Progress Report for CDFA Contract Number: 04-0486

Project Title: Identification and utilization of cold temperature induced grapevine metabolites to manage Pierce's disease.

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Time Period: January 2010-February 2011

Project Objectives:

1) Over express the grapevine thaumatin-like protein (TLP) in transgenic grapevines. Prepare anti-TLP antibodies to quantify TLP in transgenic xylem sap using ELISA.

We have cloned and expressed grapevine TLP in *E. coli* (Figures 1 and 2). When *Xf* is incubated with TLP it shows less growth than compared to *Xf* incubated with water or a potasium buffer, data was shown in the 2010 PD/GWSS Conference proceedings. This is a promising result, which we think validates our approach. We are now in the process of making the appropriate TLP-Agrobacterium constructs to submit to the UC Davis plant Transformation facility. Based on our experience in obtaining transgenic grapevines expressing Xf hemagglutinin proteins, we anticipate that it will take approximately 16 months before we will have sufficient transgenic materials expressing TLP.

We are also in the process of making a larger quantity of pure recombinant TLP to be given to the Comparative Pathology Laboratory at UC Davis for polyclonal antibody production. TLP-specific antibodies will subsequently be used for quantification of TLP in the transgenic grapevines.

2) Inoculate TLP-expressing grapevines with Xf and determine the incidence and severity of PD in transgenic versus non-transgenic *V. vinifera*.

This is dependent on first acquiring grapevines transformed with TLP, which is a goal for the coming year.

3) a. Fractionate and chemically characterize the phenolic compounds that are present in xylem sap from cold-exposed grapevines.

We have collected xylem sap from vineyards located in Placerville, CA and Winters, CA during the months of January through April 2010, and are again finishing our collection of sap from January through April of 2011. We have been collecting sap from Cabernet Sauvingon, clone 8 vines grafted on 110 rootstock and Pinot Noir clone 2A grafted on 101-14 rootstock. The Andy Waterhouse lab in the Viticulture and Enology Department at UC Davis has been analyzing these

xylem sap samples by HPLC/MS to determine which phenolics are present. Initial analysis of phenolic compounds in the 2010 collection was reported in detail in the 2010 PD/GWSS Conference Proceedings.

b. Compare the phenolic content of xylem sap of grapevines treated with ABA under non-freezing conditions to phenolics in cold-exposed xylem sap.
If the applications of ABA to field grown vines shows promise as a means of inducing the cold curing process under non-freezing conditions then we will collect sap from ABA-treated field vines at the time of application in November and bi-monthly collections thru the winter. Saps will be analyzed by the Waterhouse lab for presence and relative quantities of phenolics.

c. Determine if these compounds affect Xf growth/survival *in vitro.*
The paper by Maddox et. al. published in October of 2009 in the journal Current Microbiology examined the effect that different polyphenolic compounds on *Xf* growth *in vitro*. One of the compounds they tested was trans-resveratrol. They found that this compound inhibited the growth of *Xf in vitro*, which is interesting because in our analysis of "cold sap" we found that resveratrol was present, while it was absent in "warm sap. However the concentrations used in the Maddox study may not reflect the actual concentrations of phenolics that are present in grapevine xylem sap. The determination of actual concentrations of various phenolic compounds in sap is a time-consuming endeavor which the Waterhouse lab is now in the process of undertaking.

We will further assess the potential toxicity of the various grapevine saps we have collected over the past 2 years on Xf in *in vitro* assays. Results of these toxicity studies will be available by fall, 2011.

4) Determine if foliar and drench applications of ABA can increase PD-curing rates in field-grown vines under non-freezing conditions.
Our previous research showed that ABA applications to greenhouse grown Cabernet Sauvignon and Pinot Noir grapevines infected with *Xf* increased overwinter curing rates in Davis, CA, a location with relatively mild winter temperatures that do not typically induce PD cold curing. Our previous work also showed that these vines had higher levels of polyphenolics than did vines growing in Davis which did not receive ABA applications (Meyer and Kirkpatrick, 2011). In October of 2010 we applied foliar sprays and root drenches of ABA to *Xf* infected vines growing in Napa. We will evaluate the potential effect of these applications of ABA on the incidence and severity of PD symptoms in treated-infected and infected, non-treated vines in August, 2011.

Research Accomplishments: We have successfully cloned and expressed grapevine TLP in *E. coli*. We performed a time course experiment in which *Xylella fastidiosa* was combined with dialyzed TLP, and then plated onto PD3 media at intervals over two days. As controls we also performed the same procedure with *Xf* and water, *Xf* with empty vector supernatant (*E. coli* that was not transformed with TLP), and *Xf* with a potassium buffer. We plated this suspension directly after combining (0 hours), 16 hours, 24 hours, 40 hours and 48 hours after combination. These plates were incubated at 28C for 10-14 days. Our results showed that the early platings showed no differences in *Xf* growth. By 48 hours post incubation, the *Xf* and water control as

well as the *Xf* and potassium buffer control still produced numerous *Xf* colonies. The *Xf* cells that were incubated with the empty vector supernatant showed growth, but less than the water and potassium buffer. *Xf* cells that were incubated with dialyzed TLP did not show any growth.

We have analyzed sap samples collected from Placerville, Ca (during the months of January and February) where PD cold curing occurs, as well as sap from Winters, CA where PD cold curing does not take place. We are working towards producing accurate polyphenolic profiles for Cabernet Sauvignon, clone 8 on 110R rootstock and Pinot Noir, clone 2A on 101-14 rootstock. In the Placerville (cold) Pinot Noir samples, a number of phenolic compounds were identified: B procyanidins, Catechin, Epicatechin, Trans-Resveratrol, Cafetaric acid, and a Resveratrol tetramer. Cabernet Sauvignon samples produced an identical polyphenolic profile except that the Resveratrol tetramer was not present. Interestingly, the warm Pinot Noir sap lacked characteristic peaks for Trans-Resveratrol as well as the Resveratrol tetramer. The fact that Resveratrol is present in vines that experience "cold curing" while it is absent in vines that do not undergo "cold curing" suggests that Resveratrol may play a role in the curing process. We will analyze the MS/HPLC data after two winters of collecting sap, and should be able to provide a more accurate picture of what happens in regards to polyphenolic synthesis during the winter.

Publications:

1) Meyer, M. and B.C. Kirkpatrick. 2011. Exogenous applications of abscisic acid increase curing of Pierce's disease-affected grapevines growing in pots. Plant Disease 95: 173-177.

2) We have completed the first draft of a paper describing the work we have done cloning TLP in *E. coli*, and the results of toxicity screens that were performed with recombinant TLP.

Research Presentations: We presented this work at the 2009 and 2010 Pierce's Disease Research Symposium both in the Symposium Proceedings and in poster form.

Kirkpatrick, B. 2010. Identification and utilization of cold temperature induced grapevine metabolites to manage Pierce's disease. PD/GWSS Research Symposium Proceedings, san Diego, California December 15-17. pgs 191-195

Research Relevance: This research shows promise as a means of controlling *Xylella fastidiosa.* Both the TLP and polyphenolic aspects of this project have shown promise as agents that can inhibit the growth of *Xf*. We are hopeful that further evaluating these results in grapevines growing in the field may lead to a novel approach to managing PD. Furthermore, because TLP is naturally found in grapevines, the public may be more open to the idea of the overproduction of TLP in modified grapevines as opposed to the introduction of an exogenous, non-grapevine gene product. Similarly, if the application of the plant hormone ABA shows an increase in anti-*Xf* activity the general public may accept this more readily than a genetically modified grapevine. However, there are significant differences between treating Xf-infected grapevines growing in 1 gallon pots in the lath house compared to treating large PD-affected vines growing in the field. Even if the application of ABA to field infected vines shows promise we will have to determine the costs involved with purchasing and applying ABA in the field. ABA applications on

grapevines growing in California have been registered, largely for increasing the color of table grapes.

Layperson Summary: We have succeeded in producing recombinant TLP in *E.* coli. W*e* have also observed that grapevine TLP produced by *E. coli* has a deleterious effect on *Xylella fastidiosa* when it is grown in the laboratory. This confirms our objective of expressing TLP in higher amounts in grapevines as a valid course of action. We have been characterizing the phenolic compounds in cold xylem sap and comparing these to warm xylem sap. We have noticed a number of differences, specifically the presence of the phenolic compound trans-resveratrol in cold sap and its absence in warm sap. This leads us to believe that trans-resveratrol may play a role in the cold curing process. We added trans-resveratrol to solid media used to grow *Xylella fastidiosa* and observed that the Temecula strain is inhibited at concentrations lower than at which the Fetzer strain is inhibited. This suggests that phenolic compounds play a role in the cold curing process. We are examining if root and foliar applications of the plant hormone ABA might be able to increase the winter PD curing rate of infected vines growing in regions with comparatively mild winters, such as Napa valley.

Status of Funds: As per April, 2011 approximately 1/3 of the appropriated funds were unspent.

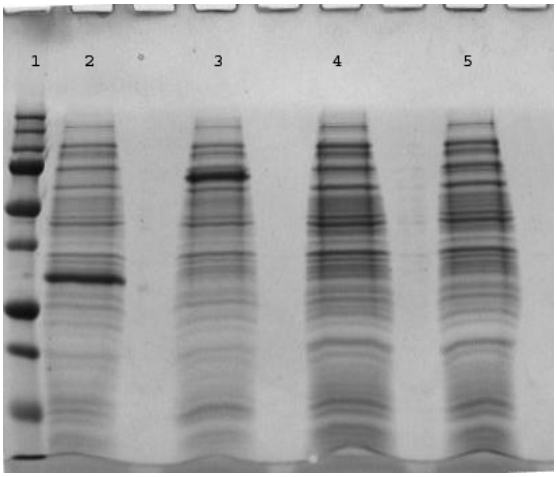

Figure 1: SDS-PAGE of recombinant *Vitis vinifera* 'Cabernet Sauvignon' TLP protein expression analyzed by SDS-PAGE. Lane 1: Dual Color SDS-ladder (lower band-25 kD; upper band-75 kD); Lane 2: Induced recombinant Cabernet Sauvignon TLP; Lane 3: Induced recombinant polygalacturonase (PG) (positive control); Lane 4: non-induced recombinant Cabernet Sauvignon TLP; Lane 5: non-induced recombinant polygalacturonase (PG).

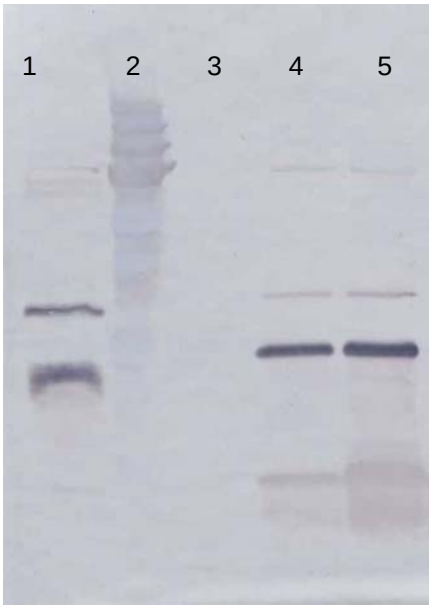

Figure 2: Western blot of SDS-PAGE of recombinant *Vitis vinifera* 'Cabernet Sauvignon' TLP protein expression analyzed by SDS-PAGE. Lane 1: CS3 raw lysate pellet (positive control); Lane 2: SDS Dual color ladder; Lane 3: Non-induced CS3 (negative control); Lane 4: CS3 dialysis purified pellet; Lane 5: CS3 dialysis purified supernatant.

References cited:

Chan, Marion Man-Ying, "Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin." Biochemical Pharmacology, January 2002, p. 99-104.

Cowan, Marjorie Murphy. "Plant Products as Antimicrobial Agents" Clinical Microbiology Reviews, October 1999, p. 564-582, Vol. 12, No. 4.

Frément, Lucie. "Biological effects of resveratrol" Life Sciences Volume 66 No.8, January 2000, p. 663-673.

Jeandet, Phillipe; Bessis, Roger; and Bernard Gautheron. "The Production of Resveratrol (3,5,4'-trihydroxystilbene) by Grape Berries in Different Developmental Stages" Am. J. Enol. Vitic. 42:1:41-46 (1991).

Kantz, K. and V.L. Singleton. 1990. Isolation and determination of polymetric polyphenolics using Sephadex LH-20 and analysis of grape tissue extracts. Am. J. Enol. and Viticul. 41:223-228.

Kirkpatrick, B. 2008 "Identifying factors mediating resistance to almond leaf scorch disease." In Proceedings of the 36th Almond Industry Conference, Almond Board of California, pp. 159-164.

Kirkpatrick, B. and T. Voegel, 2008. "Isolation, characterization and genetic manipulation of Xf hemagglutinin genes." Proceedings of the Pierce's disease Research Symposium, Calif. Dept. Food and Agriculture, pp. 176-179.

Kuwabara, C., D. Takezawa, T. Shimada, and K. Arakawa. 2002. "Abscisic acid and cold-induced thaumatin-like protein in winter wheat has an antifungal activity against snow mold, *Microdochium nivali"*. Physiolgia Plantarum 115: 101-110.

Langcake, P amd Pryce, RJ. "The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury Physiological Plant Pathology" p. 77-86 (1976).

Maddox, C. E., L. M. Laur, and L. Tian "Antibacterial Activity of Phenolic Compounds Against the Phytopathogen *Xylella fastidiosa"* Curr Microbiol. 2010 January; 60(1): 53–58.

Meyer, M. and B.C. Kirkpatrick. 2011. Exogenous applications of abscisic acid increase curing of Pierce's disease-affected grapevines growing in pots. Plant Disease 95: 173-177.

Meyer, M. and B. Kirkpatrick. "Identification of mechanisms mediating cold therapy of Xf-infected grapevines." Proceedings of the Pierce's disease Research Symposium, Calif. Dept. Food and Agriculture; 2005 pp242-246; 2006 pp. 236-239; 2007 pp. 208-211; 2008 pp. 167-171.

Purcell, A.H.. 1980. "Environmental therapy for Pierce's disease." Plant Disease 64:388-390.

Reddy, J.G., S.L. Reddy, D.L. Hopkins and D.W. Gabriel. 2007. "TolC is required for pathogenicity of Xf in *Vitis vinifera* grapevines." MPMI 20: 403-410.

Toshitsugu Taguri, Takashi Tanaka and Isao Kouno, "Antimicrobial Activity of 10 Different Plant Polyphenols against Bacteria Causing Food-Borne Disease", Biol. Pharm. Bull., Vol. 27, p. 1965-1969 (2004).

Waterman, P.G. and S. Mole. 1994. "Analysis of phenolic plant metabolites". Blackwell Scientific Publications, pp. 238.