I. PROJECT TITLE.
TOOLS TO IDENTIFY PGIPS TRANSMITTED ACROSS GRAPEVINE GRAFTS

II. PRINCIPAL INVESTIGATORS.

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Reporting Period: The results reported here are from work conducted 1 October 2010 to 1 March 2011.

III. OBJECTIVES.

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.
2. Calibrate the antibodies produced by the hybridoma clones to determine effective dilutions for use in detecting the pPGIP protein.
3. Use the antibody to detect transgenic pear PGIP in xylem sap of own-rooted and grafted grapevines.

IV. Summary of major research accomplishments and results for each objective.

Objective 1: Purification of pear PGIP from transgenic Arabidopsis leaves and pear fruit.

The generation of a monoclonal antibody requires purified protein to be used as the antigen. PGIPs are plant cell wall proteins that require plant specified glycosylation for activity (Powell et al., 2000). The project "Optimizing grape rootstock production of and export of inhibitors of *Xylella fastidiosa* (*Xf*) polygalacturonase (PG) activity" (Labavitch, 2008) generated transgenic *Arabidopsis thaliana* plants expressing the pPGIP protein fused to a C-terminal histidine tag for purification. Leaves from these transgenic plants yielded a minimal amount of total protein, as determined by Bradford assays. Work to refine the transgenic protein purification process is ongoing.

pPGIP extraction has begun from fresh pear fruit flesh using the protocols in Stotz et al. (1993) with modifications. In the summer of 2010, peeled, cored, and sliced pear fruit (2 kg) were homogenized in 2 L of extraction buffer (1 M sodium acetate, pH 6, 1 M NaCl, 1 % [w/v] polyvinylpolypyrrolidone, 0.2% [w/v] sodium bisulfite) and then stirred at 4°C for 1 hour before filtration through three layers of Miracloth. The liquid fraction was collected by centrifugation (10,000 rpm, 20 min, 4°C). Total protein precipitating between 50% and 100% ammonium sulfate saturation was collected and resuspended in 100 mM sodium acetate, pH 6, and extensively dialyzed at 4°C against 10 mM sodium acetate, pH 6, using 6000-8000 molecular weight cut-off membranes. The dialyzed ammonium sulfate fraction was mixed with an equal volume of 2x ConA buffer (200 mM sodium acetate, pH 6, 2 M NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 2 mM $MnCl_2$). A small volume was applied to a Concanavalin A-sepharose column which was then washed with several volumes of 1x ConA buffer. Bound protein was eluted with 250 mM and 1 M methyl-α-D-mannopyranoside. The initial column chromatography purification has not separated the glycosylated pPGIP protein from the dialyzed fraction, potentially due to improper binding to the Con A matrix.

pPGIP activity was measured throughout purification by radial diffusion assays (Taylor and Secor, 1988). Samples of the initial pear homogenate were able to fully inhibit a PG (*Bc*PG) mixture from *Botrytis cinerea* culture filtrates. The pPGIP purification preparations resulted in 66% and 59% reductions in BcPG activity after ammonium sulfate precipitation and subsequent dialysis, respectively.

We concluded that the preparation was too dilute to process further. The isolation procedure has been modified and will advance once we find a good source of sufficient green pear fruit.

We are requesting a one year extension for this project because it was not possible to begin work on the project until quite late in 2010. The account for the project only became available on 21 September, 2010, well after the peak collection time for pear fruit of the appropriate ripeness for purification of the pPGIP protein needed to generate the antibody. The start of the project was delayed because the original request was substantially modified at the request of the CDFA and GWSS board: salary support (1%) for the PI was essentially removed and the term of the budget was cut in half.

The preparation of the protein is expected to take about 2 months and the generation of the antibody will take the remainder of the year.

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

Will commence once the antibody has been generated.

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

Will commence once the antibody has been generated.

V. PUBLICATIONS OR REPORTS RESULTING FROM THE PROJECT.

None

VI. PRESENTATIONS ON RESEARCH.

Poster presentation by Zachary Chestnut at the PD-GWSS annual meeting, San Diego CA. December 2010.

VII. RESEARCH RELEVANCE STATEMENT.

Pierce's Disease (PD) incidence has been associated with the spread of *Xylella fastidiosa* (*Xf*), throughout the xylem vasculature of infected grapevines. The spread of bacteria from one vessel to the next utilizes bacterial cell wall modifying enzymes to degrade the pit membranes separating adjacent vessels (Pérez-Donoso et al., 2010). One enzyme, a polygalacturonase (*Xf*PG), has been well characterized and is a PD virulence factor (Roper et al., 2007). Several previous projects have analyzed the effectiveness of PG-inhibiting proteins (PGIPs) in minimizing the detrimental effects of pathogen and pest attack on various plants. Two currently funded projects use pear fruit PGIP (pPGIP) to restrict *Xf* movement: "Optimizing grape rootstock production and export of inhibitors of *X. fastidiosa* PG activity" (PI Labavitch) and "*In planta* testing of signal peptides and antimicrobial proteins for rapid clearance of *Xylella*" (PI Dandekar).

This project was developed to generate a monoclonal antibody that selectively recognizes the pear fruit pPGIP protein. The monoclonal antibody is a necessary tool for both aforementioned research projects and the related project "Field evaluation of grafted grape lines expressing PGIPs" (PI Powell) and will allow for detection and quantification of pPGIP specifically without cross-reactive interference from the native PGIP. 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.

The original request was for 2 years to generate monoclonal antibodies to the pear fruit PGIP and a second PGIP that are being tested in field trials. The trials are designed to test the feasibility and efficacy of grafted rootstock lines expressing pPGIP. The monoclonal antibody will be used to make quantitative comparisons of the amounts of pPGIP in the tissues.

References

Pérez-Donoso A, Sun Q, Roper MC, Greve LC, Kirkpatrick B, Labavitch JM. 2010. Cell Wall-Degrading Enzymes Enlarge the Pore Size of Intervessel Pit Membranes in Healthy and *Xylella fastidiosa*-Infected Grapevines. Plant Physiol. 152: 1748-1759.

Fergus CL. 1952. The Nutrition of *Penicillium digitatum* Sacc. Mycologia. 44: 183-199.
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.
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, 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.
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.
Taylor RJ and Secor GA. 1988. An Improved Diffusion Assay for Quantifying the Polygalacturonase Content of *Erwinia* Culture Filtrates. Phytopathology. 78: 1101-1103.

VIII. LAY SUMMARY OF CURRENT YEAR'S RESULTS.

The CDFA Pierce's Disease and Glassy-winged Sharpshooter Board's Research Scientific Advisory Panel review in 2007 and subsequent RFPs have given top priority to delivery of PD control candidates, including polygalacturonase-inhibiting proteins (PGIPs), from grafted rootstocks. Four currently funded projects (two research projects and two field trials) use expression of PGIPs as a control strategy to limit the spread of *X. fastidiosa* in the xylem network and thereby limit PD symptom progression in infected vines. A monoclonal antibody recognizing the pear fruit PGIP, the protein expressed in the grape lines in the four projects, is being developed to detect, quantify, and observe the localization of the protein in transformed grapevines and in grafted vines with transformed rootstocks. Pear PGIP protein is being isolated from mature green 'Bartlett' pear fruit tissue. Monoclonal antibody production by Antibodies, Inc. will begin once sufficient quantities of properly glycosylated, active PGIP have been purified.

X. fastidiosa utilizes a key enzyme, polygalacturonase (*Xf*PG), to spread from one xylem vessel to the next, eventually leading to the development of Pierce's Disease symptoms in infected vines. Plant proteins, called PG-inhibiting proteins (PGIPs), selectively inhibit PGs from bacteria, fungi, and insects, including the *Xf*PG. Our work has identified a PGIP from pear fruits as partially inhibiting 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 to detect, quantify, and characterize the protein's role in *Xf*PG inhibition in transformed grapevines. We are purifying sufficient pear PGIP protein to produce a monoclonal antibody that recognizes this protein.

IX. STATUS OF FUNDS.

About $13,000 remains of the original funds because the work has been limited thus far. A one year extension has been requested. Most of the budget was to support the actual costs of the monoclonal antibody preparation by Antibodies, Inc.

X. SUMMARY AND STATUS OF INTELLECTUAL PROPERTY PRODUCED DURING THIS RESEARCH PROJECT.

None applicable.