BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

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Reporting Period: The results reported here are from work conducted from October 2006 through September 2007.

ABSTRACT
Progress continues on Pierce's disease (PD) resistant winegrapes and has been greatly accelerated by the incorporation of marker-assisted selection (MAS) for the Pierce's disease resistance gene, *PdR1* (see companion report). The use of MAS and our acceleration of the seed to seed breeding cycle to three years have allowed very rapid progress towards PD resistant winegrapes. Populations from the 2006 crosses were screened with MAS for both PD and powdery mildew (*Run1*) where appropriate and only those with the markers were planted in the field. The 2007 crosses were made to: 1) produce populations with *PdR1* and 93.75% *vinifera*; 2) broaden the base of *vinifera* wine grape types with *PdR1* at the 87.5% *vinifera* level; 3) broaden the base of *vinifera* wine grapes with *PdR1* from the 8909-17 allele; 4) produce *vinifera* wine grape types with resistance from two other non-*PdR1 V. arizonica* resistance sources (b42-26 and b40-14); and 5) to produce additional rootstock lines with *PdR1* and *XiR1* (the *Xiphinema index* resistance gene). *Vinifera* wine grape types with *PdR1* and 87.5% *vinifera* in were planted in a Beringer, Napa county trial. Finally, small scale wine lots were made from four of eight selected 87.5% *vinifera PdR1* containing wine grape types. Fruit evaluation and must analysis were performed on numerous other promising progeny at this level.

INTRODUCTION
The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xf* resistance (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005), and in possession of unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, to allow the introduction of extremely high levels of *Xylella* fastidiosa(*Xf*) resistance into commercial grapes. They have produced seed that is 93.75% *V. vinifera*, from winegrape cultivars, with resistance from our b43-17 *V. arizonica*/*candicans* resistance source. There are two sources of *PdR1*, 8909-08 and 8909-17, both siblings of b43-17. These selections have been introgressed into a wide range of winegrape backgrounds over multiple generations, and resistance from southeastern United States (SEUS) species is being advanced in other lines. However, the resistance in these later lines is complex and markers have not been developed to expedite breeding.

OBJECTIVES
1. Breed PD resistant winegrapes through backcross techniques using high quality *V. vinifera* winegrape cultivars and *Xf* resistant selections and sources characterized from our previous efforts.
2. Continue the characterization of *Xf* resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations.

RESULTS
Objective 1 The breeding cycle for the development of PD resistant grapes has been reduced to three years (seed to seed) using marker-assisted selection (MAS) with the b43-17 resistance sources and their progeny. The 2006 crosses, number of seedlings produced and the number of seedlings that went to the field are presented in the 2006 Proceedings and in March 2007 Report. Our goal at this point is to introgress our PD and *PdR1* resistance sources into a large number of *V. vinifera* winegrapes backgrounds. Until we get to the backcross 4 (BC4) (96.8% *V. vinifera*), there is not much point to growing very large numbers of progeny from any given cross. With the now standard three-year seed-to-seed cycle we will plant BC4 progeny in 2010.

Table 1 presents the crosses made and seed produced in 2007. The goals of this year's crosses were to: 1) Use the *PdR1* allele from the 8909-08 to make 93.75% *vinifera* level progeny; 2) Broaden the *vinifera* winegrape lines in the 8909-08 resistance source at the 87.5% *vinifera* level; 3) Combine *PdR1* with the powdery mildew resistance gene *Run1* at the 87.5% *vinifera* level; 4) Use 8909-17 and 8909-08 based resistance with diverse *vinifera* winegrapes to produce resistant progeny at the 75% *vinifera* level; 5) Use the 8909-15 resistance source with a broad range of *vinifera* winegrapes; and 6) Produce rootstocks with *PdR1* and broad-based nematode resistance.

Five groups of plants were greenhouse screened for *Xf* resistance in 2007. Group A tests (160 genotypes) were done to verify the expression of *PdR1* from b43-17 in the 04190 (*V. vinifera* F2-7 x 8909-08) population, and confirm *PdR1* in parents used in 2006. The Group B tests (76 genotypes) examined progeny of Midsouth and BD5-117 crossed to advanced *vinifera* wine

types. Both of these parents continue to produce resistant progeny, but very few and in ratios that suggest a complex inheritance. The progeny of Haines City (n=9) were all resistant by ELISA in the greenhouse screen, but do not contain *PdR1*. Eleven genotypes from Olmo's e-series BC2 that carry *Run1* (the powdery mildew resistance marker) were also tested, none were resistant and they seemed more sensitive to PD than typical *vinifera*. Group C tested the use of b43-17 as a rootstock and interstock to examine *Xf* transmission and expression through a graft union. b43-17 did not induce PD resistance in Chardonnay, but preliminary results found that it and A8909-05 (*V. rupestris* x *M. rotundifolia*) prevented PD expression in Chardonnay scions when they were used as interstocks. Group D tests (120 genotypes) focused on mapping population progeny to verify recombinants and establish resistance ratios. Recombinants from 04190 were tested and aided fine-scale *PdR1* mapping efforts; three recombinants from the 04191 (*vinifera* F2-7 x 8909-17) were also tested. The 05347 population (*vinifera* F2-35 x *V. arizonica* b42-26) was also tested (n=60) to establish the R/S ratios derived from b42-26, which is quantitatively inherited and provides an alternative and strong source of PD resistance. Progeny from these tests will be used for mapping studies and as parents with a non-*PdR1* resistance. Group E tests (150 genotypes) included additional 04190 progeny and remnants from the 9621 (8909-15 x 8909-17) that had not been tested. This group also tested advanced *PdR1* carrying parents, which were used in the 2007 crosses based on marker data alone, to confirm their resistance.

Objective 2 Although resistance from other backgrounds is complex and quantitative, which results in few resistant progeny from crosses to *vinifera* cultivars, we continue to advance a number of lines. In order to better understand the limits of other PD resistance sources the following resistance sources are being studied:

***V. arizonica* b42-26 –** *Xf* resistance in the 0023 (D8909-15 (*V. rupestris* x b42-26) x *vinifera* B90-116) population is strong, but is quantitatively inherited. Quantitative trait locus (QTL) analysis has identified a major QTL that accounts for about 20% of the variability (preliminary results). Previous efforts with the 0023 were focused on table grape breeding, and found that the 0023 population (F1, 1/4 b42-26) had about 30% resistant progeny. This population has a large number of weak genotypes, few females with viable seeds, and generally lacks fertility. The progeny of a cross of a resistant 0023 genotype crossed back to *vinifera* (BC1) were tested and only 7% were resistant. We are now testing 05347 (*vinifera* F2-35 x b42-26) to examine the b42-26 resistance source in a less complex background (without the confounding effect of *V. rupestris*). Crosses using elite *V. vinifera* wine type pollen were made to a number of females in this population in Spring 2007 (Table 1f).

***V. arizonica* b40-14 –** In 2006 we crossed F2-35 x b40-14, produced 1,385 seeds and established 198 seedlings for testing. We are planning on using this population, or one generated from its progeny for mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance (Table 1f).

Field and Wine Evaluations The A81 series (BC1, 75% *vinifera*) F8909-08 allele type of *PdR1* is in its second year of field testing at the Beringer Yountville test site; ELISA and visual symptom results have been consistent with greenhouse assays. Selections from the 045554 (BC2, 88% *vinifera*) have been made onto Dog Ridge (currently the only certified PD resistant rootstock) and were planted at Yountville on May 14, 2007. These genotypes have been marker tested and their PD resistance status confirmed by greenhouse testing. Twelve genotypes were resistant, four were recombinants (one resistant and three susceptible in the greenhouse test). They will be inoculated in Spring 2008.

Three of eight advanced red wine selections (U0501-12, U0502-01 and -10) containing *PdR1* that are 87.5% *vinifera* from crosses with Syrah and Chardonnay were replicated for small-scale fermentation this Fall. About four liters of wine from each were produced along with similar amounts of Cabernet Sauvignon and Pinot noir as *V. vinifera* controls and Midsouth and Lenoir as standard PD resistant controls. These selections were evaluated for their productivity, flowering and ripening dates, and berry and cluster weights. Vine, fruit and juice analyses are presented in Tables 2a, 2b and 2c, and images of the leaves and fruit are in Figure 1.

Numerous other genotypes from crosses involving elite *vinifera* wine cultivars were examined for fruit evaluation and must analysis. ETS Laboratories (www.etslabs.com) of St. Helena kindly donated their fruit analysis and phenolics panel, which uses a wine-like extraction to model a larger fermentation. Surprisingly, none of the U05 series analyzed contained significant levels of diglucoside anthocyanins, which are negative quality markers for hybrid wines with American grape species and which would create problems with exporting wines to the EU. Cuttings of the best of these will be taken in the winter so that we can get small-scale wine lots made for evaluation in 2009. Plants will be grown in both Davis and Napa when possible. A new MS student is examining the reasons for the lack of diglucoside anthocyanins in these selections to determine whether the *arizonica*-resistance sources possess these anthocyanins. A second MS student is in the second year of examining what wine quality parameters are useful at micro (2L), mini (20L) and macro (2,000L) scales. Determining how to best select for wine quality at the micro scale will be critical as we begin to evaluate large populations of PD resistant and 96% vinifera selections.

Powdery Mildew - Any new PD resistant variety should also be resistant to powdery mildew. We have been exploring powdery mildew resistance in a number of backgrounds including Olmo's VR (*vinifera* x *rotundifolia*) hybrids, which form the base of international efforts at characterizing *Run1*, the *rotundifolia*-based locus responsible for resistance to powdery mildew. Current season field evaluations of the 2006 crosses show the markers correlating perfectly with field resistance on leaf and cane. Berry and cluster observations will begin in Fall 2008. The goal with these individuals is to cross our advanced PD resistant selections with selections from these powdery mildew resistant progeny. We also made crosses to examine powdery mildew in two other backgrounds, Villard blanc and Tamiami. Powdery mildew resistance markers are being developed for these resistance sources by labs in Germany and the US.

CONCLUSIONS

This project continues to breed PD resistant winegrapes with the primary focus on the *PdR1*resistance source so that progress can be expedited with MAS. Populations with *Xf* resistance from other sources are being maintained and expanded, but progress is slower with these sources. We continue to supply plant material, conduct greenhouse screens and develop new mapping populations for our companion project on fine-scale mapping of PD resistance leading to the characterization of the *PdR1* resistance. Fall 2007 will see the first testing of wine from advanced selections with 87.5% *vinifera* from winegrapes.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Additional support from the Louis P. Martini Endowed Chair in Viticulture, and the donated wine analyses from ETS Labs, St. Helena are also gratefully acknowledged

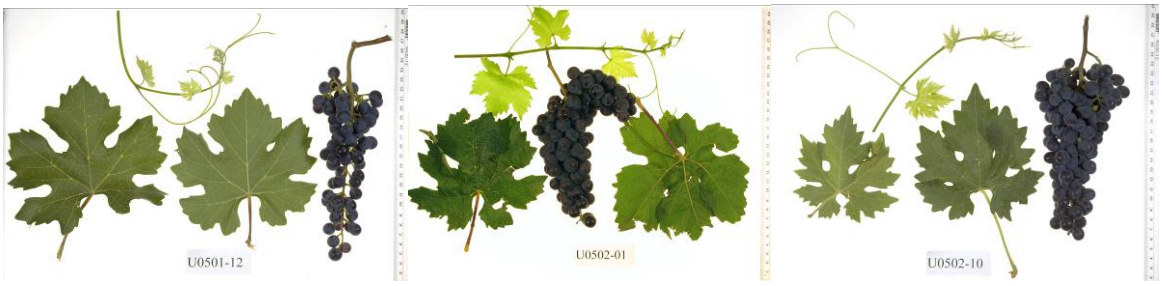

Figure 1. Pictures of the 87.5% *vinifera* PD resistant wine grape selections used for small-scale winemaking at UCD in 2007.

Table 1. 2007 crosses and numbers of seed produced.

Resistant Type	*Vinifera* Parent of Resistant Type	*Vinifera* Types used in 2007 crosses	# Seeds Produced
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.			
U0501	Syrah	F2-7, F2-35	478
U0502	Chardonnay	F2-7, F2-35	2,769
U0503	Sauvignon blanc	Chardonnay, Palomino, Semillon	126
U0505	Cabernet Sauvignon	Chardonnay, F2-7, LCC, Merlot, Palomino, Petite Syrah	3,229
1b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 87.5% *V. vinifera* parentage			
05310	Alicante Bouschet	Burger, Carignane, LCC	1,666
05312	Cabernet Franc	Zinfandel	194
05317	Tempranillo	Burger, LCC	371
05319	Zinfandel	Cabernet Franc, LCC	144
A81-17	A38-7	Carignane, Grenache noir, LCC	705
1c. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Run1* powdery mildew resistance.			
U0501, U0504	Syrah	e-series, e78 and e88 allele patterns	499
U0502	Chardonnay	e-series, e78 and e88 allele patterns	837
U0505	Cabernet Sauvignon	e-series, e78 and e88 allele patterns	642
A81-17	A38-7	e-series, e78 allele pattern	603
1d. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Vitis* PM resistance source.			
U0505, A81-17	Cabernet Sauv, A38-7	Villard blanc	348
1e. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 75% *V. vinifera*.			
04373-02	F2-35 (Cab x Carignane)	Alicante Bouschet, Aligote, Carignane, Chardonnay, Zinfandel	597
04373-08	F2-35 (Cab x Carignane)	Aligote, Cabernet Franc, Carignane	938
04373-64	F2-35 (Cab x Carignane)	Grenache noir	293
1f. Other resistance sources. R89 is 50% *vinifera*, 25% resistance source. 05347 is 75% *vinifera* and 25% resistance source			
R89 (b40-14)	NR	Airen	238
05347 (b42-26)	F2-35	Aligote, Chardonnay, Grenache noir, Zinfandel	1,877
1g. Rootstock crosses to combine PD and nematode resistance.			
9621-257	9365-85		653
9365-43	9621-161		112

Table 2a. Phenotypic observations of reference varieties and select progeny with the *PdR1* resistance source.

Genotype	Parentage	Percent *vinifera*	2007 Bloom Date	Berry Color	Berry Size (g)	Ave Cluster Wt. (g)	Ripening Season	Prod 1=v low 9=vhigh
Cab. Sauv.	Cab. Franc x S. blanc	100%	5/20/07	B	1.0	168	mid-late	6
Pinot noir	Historic	100%	5/7/07	B	1.1	259	Early	6
U0501-12	A81-138 x Syrah	87.5%	5/7/07	B	1.0	90	mid-late	4
U0502-01	A81-138 x Chardonnay	87.5%	5/1/07	B	1.6	128	mid-late	4
U0502-10	A81-138 x Chardonnay	87.5%	5/1/07	B	1.4	160	very early	7
Lenoir	*V. aestivalis* hybrid	<50%	5/12/07	B	0.8	201	Late	7
Midsouth	DGxGalibert 255-5	<50%	5/5/07	B	2.2	211	mid-late	6

Table 2b. Analytical evaluation of reference varieties and advanced selections with the *PdR1* resistance source. Diglucoside anthocyanins were detected in Midsouth and Lenoir. All analysis courtesy of ETS Laboratories, St. Helena, CA.

Genotype	L-malic acid (g/L)	°Brix	potassium (mg/L)	pH	TA (g/100mL)	YAN (mg/L (as N)	catechin (mg/L)	tannin (mg/L)	Total antho-cyanins (mg/L)
Cab. Sauvignon	2.19	24.9	2460	3.65	0.62	227	59	250	404
Pinot noir	2.43	26.5	2190	3.83	0.49	279	321	842	568
U0501-12	4.20	29.4	2900	3.87	0.68	420	88	802	979
U0502-01	2.90	25.9	2530	3.77	0.61	301	91	564	380
U0502-10	4.92	23.7	2220	3.48	0.85	301	87	588	845
Lenoir	4.32	26.9	2920	3.67	0.75	164	195	341	1801
Midsouth	4.60	18.2	2220	3.49	0.81	278	32	230	971

Table 2c. Sensory evaluation of reference varieties and advanced selections with the *PdR1* resistance source.

Genotype	Juice Hue	Juice Intensity	Juice Flavor	Skin Flavor	Skin Tannin (1=low, 4= high)	Seed Color (1=gr, 4= br)	Seed Flavor	Seed Tannin (1=high, 4= low)
Cab. Sauv.	pink-brown	light-med	fruity-CS	fruit jam	2	3	nutty-full	4
Pinot noir	pink-brown	medium	hay, honey	mildly fruity	1	4	spicy	4
U0501-12	red	med-dark	fruity	fruit jam	2	4	neutral	2
U0502-01	pink-brown	medium	fruity-PN	sweet fruit	1	3	spicy	1
U0502-10	pk-red-orng	med-dark	slight vegetal	mildly fruity	1	4	nutty,spicy	1
Lenoir	red	dark	mildly fruity	fruity	1	4	nutty	1
Midsouth	red-orange	med-dark	veg-fruity	neutral	1	4	neutral	4