- **Title of report:** Interim Progress Report for CDFA Agreement Number 10-0278.
- **Title of Project:** Tools for Identifying PGIP transmission from grapevine rootstock to scion
- **Principal Investigators:**

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- **Time Period Covered by Report:** The results reported are from work conducted October 2012 to March, 2013.
- **Objectives:**
  - ***Objective 1*** - Using existing fresh pear flesh, prepare pPGIP protein and provide it to Antibodies, Inc. to develop mouse hybridoma lines expressing monoclonal antibodies against the pear PGIP.
  - ***Objective 2*** - Calibrate the antibodies produced by the hybridoma clones to determine effective dilutions for use in detecting the pPGIP protein.
  - ***Objective 3*** - Use the antibody to detect transgenic pear PGIP in xylem sap of own-rooted and grafted grapevines.
- **Description of Activities:**
  - ***Objective 1: Purification of pear PGIP from transgenic Arabidopsis leaves and pear fruit.***

  Because of budget limitations, we abandoned purification of the pear PGIP from transgenic Arabidopsis leaves engineered to express a tagged version of the protein.

  We purified sufficient active pear fruit PGIP (pPGIP) from immature green pears for evaluation of the antibodies being prepared by Antibodies Inc. Approximately 195 μg of protein was obtained and is active against PGs produced in culture by the Del 11 strain of *B. cinerea*, as expected. Figure 1 shows results from a previous report documenting the purity of the protein. As described in Objective 2, we decided not to use this protein itself to develop monoclonal antibodies because of its extensive glycosylation, typical of plant proteins.

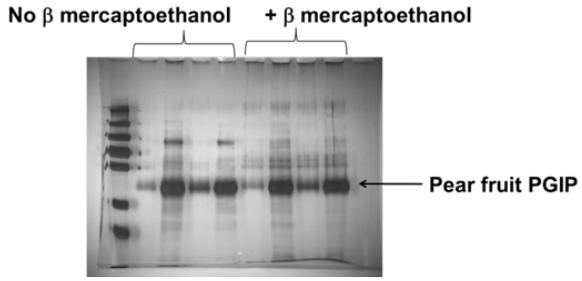

Figure 1. Silver stained SDS-PAGE gel showing pPGIP collected from cation exchange column fractions. Loading dye containing β mercaptoethanol causes a reduction of, presumably, multimeric PGIP proteins (90 kDa). The 90 kDa band in the presence of β mercaptoethanol is resolved into the 45 kDa pPGIP bands. Differences in glycosylation may account for PGIP sub-bands around 45 kDa.

- ***Objective 2 - Calibrate the antibodies produced by the hybridoma clones to determine effective dilutions for use in detecting the pPGIP protein.***

Based on the concern noted above that authentic pPGIP protein may not permit generation of sufficiently specific anti-pPGIP monoclonal antibodies, we worked with Richard Krogsrud, CEO of Antibodies Inc., to identify hydrophilic peptide sequences in the pPGIP protein sequence that could be used as antigens. We selected 3 peptides (Figure 2) that would be specific to pPGIP and would be likely to assure that the antibodies would not recognize other PGIPs. We decided to mix the three peptides when they are administered to the mouse cells to optimize the chances of getting a specific and robust antibody. In addition, we identified a peptide that has a sequence that is conserved in PGIPs. We selected this peptide to generate a new polyclonal antibody that can be used to detect other PGIPs in addition to the pPGIP. The peptides have been synthesized through subcontractors used by Antibodies Inc.. We expect that the peptides will be introduced into the cells by the third week of March, 2013.

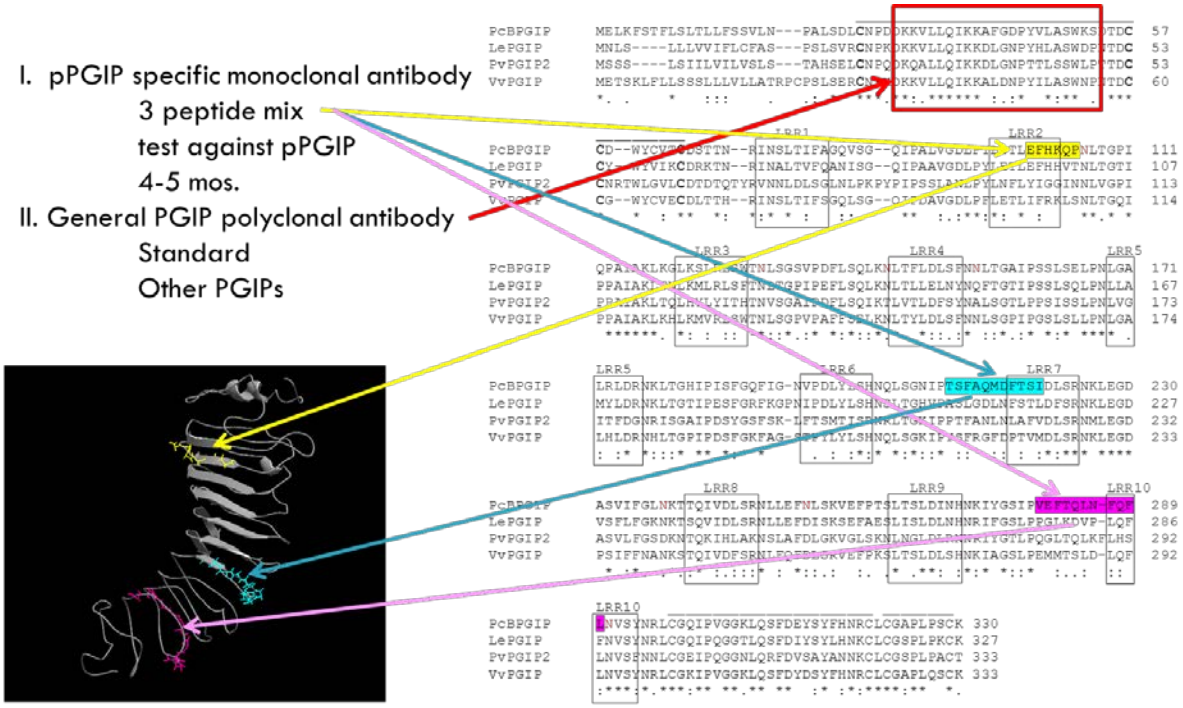

Figure 2. Amino acid sequence of pear (pcPGIP), tomato (LePGIP), common bean (pvPGIP) and grape (vvPGIP) showing the location of the leucine-rich repeats (LRR) and the three pPGIP specific peptides (in yellow, blue and pink) and the peptide common to all PGIPs (in red). Locations on the predicted 3-D structure of PGIP are shown.

- ***Objective 3 - Use the antibody to detect transgenic pear PGIP in xylem sap of own-rooted and grafted grapevines.***

Will commence once the antibodies have been received at UC Davis.

- **Publications:**

None.

- **Research relevance statement, indicating how this research contributes towards finding solutions to Pierce's disease in California:**

In response to the strategy recommended by the Advisory Board to enhance the resistance of grapevines to PD, several field trial projects have used alternative approaches to optimally express plant genes for particularly effective PGIPs targeting the *X. fastidiosa* PG (*Xf*PG) in transgenic grape rootstocks. This project was designed to generate a monoclonal antibody that specifically recognizes the pPGIP protein. The monoclonal antibody is a necessary tool for the multiple field trial projects evaluating the efficacy of pPGIP as an anti-Xf strategy. The antibodies will allow for detection and quantification of pPGIP without cross-reactive interference from the native PGIP and will allow comparisons between groups. Plants can, therefore, be more efficiently screened for the presence of the pPGIP protein, whether directly produced in, or transported to the plant tissue of interest from grafted rootstocks.

The goal of the project is to provide the resources needed for the field trial projects that are designed to help the California grape industry develop a strategy that uses plant genes to limit the damage caused by *Xf* and to mobilize this technology with non-transgenic vines grafted on the disease limiting rootstocks. The project's outcomes should provide growers with plants that resist PD and produce high quality grapes.

- **Layperson summary:**

X. fastidiosa (*Xf*), the bacteria that causes Pierce's Disease (PD) in grapevines, utilizes a key enzyme, polygalacturonase (XfPG), to spread from one grapevine xylem vessel to the next, eventually leading to the development of PD symptoms because the bacteria multiply and interrupt the flow of nutrients and water through the vessels in the plant. Plant proteins called PG-inhibiting proteins (PGIPs) selectively inhibit PGs from bacteria, fungi, and insets. Our work (Abu-Goukh et al., 1983) identified a PGIP from pear fruit that at least partially inhibits the XfPG and we demonstrated reduced PD symptom development in grapevines expressing the pear fruit PGIP. Current projects, including field trial evaluations, require a monoclonal antibody specifically recognizing the pear fruit PGIP protein in order to detect, quantify, and characterize the PGIP protein delivered to the scion portion of grafted plants from rootstocks expressing the pear fruit PGIP (Aguero et al., 2005). The monoclonal antibody will allow the researchers to compare the amounts of the PGIP protein at different times and places and thereby determine the protein's role in XfPG inhibition in grapevines. We have purified active pear PGIP from green pear fruit to evaluate the specificity of monoclonal and polyclonal antibodies prepared by a firm which specializes in antibody production to meet the needs of the collaborating groups and we have set up an alternative strategy to generate more robust monoclonal antibody candidates.

- **Status of funds:** From the original $14,070 award, as of 28 February 2013, $4,809 has been spent plus $7946 is contracted to be paid to Antibodies Inc. as they deliver the antibodies and clones over the coming 6 months. The remaining funds ($1315) will go toward supplies when testing the antibodies.
- **Summary and status:**

The ability to compare multiple PGIPs to determine an optimal inhibitor for specific PGs is key for developing transgenic grape rootstocks as strategies against pathogens that utilize PG(s) for virulence. Several field trials have been supported by the CDFA and GWSS boards for evaluating resistance strategies involving PGIPs. An important tool for making these comparisons is needed to measure the amount of PGIP. Monoclonal antibodies are the tools that allow this comparison. We prepared authentic pPGIP protein from pear fruit but reconsidered using it to generate the antibodies. However, this protein will be useful for future testing of the

strength and specificity of the antibodies we are having made to PGIP-specific peptides. We encountered difficulties in 2011/12 with Antibodies Inc., due to their internal reorganization and we tried to work with an off-shore company to make the antibodies. This interaction did not prove to be productive and so we went back to Antibodies Inc. in late 2012, to see if they could now provide the service. They have been very helpful and the peptides for the antibodies have been synthesized and are in the pipeline for monoclonal and polyclonal antibody production. However, because of these delays due to the issues finding an appropriate partner to produce the antibodies, we are asking for a no-cost extension so we can receive the antibodies (expected at the beginning of Summer, 2013) and then test for specificity etc. Attached is our no-cost extension request that has been filed with UC Davis and the CDFA.

REFERENCES CITED:

Abu-Goukh AA, Greve LC Labavitch JM 1983. Purification and partial characterization of "Bartlett" pear fruit polygalacturonase inhibitors. Plysiological Plant Pathology 23:111-122.

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.

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COOPERATIVE EXTENSION

No Cost Extension Request

Title: Tools for Identifying PGIP transmission from grapevine rootstock to scion

PI: Ann L.T. Powell

CoPIs: Abhaya Dandekar and John Labavitch

Original Duration: 1 year

Original Start Date: 1 July 2010

Requested new end date: 30 June 2014

SPO number: 201013014, Contract number: 10-0278

Total original budget: $14,070

Justification for request:

Antibodies Inc. is in the midst of preparing clones for the generation of monoclonal antibodies and a new polyclonal antibody to pPGIP. The preparation of these was the objective of the initial proposal. In 2011/12, Antibodies Inc. had an internal reorganization and was not able to respond to our requests. We pursued other options through overseas companies, but none could provide appropriate services. Early in 2013, we reinitiated contact with Antibodies Inc. and they are now able to produce the cell lines and polyclonal antibody. On 13 March 2013, Richard Krogsrud (Antibodies Inc. CEO) responded:

Hi Ann,
The peptides and conjugates will be in next week (promised). Jenny will have mice ready for the hybridoma (injection of the cocktail of 3 peptides) and rabbits for the injection of the long peptide.
Thanks for asking,
I was just looking at that schedule yesterday. It's a week later than originally planned.
I'll let you know and then try to keep you informed of progress.
Regards,

We received a quote from Antibodies Inc. to do this work and expect to pay them $7946 in two installments over the next 6 months as the work is performed.

Therefore, in order to be sure to obtain the cell lines and antibodies and analyze the cross-reactivity with the authentic pPGIP protein we have prepared from pear fruit, we are requesting a 6 month extension.

Budget for Contract Extension Time Period – July 1, 2013 to June 30, 2014

	Amount in original budget ($)	Amount spent to date (1 July 2010-28 Feb 2013) ($)	Amount to be spent through current ending date 30 June 2013	Amount to be spent in extension to 31 Dec 2013 ($)
Personnel				
Professional	950	771	0	0
SRA/Tech				
Lab Assistant				
Other				
Employee Benefits	29	204	0	0
SUBTOTAL (Personnel + Benefits)	979	975	0	0
Supplies and Expenses (Incl. Antibodies Inc. charges)	13,091	3,834	0	9,261
Equipment				
Travel				
Computer Time				
Other				
Indirect Costs*				
SUBTOTAL (Supplies, Expenses, Equipment, etc.)	13,091	3,834	0	9,261
TOTAL	14,070	4,809	0	9,261

Sincerely,

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