

- **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 March 2013 to July 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.

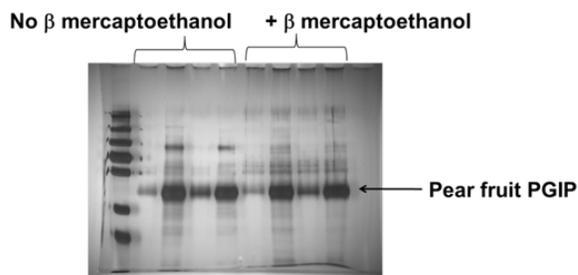


Figure 1. Silver stained SDS-PAGE gel showing pPGIP collected from cation exchange column fractions. Loading dye containing β mercaptoethanol causes a reduction of, presumably, multimeric PGIP proteins (90 kDa). The 90 kDa band in the presence of β mercaptoethanol is resolved into the 45 kDa pPGIP bands. 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 result in the 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 from previous report) that would be specific to pPGIP and would be likely to assure that the antibodies would not recognize other PGIPs. We intended to mix the three peptides when they are administered to the mouse cells to optimize the chances of getting a specific and robust antibody. We also identified a peptide from the conserved amino end of the 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. although one of the pPGIP-specific peptides has been recalcitrant to conjugation. Antibodies Inc. has gone ahead and developed hybridomas using the other two pPGIP-specific peptides. We have not yet received antibodies back from the hybridomas at Antibodies Inc. to check. Staff at Antibodies Inc. has said the monoclonal antibodies may be available at the end of July.

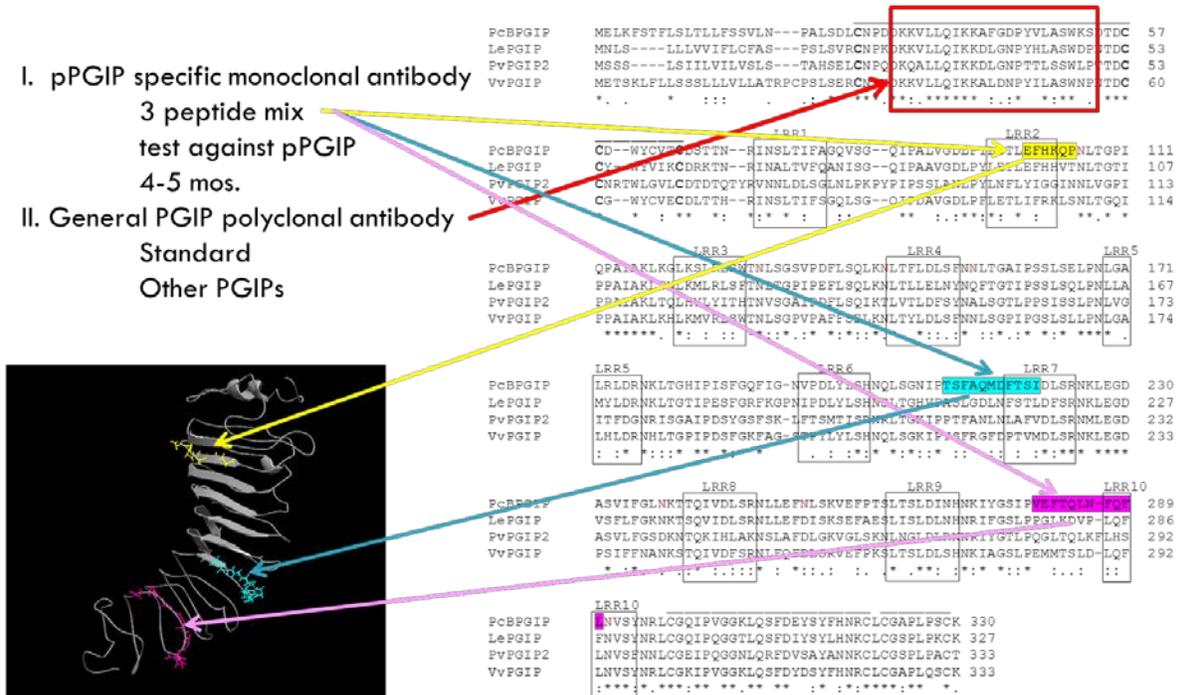


Figure 2. Amino acid sequence of pear (pcPGIP), tomato (LePGIP), common bean (pvPGIP) and grape (vvPGIP) showing the location of the leucine-rich repeats (LRR) and the three pPGIP specific peptides (in yellow, blue and pink) and the peptide common to all PGIPs (in red). Locations on the predicted 3-D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

In May 2013, we received the first test bleed and pre-immune sera from the polyclonal antibody preparations generated against the general PGIP peptide. This antibody preparation is considered a general PGIP antibody because it is was generated in response to a conserved region at the amino end of the PGIP proteins. We have used the pre-immune serum on a western blot with protein extracts from tomato plants expressing pPGIP and the purified pear fruit pPGIP

protein described above. The antibody specifically recognizes the purified pPGIP protein from pear fruit as well as the pPGIP protein expressed in the tomato Cuatomate line 3-15 (Figure 3a).

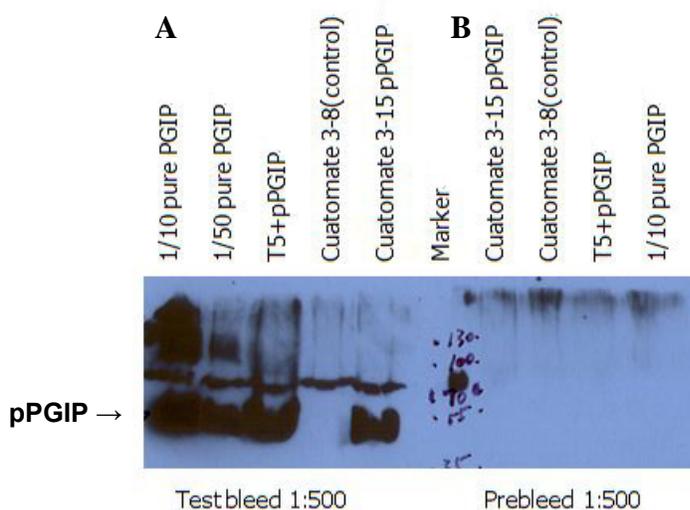


Figure 3. Image of western blot of proteins cross-reacted with antibodies from the first test bleed serum in response to the general PGIP peptide (A) and the pre-bleed serum from the rabbits (B).

The antibody preparation does not detect tomato PGIPs in the Cuatomate material 3-8, which is not transformed to express pPGIP. With the antibodies from the first test bleed, we were able to detect strongly just the pPGIP band in the same protein preparations used to check the pre-immune serum (Figure 3b). We detected no cross-reactivity with the pre-immune serum (Figure 3b). On 18 July, 2013, the final bleed sera were received from Antibodies Inc., and brought to UC Davis. They will be aliquoted, stored and distributed.

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

Activities for this objective commence once the monoclonal antibodies have been received at UC Davis. The western blot with the new polyclonal antibodies in Figure 3 contains proteins from the leaves of a tomato line expressing pPGIP; similar results have been obtained with xylem sap collected from the cut stem of the same plants. Efforts to collect xylem sap from pPGIP-expressing grapevines has yielded only a very small amount of protein and the expected greater sensitivity of the monoclonal antibodies is necessary to detect this pPGIP from grapevine xylem exudate.

- **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 insects. 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 CDFR 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 went back to Antibodies Inc. in late 2012, and asked for their help in designing pPGIP-specific peptides for monoclonal production. They also helped us design a more general peptide that can be used to detect multiple PGIPs. This peptide has been successful in producing a new preparation of polyclonal antibodies that is reasonably specific in recognizing pPGIP. The monoclonal antibodies for two of the pPGIP-specific antibodies is expected by the end of summer, 2013.

REFERENCES CITED:

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

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