2
Rice	*Oryza sativa* cv. Roma	OsPGIP2	17.5	9.3	**1.6**	-3.1	-6.1	-8.8
Common bean	*Phaseolus vulgaris* cv. Pinto	PvPGIP2	22.7	17.6	**12.9**	10.2	8.5	7.1
Peach	*Prunus persica*	PpePGIP	28.7	21.9	**14.9**	10.3	7.5	5.3
Chinese Firethorn	*Pyracantha fortuneana*	PfPGIP	16.9	11.7	**6.6**	3.4	1.4	-0.3
Bartlett pear	*Pyrus communis* cv. Bartlett	PcBPGIP	23.1	16.1	**9.3**	5.0	2.6	0.7
Tomato	*Solanum lycopersicum* cv. VFNT Cherry	LePGIP	29.8	23.4	**17.0**	12.8	10.1	7.7
Grape	*Vitis vinifera* cv. Pinotage	VvPGIP	30.5	24.0	**17.7**	13.6	11.1	8.7
		*Xf*PG	41.0	31.3	**22.2**	16.4	11.9	6.8

Objective 2: Express PGIPs and test for optimal inhibition of XfPG

The 14 candidate PGIPs will be tested for their ability to inhibit *Xf*PG. The previously reported plant transformation strategy (Labavitch 2008) is being used to generate plant transformation vectors containing a PGIP sequence under control of the CaMV 35S constitutive promoter and linked to a C-terminal poly-His tag for protein purification. These plant proteins are highly glycosylated (**Figure 3B**) and therefore require expression in a plant-based system. Arabidopsis lines transformed to express each PGIP will be used to obtain the proteins necessary for *in vitro* radial diffusion assays, testing the inhibitory capacity of each PGIP. Cloning each of the candidate PGIPs from its source species into the proper plant transformation vector is in progress (**Table 2**). The stably expressing Arabidopsis lines will provide PGIPs for inhibition assays against the PD causing *Xf*PG, as well as against PGs from other pathogens and pests linked to many plant diseases and resulting crop losses.

Table 2. Research progress for cloning the 14 candidate PGIPs. "X" = completed checkpoint, "O" = work in progress, "-"= checkpoint to be completed.

Protein	Cloning Progress Checkpoints				
	Plant tissue acquired	PGIP cDNA isolated	Transformed into *E. coli*	Transformed into *A. tumefaciens*	PGIP ready for plant transformation
AtPGIP1	X	X	X	O	-
AtPGIP2	X	X	X	O	-
BnPGIP1	O	-	-	-	-
CaPGIP	X	O	-	-	-
CsiPGIP	X	O	-	-	-
FaPGIP	X	X	-	-	-
OsPGIP1	X	O	-	-	-
OsPGIP2	X	O	-	-	-
PvPGIP2	X	O	-	-	-
PpePGIP	O	-	-	-	-
PfPGIP	X	O	-	-	-
PcBPGIP	X	X	X	X	O
LePGIP	X	X	X	X	O
VvPGIP	O	-	-	-	-

The *in vitro* assays require optimal expression and activity of *Xf*PG, a topic covered below. We are developing another assay to test each candidate PGIP's ability to inhibit *Xf*PG *in planta*. This assay will provide an environment more similar to the potential PG-PGIP interaction taking place in the plant apoplastic space. Separate plant transformation vectors carrying a candidate PGIP and the *Xf*PG coding sequence will be used to transiently co-express both proteins in tobacco leaves by *Agrobacterium tumefaciens* pressure infiltration. An analogous assay was used to test the effectiveness of a grape PGIP (VvPGIP) in inhibiting a PG from *B. cinerea* (Joubert et al., 2007). Both the PG and PGIP were transiently expressed in tobacco leaves by co-infiltration of *A. tumefaciens* clones carrying the genes of interest. Expression of the PG alone resulted in PG-dependent lesions which were visible and could be measured. Co-infiltration of PG and PGIP-expressing clones resulted in inhibition of PG-mediated lesion development (**Figure 2**). *Xf* is known to cause local lesions in tobacco leaves after infection. Two plant transformation constructs have been developed containing *Xf*PG for this experiment: one with the native coding sequence and one with an apoplastic targeting sequence attached upstream of the coding region to ensure *Xf*PG secretion by the plant cells. We expect to see results similar to those from the earlier work: local lesions induced by the expression of *Xf*PG will be lessened when the *Xf*PG is co-expressed with an inhibiting PGIP. This will support rapid comparisons of the effectiveness of each PGIP in inhibiting *Xf*PG *in planta*.

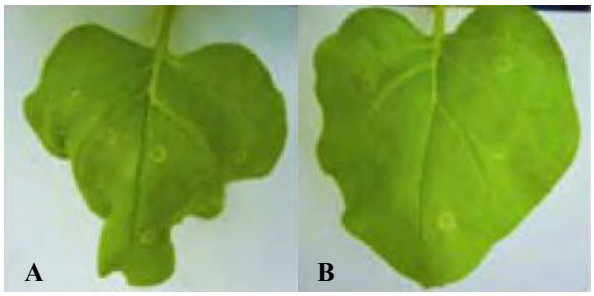

Figure 2. Co-expression of *Bcpg2* with either (A) empty vector control or (B) *Vvpgip* in *N. benthamiana* leaves at 24 h postinfiltration. Leaves were infiltrated with the two *A. tumefaciens* strains in a 1:1 ratio. Figure from Joubert et al., 2007.

Objective 2: XfPG cloning and expression

The *Xf*PG gene was subcloned from the pET29b vector into pMT/BiP/V5-HisA, a vector compatible with the Drosophila protein expression system used by R. Booth. The construct was confirmed by performing digestions with EcoRI, XhoI, EcoRV, and sequencing by the UC Davis DNA Sequencing facility. Transfections were performed with the confirmed *Xf*PG construct or an expression vector containing a GFP marker as the positive control; non-transfected cells were tested as the negative control. Cellular components (pellet) and supernatant (SN) from the transfected lines were collected. Protein expression was validated by SDS-PAGE and Western Blot analyses (**Figure 3**). The *Xf*PG protein has an apparent molecular weight of 70 kD, slightly greater than expected, possibly due to the effects of glycosylation. Crude extracts will be assayed for PG activity while further steps to purify the protein using the attached His-tag will provide *Xf*PG for future *in vitro* PGIP inhibition assays.

Figure 3. Lanes 1-4 are protein collected from the SN of the transfection reaction. Lanes 5-8 are protein collected from the pellet. Lanes 1, 5: negative control; lanes 2, 3, 6, 7: *Xf*PG transfection; lanes 4, 8: positive control of GFP marker-expressing cells.

Objective 2: Model PGIP and XfPG interactions to aid in optimal PGIP prediction for PD defense

The interaction between PG and PGIP proteins influences whether the plant PGIP is able to successfully inhibit the pathogen virulence factor, *XfPG*. The crystal structure of PvPGIP2 (Di Matteo et al., 2003) has facilitated structural inquiries into what regions of the PGIP are responsible for PG inhibition. One study found that a single amino acid, Q224, is responsible for *Fusarium moniliforme* PG (*Fm*PG) inhibition by PvPGIP2 by comparison to PvPGIP1, which is unable to inhibit *Fm*PG (Leckie et al., 1999). While sequence variation can account for some of the specificity, the ability of PvPGIP2 to have competitive, non-competitive, and mixed modes of inhibition for *Fm*PG, *A. niger* PGII, and *B. cinerea* PG1, respectively, suggests that additional recognition and specificity sequences or motifs occur (Federici et al., 2001; King et al., 2002; Sicilia et al., 2005). PGIPs are heavily glycosylated proteins with 7 potential N-linked glycosylation sites on PcBPGIP (Lim et al., 2009) thereby adding 14.5 kD to the molecular weight (Powell et al., 2000). It has been hypothesized that differing glycosylation patterns also affect PGIP specificity.

Homology modeling efforts by D. King created *in silico* interactions between the predicted structures of *Xf*PG and each of the 14 candidate PGIPs to visualize the possible interactions and predict the likelihood of a successful inhibition. Each structural model was created by threading the PGIP amino acid sequence onto the PvPGIP2 crystal structure. Models were then optimized with molecular mechanics, MM3, using the Swiss PDB Viewer DeepView 3.7 and the modeling suite BioMedCAChe 6.1 (**Figure 4A**). Glycosylated versions of the models were created by attaching three $Man_3XylGlcNAc_2$ and four $Man_3XylGlcNAc_2Fuc$ groups to the appropriate sites (NxS/T) on the optimized protein structures as previously determined for PcBPGIP (**Figure 4B**; Lim et al., 2009). The putative *Xf*PG model was visualized and optimized with the same techniques used for each PGIP.

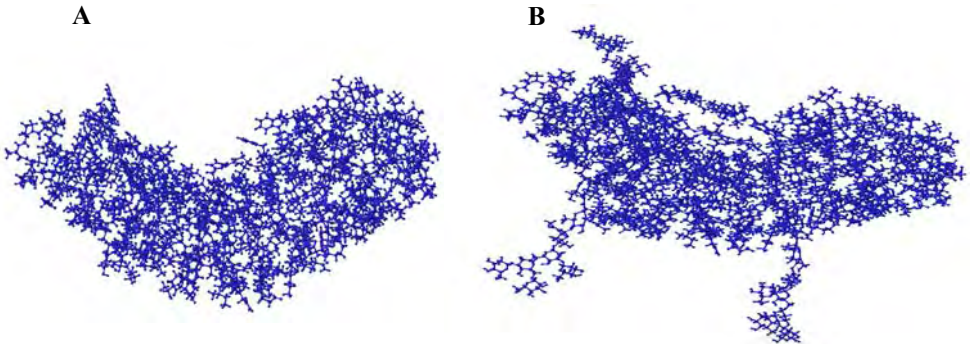

Figure 4. Homology models of (A) the PcBPGIP protein and (B) the protein with N-linked glycosylations. The concave face of the PGIP is thought to interact with PGs.

The inhibitory capability of each PGIP was determined through a series of dynamic reaction simulations where the effectiveness of the potential inhibition was measured by the ability of amino acids or glycosylations on the PGIPs to block key amino acids on the surface of the *Xf*PG that are responsible for cleaving the modeled polygalacturonan (PGA) substrate. The *Xf*PG model was put through a series of simulations with the PGA in its active cleft and keeping various groups of amino acid residues on the outer β sheet locked in place until immobilizing a particular group of residues inhibited the enzyme's *in silico* cleavage of the substrate (**Figure 5A**). It was determined that two clusters of amino acids, 63-74 & 223-226, control the ability of *Xf*PG to cleave its substrate. Dynamic reaction simulations were carried out with the PG, PGIP, and PGA substrate to determine if the PGA was cleaved and therefore, to what extent the PGIP inhibited the PG (**Figure 5B**). The dynamic reaction simulations were supplemented by preliminary surface chemistry mapping in BioMedCAChe to determine

if compatible acid/base regions were present on the LRR face of the PGIP and the previously demonstrated controlling region of *Xf*PG. Both techniques identified PcBPGIP, CsiPGIP, and OsPGIP1 as the potentially most effective inhibitors of *Xf*PG.

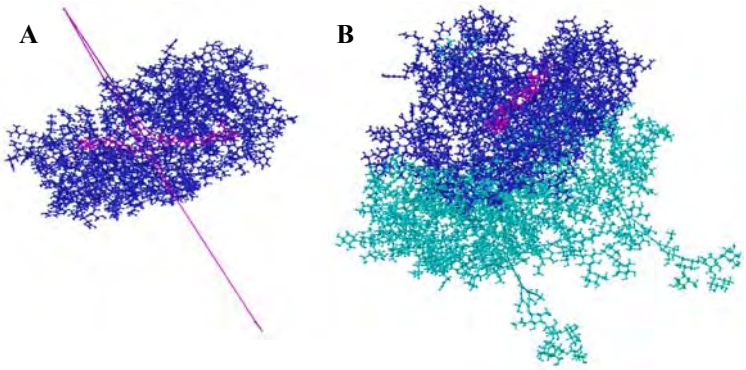

Figure 5. *Xf*PG homology models. (A) *Xf*PG (blue) with PGA (pink) being cleaved in its active site. (B) Dynamic reaction simulation of PcBPGIP (green) inhibiting substrate cleavage by blocking certain residues away from the cleft of *Xf*P.

Objectives 3 and 4
No activity planned for this reporting period.

CONCLUSIONS

1. Fourteen selected PGIPs have been identified that are likely candidates to effectively inhibit *Xf*PG.
2. Cloning has progressed to obtain each of the selected PGIPs in a format so their *in planta* and *in vitro Xf*PG inhibiting activities can be tested.
3. Molecular modeling has progressed so that differences in ability to inhibit *Xf*PG can be related to unique conformational properties of the selected PGIPs.
4. *Xf*PG has been expressed in Drosophila cells to obtain material for *in vitro* analysis of the inhibition activity of the selected PGIPs.
5. Relevant federal agencies have been consulted for regulatory issues related to commercial product development.

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.

Cervone F, DeLorenzo G, Pressey R, Darvill AG, Albersheim P. 1990. Can *Phaseolus* PGIP inhibit pectic enzymes from microbes and plants? Phytochemistry 29: 447-449.

Di Matteo A, Federici L, Mattei B, Salvi G, Johnson KA, Savino C, De Lorenzo G, Tsernoglou D, Cervone F. 2003. The crystal structure of polygalacturonase-inhibiting protein (PGIP), a leucine-rich repeat protein involved in plant defense. Proc. Natl. Acad. Sci. U.S.A. 100: 10124-10128.

Federici L, Caprari C, Mattei B, Savino C, Di Matteo A, De Lorenzo G, Cervone F, Tsernoglou D. 2001. Structural requirements of endopolygalacturonase for the interaction with PGIP (polygalacturonase-inhibiting protein). Proc. Natl. Acad. Sci. U.S.A. 98: 13425-13430.

Joubert DA, Kars I, Wagemakers L, Bergmann C, Kemp G, Vivier MA, van Kan JAL. 2007. A polygalacturonase-inhibiting protein from grapevine reduces the symptoms of the endopolygalacturonase BcPG2 from *Botrytis cinerea* in *Nicotiana benthamiana* leaves without any evidence for in vitro interaction. Mol. Plant Microbe Interact. 20: 392-402.

King D, Bergmann C, Orlando R, Benen JAE, Kester HCM, Visser J. 2002. Use of amide exchange mass spectrometry to study conformational changes within the endopolygalacturonase II-homogalacturonan-polygalacturonase inhibiting protein system. Biochemistry 41: 10225-10233.

Labavitch JM. 2006. The pit membrane barrier to *Xylella fastidiosa* movement in grapevines: Biochemical and physiological analysis. Proceedings of the 2006 Pierce's Disease Symposium, p. 280-282.

Labavitch JM. 2007. The pit membrane barrier to *Xylella fastidiosa* movement in grapevines: Biochemical and physiological analyses. Proceedings of the 2007 Pierce's Disease Symposium, p. 256-260.

Labavitch JM. 2008. Optimizing grape rootstock production and export of inhibitors of *Xylella fastidiosa* polygalacturonase activity. Proceedings of the 2008 Pierce's Disease Symposium, p. 214-219.

Labavitch JM, Backus EA, Morgan D. 2006. The contribution of the pectin-degrading enzyme polygalacturonase (PG) in transmission of *Xylella fastidiosa* to grape and the use of PG-inhibiting proteins for transgenic resistance to Pierce's Disease. Proceedings of the 2006 Pierce's Disease Symposium, p. 287-289.

Leckie F, Mattei B, Capodisca C, Hemmings A, Nuss L, Aracri B, De Lorenzo G, Cervone F. 1999. The specificity of polygalacturonase-inhibiting protein (PGIP): a single amino acid substitution in the solvent-exposed β-strand/β-turn region of the leucine-rich repeats (LRRs) confers a new recognition capability. EMBO J. 18: 2352-2363.

Lim JM, Aoki K, Angel P, Garrison D, King D, Tiemeyer M, Bergmann C, Wells L. 2009. Mapping glycans onto specific N-linked glycosylation sites of *Pyrus communis* PGIP redifines the interface for EPG-PGIP interactions. J. Proteome Res. 8: 673-680.

Powell ALT, van Kan J, ten Have A, Visser J, Greve LC, Bennett AB, Labavitch JM. 2000. Transgenic expression of pear PGIP in tomato limits fungal colonization. Mol. Plant Microbe Interact. 13: 942-950.

Roper MC. 2006. The characterization and role of *Xylella fastidiosa* plant cell wall degrading enzymes and exopolysaccharide in Pierce's Disease of grapevine. Ph.D. dissertation. University of California, Davis. 128 pages.

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.

Shackel KA, Celorio-Mancera MP, Ahmadi H, Greve LC, Teuber LR, Backus EA, Labavitch JM. 2005. Micro-injection of lygus salivary gland proteins to simulate feeding damage in alfalfa and cotton. Arch. Insect Biochem. Physiol. 58: 69-83.

Sicilia F, Fernandez-Recio J, Caprari C, De Lorenzo G, Tsernoglou D, Cervone F, Federici L. 2005. The polygalacturonase-inhibiting protein PGIP2 of *Phaseolus vulgaris* has evolved a mixed mode of inhibition of endopolygalacturonase PG1 of *Botrytis cinerea*. Plant Physiol. 139: 1380-1388.

Stotz HU, Powell ALT, Damon SE, Greve LC, Bennett AB, Labavitch JM. 1993. Molecular characterization of a polygalacturonase inhibitor from *Pyrus communis* L. cv Bartlett. Plant Physiol. 102: 133-138.

Thorne ET, Young BM, Young GM, Stevenson JF, Labavitch JM, Matthews MA, Rost TL. 2006. The structure of xylem vessels in grapevine (*Vitaceae*) and a possible passive mechanism for the systemic spread of bacterial disease. Am. J. Bot. 93: 497-504.

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