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USDA-UC PD Grant Progress Report
Funding period (July 1, 2010- June 30, 2012)

I. **Grapevine xylem phenolic composition: Correlation with susceptibility to Pierce's Disease and induction trials**

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III. **List of objectives and description of activities conducted to accomplish each objective**

The objectives of the 2 year project are listed below:

1. Identify phenolic composition of xylem fluid in different species (from tolerant native vines to susceptible *Vitis vinifera*) to see if there is a correlation between composition and disease resistance over four seasons.
2. Identify phenolic composition of xylem fluid in different *Vitis vinifera* species (most susceptible to least susceptible) to see if there is a correlation between composition and disease resistance.
3. Compare phenolic composition of xylem fluid with *Xylella fastidiosa* (*Xf*) populations.
4. In preliminary experiments, year two: induce t-resveratrol in grapevine tissue and measure xylem sap concentration before and after treatment.

The accomplishments of the objectives are listed below:

1,2. To date, all seasonal sampling of *Vitis vinifera* canes have been completed at four discrete time points, Fall 2010, Winter, Spring and Summer 2011. Two resistant native vines, *Muscadinia rotundifolia* "Trayshed" and *Vitis arizonica* "643.17" were sampled twice, once in November 2010 and again in June 2011. Varietals used in this study as well as locales are listed in Supplement, Table 5. and a map of the sites is shown in Supplement, Figure 2. Xylem fluid was extracted using a pressure bomb and stored at -80ºC. All samples were analyzed after the fourth collection by high pressure liquid chromatography with diode array and mass spectrometry detection. Twenty eight phenolic compounds have been tentatively identified (Table 1) in *Vitis vinifera* xylem fluid. Phenolic compounds have been quantified in five cultivars over a one year period (one sampling per season) from both climate regions. Flavonols, secondary metabolites that are a stress-responsive class of polyphenolics were quantified separately. The cultivars include; Chardonnay, Zinfandel, Cabernet Sauvignon, Barbera and Petite Sirah.

Phenolic Compounds			
	Identification method		
	Reference Standard	Lab isolation	Literature, m/z, CID
monogalloylglucose			√
gallic acid	√		
caftaric acid	√		
coutaric acid	√		
fertaric acid	√		
catechin	√		
epicatechin	√		
Pro B1		√	√
Pro B2		√	√
Pro B3		√	√
Pro B4		√	√
catechin gallate	√		
epigallocatechin	√		
rutin	√		
quercetin-3-O-glucoside	√		
quercetin-3-O-glucuronide			√
quercetin-3-O-galactoside			√
astilbin (dihydroquercetin-3-O-rhamnoside)		√	√
isorhamnetin	√		
isorhamnetin glycoside			√
myricetin glycoside			√
myricetin-3-O-glucuronide			√
kaempferol-3-O-glucoside	√		
kaempferol-3-O-glucuronide			√
kaempferol-3-O-galactoside			√
engeletin (dihydrokaempferol-3-O-rhamnoside)			√
t-resveratrol	√		
resveratrol tetramer			√

Table 1. Tentative identification of twenty eight phenolic compounds in xylem fluid from *Vitis vinifera* cultivars. The green shaded area corresponds to those compounds belonging to the flavonol phenolic group. Identification was based on certified reference standards,

laboratory purification, existing literature or a combination thereof using mass spectra, fragmentation, UV-Vis spectra and retention times as criteria for identification.

3. All vines were asymptomatic based on visual assessment during our sampling period. It was therefore deemed unnecessary at this time to quantify *Xf* titers in xylem fluid. Previously, three cultivars in our study, Chardonnay, Zinfandel and Cabernet Sauvignon (potted vines) were inoculated by Dr. David Gilchrist with *Xf*-Temecula-PD strain. After five months bacterial titers were correlated with relative symptomology (Gilchrist and Lincoln, 2010 and Supplemental Figure 3). Dr. Gilchrist has agreed to inoculate Barbera and Petite Sirah potted grapevines with *Xf*-Temecula-PD strain and calculate titers. Thus, all five cultivars in our study will have titer values associated with their relative susceptibility to PD. This is of value to our study as we can better determine if a correlation exists between relative susceptibility and the quantified phenolic compounds.

4. **Induction trials are scheduled on Cabernet Sauvignon vines at the Yolo County site for the month of April 2012**. Two compounds which induce systemic acquired resistance (SAR) will be applied to vines approximately four weeks after budbreak between the stages of "3 leaves unfolded" and "visible inflorescences". Acibenzolar-S-Methyl (Benzo-(*1,2,3*)-thiadiazole-7-carbothioic acid S-methyl ester or BTH) (Bion, Syngenta, NC), a synthetic plant elicitor and methyl jasmonate, a naturally occurring elicitor are known to increase secondary metabolites in grapevines. BTH was found to increase phenolic content of several classes of polyphenols when applied to grapes (Ruiz-Garcia et. al. 2012). Previously, BTH was found to increase phenolic levels in strawberries which raised their resistance to powdery mildew (Hukkanen et. al. 2007). Increased concentrations of stilbenes were found in leaves and berries after methyl jasmonate application (Larronde et. al. 2003). So, the effects of these compounds have been actively investigated in berries but nothing is known about their effect on phenolic compounds within xylem fluid. We will compare flavonol and stilbene concentration in xylem fluid before and after treatments to determine if a treatment with either agent increases phenolic levels, especially flavonols and stilbenes. (Our proposal for continued funding suggested this work would not be accomplished this year, but the limited study originally included for this period will, in fact, be completed.)

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

Our research to date on phenolic xylem fluid composition has shown that there are seasonal differences within a variety, an example given in Table 2, as well as profile differences between cultivars (data not shown). Perhaps more importantly, cold winter vines were found to have higher yearly concentrations of xylem fluid flavonols than warm climate vines. Our analysis of flavonols (Table 3) have confirmed this in five varietals (Petite Sirah not shown). As mentioned in Section III, three of the cultivars we used for our study, Cabernet Sauvignon, Chardonnay and Zinfandel were shown to have varying Xf titers five months after bacterial inoculation based on research by Dr. David Gilchrist. Cabernet had the highest titers ($5x10^6$) while Zinfandel and Chardonnay had similar titer magnitudes ($5x10^5$). This is related to relative susceptibility to PD and the symptomatology correlates with titer levels (Gilchrist and Lincoln, 2010 and Supplemental Figure 2). Here we observe that Cabernet has the lowest flavonol levels, while Zinfandel and Chardonnay were higher (Table 3). So, we are pursuing possible correlations between titer susceptibility data versus total yearly flavonol concentrations, or specific phenolic compounds and their concentration present in cold climate xylem fluid.

Table 2. Zinfandel phenolic compounds from two different climate regions. Concentrations in mg/L of xylem fluid.

Cold Climate				Zinfandel	Warm Climate			
F	W	Sp	Sm	Compound (mg/L)	F	W	Sp	Sm
		x	0.48	monogalloylglucose				1.9
16	94	6.9	33	caftaric acid	0.85	9.5	2.9	11
1.0	24		6.9	coutaric acid			1.9	1.6
21	115	21	17	catechin	22	60	11	4.2
14	58			epicatechin	18	69		
				Pro B2		14		
11	70		6.9	Pro B3	9.7	56	3	tr
0.86	2.1	2.6	1.7	rutin	1.2	tr	3.1	0.79
4.6	7.3	19	4.5	quercetin-3-O-glucuronide	2.3	2.6	11	2.4
2.7	5.2	4.2	2.5	astilbin	2.4	4.8	3.7	1.7
				isorhamnetin		1.5		
				isorhamnetin glycoside				2.0
		2.9		myricetin-3-O-glucuronide			tr	
1.5	2.1	4.1		kaempferol-3-O-glucoside	1.6		3.0	
				kaempferol-3-O-galactoside				
1.6	3.1			engeletin		1.7	1.9	
no UV	tr	0.25		t-resveratrol	tr	tr	0.69	no UV
no UV	tr	0.82		resveratrol tetramer	x	tr	tr	x

x = not quantified; tr = trace levels

Table 3. Flavonol concentrations in four cultivars from two climate regions. Given are yearly total concentration as well as Winter concentration. Total yearly stilbene concentrations are reported in the last line.

Concentration (mg/L)	Chardonnay		Zinfandel		Cabernet Sauvignon		Barbera	
	cold	warm	cold	warm	cold	warm	cold	warm
Winter Flavonol	17	9.8	11	7.5	7.4	6.3	14	8.8
Total yearly Flavonol	74	74	76	48	39	14	164	55
Total yearly Stilbene	trace	trace	1	0.69	0.05	0.57	1.2	0.49

Vineyard sites were monitored through the California Irrigation Management Information System (CIMIS). Monthly average maximum and minimum air temperatures (data not shown)

were monitored as well as daily minimum temperatures during the cold seasons (Figure 1). Duration at low temperature can be ascertained from the hourly data (Table 4). Of interest, is the duration of 32º F or below daily minimum air temperature associated with the Foothill sites. In both Fall and Winter there were more consecutive days of freezing weather than in either of the warmer climate sites.

Figure 1. Minimum air temperatures for each season recorded by CIMIS stations.

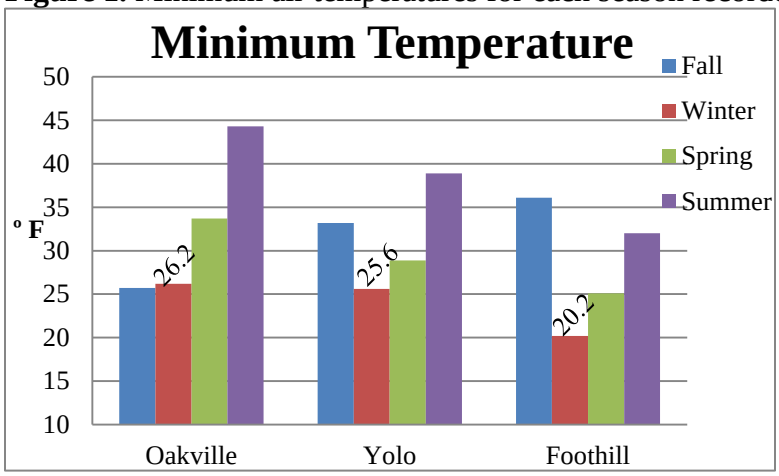

Table 4. Number of consecutive days where air temperature reached a minimum of 32º F.

	Oakville	Yolo	Foothill
Fall	4	3	8
Winter	4	5	14

Stations include: #13 – Sierra Foothill (Camino), Station #6 Sacramento Valley (Davis) and Station #77 - North Coast Valleys Region Napa County (Oakville). The Fall sampling period ranged from September, 23, 2010 – December 20, 2010. Winter sampling period ranged from December 20, 2010 – March 19, 2011, Spring sampling period ranged from March 20, 2011 – June 20, 2011 and Summer sampling period from June 21, 2011 – September 22, 2011.

V. **Publications or reports-** A manuscript is being drafted for publication based on the findings of this research.

VI. **Presentation of Research –** an abstract has been accepted to the annual American Society for Enology and Viticulture **(**ASEV) meeting on June 20-24th 2012.

VII. **Research relevance statement**
These results suggest a specific mechanism by which cold curing operates: Pierce's Disease is suppressed via the presence of particular phenolic compounds in the xylem fluid. If this is the case, and if it is possible to induce the production of these protective substances in the xylem fluid, it may be possible to prevent or treat PD in grapevines.

VIII. **Lay summary**
To our knowledge, this was the first comprehensive study of phenolic substances in xylem fluid in grapevine species. As a part of this study, *Vitis vinifera* cuttings were collected from two different regions, "warm" (non-freezing winter temperatures) temperatures and "cold" (freezing winter temperatures). "Cold" regions further being described as areas where "cold curing" of Pierce's Disease is known to occur (Kirkpatrick and Meyer, 2005-2008). Phenolic compounds were identified in five *Vitis vinifera* cultivars that spanned three categories based on Pierce's Disease susceptibility (least, intermediate and most susceptible). Sampling occurred once per season over a one year time span. Individual phenolic concentration was found to be highly

variable across cultivars and fluctuated by season. The research to date on xylem fluid has shown that cold winter vines have a higher yearly total concentration of flavonols than do the associated warm climate cultivars. Flavonols are phenolic compounds that are produced as secondary metabolites to stressful conditions such as drought, light intensity and temperature (Makris et.al. 2006 and Treutter 2010). In addition, prior work has demonstrated that specific phenolics can inhibit *Xf* and other microbes (Maddox et. al. 2009). Further investigation is underway to correlate this "cold curing" phenomenon with the increase of certain phenolic compounds elicited by the stress of cold temperatures, and to see if vine treatments could induce the production of these protective phenolic substances.

IX. **Status of funds**- To date, $39,208 has been expended and an encumbrance of $5,407 for personnel for the balance of the year has been deducted from total funds of $62,879. A balance of $18,264 remains.

X. **Summary/status of IP** – As stated in the research proposal, the first year of the project (survey of xylem phenolic composition) is not expected to yield intellectual property.

Citations

Hukkanen, A.T., H.I. Kokko, A.J. Buchala, G.J. Mcdougall, D. Stewart, S.O. Karenlampi and R.O. Karjalainen. 2007. Benzothiadiazole Induces the Accumulation of Phenolics and Improves Resistance to Powdery Mildew in Strawberries. Journal of Agricultural and Food Chemistry 55(5) 1862-1870.

Kirkpatrick, B. and M. Meyer. Identification of mechanisms mediating cold therapy of Xf-infected grapevines. Proceedings of the Pierce's disease Research Symposium, Calif. Dept. Food and Agriculture, 2005 pp. 242-246; 2006 pp. 236-239; 2007 pp. 208-211; 2008 pp.167-171.

Larronde, F., J. P. Gaudillère, S. Krisa, A. Decendit, G. Deffieux, and J. M. Mérillon. 2003. Airborne Methyl Jasmonate Induces Stilbene Accumulation in Leaves and Berries of Grapevine Plants. American Journal of Enololgy and Viticulture 54: (1) 63-64.

Maddox, C.E., L.M. Laur and L. Tian. 2010. Antibacterial Activity of Phenolic Compounds Against the Phytopathogen *Xylella fastidiousa.* Current Microbiology 60: 53-58.

Makris, D.P., S. Kallithraka and P. Kefalas. 2006. Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. Journal of Food Composition and Analysis 19: 396–404.

Ruiz-Garcia, Y., I. Romero-Cascales, R. Gil-Munoz, J.I. Fernandez-Fernandez, J.M. Lopez-Roca and E. Gomez-Plaza. 2012. Improving Grape Phenolic Content and Wine Chromatic Characteristics through the Use of Two Different Elicitors: Methyl Jasmonate versus Benzothiadiazole. Journal of Agricultural and Food Chemistry 60 (5) 1283–1290.

Treutter, D. 2010. Managing Phenol Contents in Crop Plants by Phytochemical Farming and Breeding - Visions and Constraints. International Journal of Molecular Sciences 11: 807-857.

Supplement

Table 5. Vine locations, details and sampling dates

Warm Climate: Original selections of *Vitis vinifera* were from material declared negative for known grapevine viruses by PCR and ELISA certified by Foundation Plant Services (FPS), University of California, Davis.

Site	Variety	Location	Elavation (ft)	Rootstock	Clone	Fall 2010 d/m/y	Winter 2011 d/m/y	Spring 2011 d/m/y	Summer 2011 d/m/y
	native	**Davis**							
UC Davis	Muscidinia rotundifolia "Trayshed"	N38 32.294 W-121 45.837	56	native resistant		15/11/2010	x	19/06/2010	
Yolo County	Vitis arizonica "643.17"	N38 32.294 W-121 45.837	56	native resistant		15/11/2010	x	19/06/2010	
		Yolo County							
Martinez	Pinot noir	N38 30.366 W-122 0.453	158	5C	2A	16/11/2010	07/02/2011	27/05/2011	29/08/2011
Yolo County	Barbera	N38 30.366 W-122 0.453	158	5C	2	16/11/2010	07/02/2011	27/05/2011	29/08/2011
	Zinfandel	N38 30.366 W-122 0.453	158	5C	01A	19/11/2010	07/02/2011	27/05/2011	29/08/2011
	Petite sirah	N38 30.366 W-122 0.453	158	5C	3	16/11/2010	07/02/2011	27/05/2011	29/08/2011
	Chardonnay	N38 30.366 W-122 0.453	158	5C	4	16/11/2010	07/02/2011	27/05/2011	29/08/2011
	Merlot	N38 30.366 W-122 0.453	158	5C	15	19/11/2010	07/02/2011	27/05/2011	29/08/2011
		Oakville							
Oakville Station	Cabernet sauvignon	N38 25.711 W -122 24.486	187	110R	8	03/12/2010	07/03/2011	10/06/2011	31/08/2011

Cold Climate

Site	Variety	Location	Elevation (ft)	Rootstock	Clone	Fall 2010 d/m/y	Winter 2011 d/m/y	Spring 2011 d/m/y	Summer 2011 d/m/y
		Foothills							
Boeger	Cabernet sauvignon	N38 44.657 W-120 46.556	2186	110R	8	03/11/2010	08/02/2011	16/06/2011	01/0911
Boeger	Pinot Noir	N38 45.373 W-120 42.638	2623	101-14	2A	03/11/2010	08/02/2011	16/06/2011	01/0911
SumuKaw	Zinfandel	N38 41.101 W-120 41.872	2826	101-14, 3309, 420-A, riparia		03/11/2010	08/02/2011	16/06/2011	01/0911
SumuKaw	Barbera	N38 41.169 W-120 41.916	2843	5C grafted to Nebiola		03/11/2010	08/02/2011	16/06/2011	01/0911
Lahey	Petite Sirah	N38 44.837 W-120 46.166	2406	1103P	Duero?	03/11/2010	08/02/2011	16/06/2011	01/0911
Lava Cap	Chardonnay	N38 45.231 W-120 44.677	2622	Freedom	4	03/11/2010	08/02/2011	16/06/2011	01/0911
David Girard	Merlot	N38 46.834 W-120 53.542	1300			19/11/2010	07/03/2011	16/06/2011	01/0911

Supplement

Figure 2. Regional Map showing vineyard sites

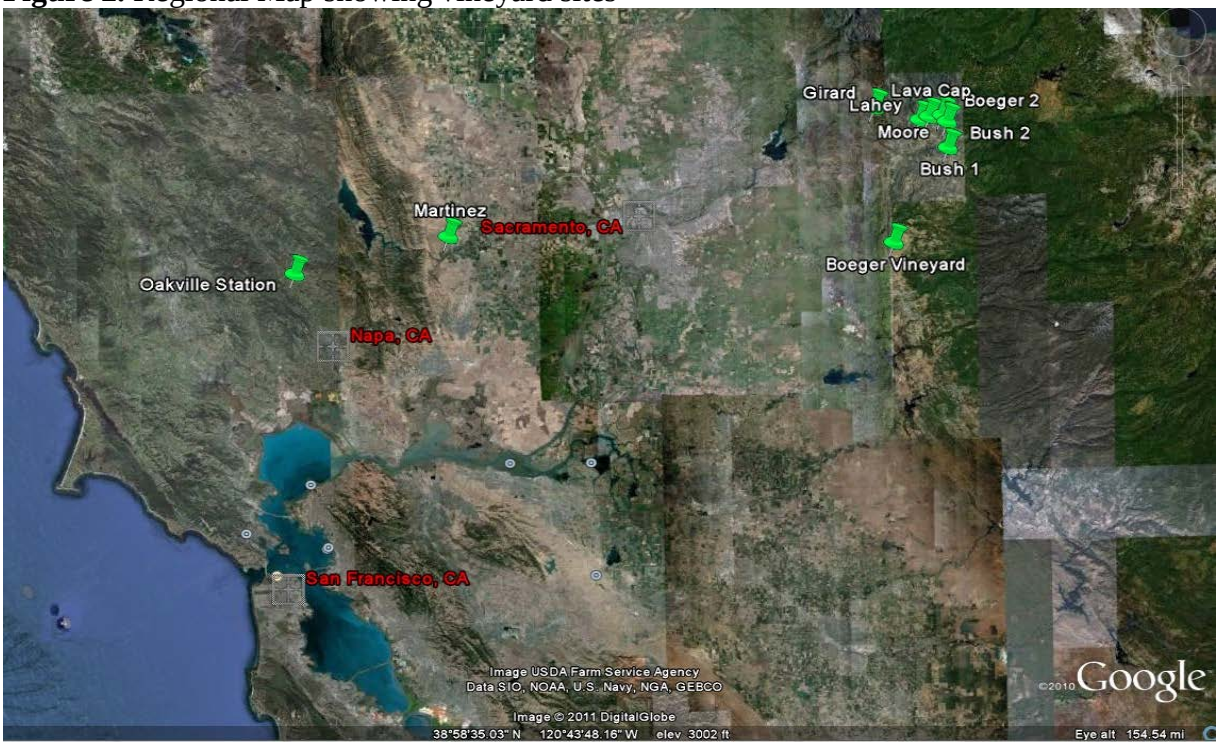

Figure 3. Data from Gilchrist and Lincoln (2010)