BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

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ABSTRACT
The use of marker-assisted selection (MAS) using DNA markers tightly linked with Pierce's disease (PD) resistance (see our companion report) and the acceleration of the seed-to-seed breeding cycle to three years have allowed very rapid progress towards the creation of PD resistant winegrapes. Seedlings from the 2008 crosses were screened for PD resistance with MAS and only those seedlings with the markers were planted in the field. The goals of the 2009 crosses were to: 1) create 97% *V. vinifera* seedlings with PD resistance using *PdR1* from *V. arizonica* hybrid– F8909-08; 2) create 75% *V. vinifera* seedlings with PD resistance from *V. arizonica* b40-14; and 3) enlarge the *V. arizonica/girdiana* b42-26 PD resistance mapping population by remaking the *V. vinifera* F2-35 x b42-26 cross. Numerous greenhouse-based PD resistance screens were performed on breeding lines, mapping populations and new PD resistant rootstocks. Selections with *PdR1* at the 87.5% and 75% *vinifera* level at our Beringer, Napa County trial were inoculated and a similar trail at the 93.75% *vinifera* level was planted. Finally, small-scale wine lots were made from three 93.75% *vinifera* and five 87.5% *vinifera PdR1* selections. Fruit evaluation and juice analysis were performed on numerous other promising progeny at the 93.75% *vinifera PdR1* level.

LAYPERSON SUMMARY
Rapid progress breeding Pierce's disease (PD) resistant winegrapes continues to be made by combining the use of MAS with *PdR1* and aggressive vine training to produce clusters in a seedling's second season to produce the next generation crosses of PD resistant populations. Wines were made this Fall from PD resistant selections that contain 94% *vinifera*. These selections resulted from the original cross of *vinifera* x F8909-08 (the *V. arizonica/candicans* resistance source from Monterrey, Mexico), followed by crossing back to a *vinifera* parent over three more generations (modified Back Cross 3 – mBC3). This Fall, wine was made from three of these 94% *vinifera*, PD resistant selections. Two years of wines have also been made from the previous generation 88% *vinifera* (mBC2) PD resistant selections with very favorable results. This year also saw the creation of the next generation back cross to *vinifera* (mBC4), which will result in 97% *vinifera* seedlings with *PdR1* resistance for planting in Spring 2010. PD resistant selections are also being tested at the Beringer ranch in Napa Valley, and wines will be made from these vines. Major advances have also been made in the production and greenhouse testing of seedling populations that will allow the characterization of PD resistance genes from multiple backgrounds (see companion project on the genetics of resistance to PD).

INTRODUCTION
The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xylella fastidiosa* (*Xf*) resistance (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005), and having 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 *Xf* resistance into commercial grapes. They have made wine from vines that are 93.75% *V. vinifera*, and possess resistance from the b43-17 *V. arizonica*/*candicans* resistance source. There are two sources of *PdR1*, 8909-08 and 8909-17 – sibling progeny 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 yet been developed to expedite breeding.

OBJECTIVES
1. Breed Pierce's disease (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 AND DISCUSSION
Objective 1 – The breeding cycle for the development of PD resistant grapes has been reduced to three years (seed-to-seed) using MAS with the b43-17 resistance sources and their progeny. The breeding goal at this point is to introgress PD and *PdR1* resistance sources into a large number of *V. vinifera* winegrape backgrounds. Now that the backcross four (BC4) (96.9% *V. vinifera*) level has been produced (seedlings will be planted in 2010) larger numbers of progeny within populations will be produced to increase chances of selecting the best winegrape quality in a PD resistant background. **Table 1** shows the crosses made in 2009. The goals of the 2009 crosses were to: 1) use the *PdR1* allele from F8909-08 to advance the *vinifera*

winegrape populations to the 96.9% *vinifera* level; 2) create populations of 93.75% *vinifera* with the *PdR1* F8909-08 resistance allele; 3) select *V. vinifera* x *V. arizonica* b40-14 progeny and produce 75% *vinifera* populations with an alternative PD resistance source; and 4) increase the *V. arizonica/girdiana* b42-26 mapping population by remaking the *V. vinifera* F2-35 x b42-26 cross.

During this period, eight groups of plants were tested in the greenhouse for *Xf* resistance (**Table 2**). Group A tests confirmed the resistance of the parents used in the 2008 crosses, previously selected on the basis of their DNA markers; initiated the exploration of the *PdR1* alleles among resistant genotypes and *vinifera* parents; and tested the progeny from one initially promising 87.5% *vinifera* VR (*vinifera* x *rotundifolia*) hybrid from Olmo's breeding program. Unfortunately, all of these VR progeny were ELISA tested as PD susceptible at over 1,000,000 cfu/ml (data not shown), confirming the complex nature of PD resistance derived from *rotundifolia*. Groups B, D and E evaluated the greenhouse-based PD resistance of the 50% *vinifera*, 25% b40-14 *V. arizonica* resistance source 07744 and 07386 populations. **Table 3** shows that for the 07744 population, 24% were classed as resistant, approximately 26% were in either of two intermediate classes and 50% were in the susceptible class. The clearly PD resistant genotypes identified in groups B & E allowed us to make 75% *vinifera* crosses in 2009 and advance this promising new PD resistance line by another generation (**Table 1c**). Group D was tested to evaluate the impact of between pot spacing on mean ELISA cfu/ml values in an 87.5% *vinifera PdR1* background. Consistent with Baumgartel (2009), tighter spacing increased the mean ELISA values relative to the standard spacing in both susceptible and resistant selections. Group F consisted of additional 9621 population recombinants that were tested to aid the fine scale mapping of *PdR1*. Concurrently, additional 2007 crosses were tested to continue the exploration of *PdR1* resistance initiated in Group A. Groups G & H focus on the F8909-17 allele of *PdR1* to elaborate differences in resistance behavior of this allele compared to the F8909-08 allele. The new PD resistant rootstocks were tested in Group G. Chardonnay was used as a susceptible scion to determine if high *Xf* levels in the scion increase *Xf* levels in the rootstock downward across the graft union. A81-17, a rigorously tested 75% *vinifera PdR1* genotype, was used as the resistant scion to determine whether grafting on *PdR1* rootstocks impacts the titer of *X. fastidiosa* found in the scion.

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/girdiana b42-26 – *Xf* resistance in the 0023 (D8909-15 (*V. rupestris* x b42-26) x *V. 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 this population focused on table grape breeding, and found that the 0023 population (F1, 1/4 b42-26) had about 30% resistant progeny. The 0023 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. In 2007, we tested the 05347 (*vinifera* F2-35 x b42-26) population to examine the b42-26 resistance source in a background without the confounding effect of *V. rupestris*. That same year, crosses using elite *V. vinifera* wine type pollen were made to a number of females in this population and 140 genotypes were planted in 2008, which flowered for the first time in Spring 2009. We planted an additional 100 05347 genotypes to the field in May 2009 and again repeated this cross, producing approximately 200 seeds, to further expand this F1 mapping population.

V. shuttleworthii Haines City – Based on encouraging greenhouse screen results for this resistance source, in 2008 the BC1 (75% *vinifera*), and BC2 (87.5% *vinifera*) using a BC1 from earlier table grape work were made. This BC1 is very resistant and has reasonable winegrape characteristics. If reliable genetic markers for *Xf* resistance from this resistance source can be developed, it will be relatively easy to incorporate this form of resistance with sources carrying *PdR1* at later stages of the program, with the goal of broadening resistance.

V. arizonica b40-14 – Over the last seven years, 45 F1 progeny of PD susceptible *V. rupestris* Wichita Refuge crossed with PD resistant *V. arizonica* b40-14 (the R89 series) have been tested. Forty-two were highly resistant and three had intermediate reactions (data not shown). In 2006, the 06339 population (*V. vinifera* F2-35 x b40-14) was made and contains 198 seedlings for testing. In 2007, *V. vinifera* cv. Airen was crossed onto two of the PD resistant R89 series genotypes and a total of 163 progeny were planted in Spring 2008. One of these is the 07744 population (F1 50% *vinifera*, 25% b40-14 – see **Tables 1c, 2 and 3**). Preliminary mapping of this population places PD resistance from b40-14 on LG14 but in a different location than *PdR1*. To date greenhouse testing has been completed for seven 06339 genotypes (F1 50% *vinifera*, 25% b40-14); they all lack PD symptoms and have low ELISA values. The progeny of the 06339 x *V. vinifera* crosses made in 2008 will be used for further mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance.

Given that low levels of *Xf* exist in resistant plants it will be important to have PD resistant rootstocks to graft with resistant scions, thus preventing failure if *Xf* moved into the rootstock. The rooting and grafting ability (with two scion varieties) of eight selections with PD resistance from *PdR1* have been tested, and they will soon be greenhouse tested for resistance and

examination of *Xf* movement across the graft union. The best selections will be tested for nematode and phylloxera resistance followed by field testing.

Field and Wine Evaluations – Field testing of advanced PD resistant selections continues at the Beringer vineyard in Yountville, CA. Natural sharpshooter vectoring is not depended on, rather each plant is needle inoculated with *Xf* each Spring. Selections from the BC3, 94% *vinifera* crosses from the 07355 (U0505-01 x Petite Sirah) and 07370 (*vinifera* F2-35 x U0502-38) populations were grafted onto Dog Ridge (currently the only certified virus-free PD resistant rootstock) in February 2009 and planted at Beringer in June 2009. These genotypes have been marker tested and their PD resistance status will be confirmed by greenhouse testing in the coming months. This Spring, selections from the 045554 (BC2, 87.5% *vinifera*) population were needle inoculated for the second time and selections from the A81 population (BC1, 75% *vinifera*) both with the *PdR1b* (F8909-08) allele were inoculated for the third time. This year the seven most promising 87.5% *vinifera PdR1* wine types (06325-42, 06325-43, U0502-01, U0502-10, U0502-35, U0502-38, U0502-41 – 2 white and 5 red; 6 reps each) grafted onto Dog Ridge and planted at the Beringer site for small-scale winemaking trials.

In 2006, at least six vines of eight 87.5% *vinifera PdR1* selections (50% Syrah or Chardonnay from the last cross) were planted for small-scale wine making tests. Wine lots of these selections made in 2007 and 2008 have shown significant promise. Sensory evaluation as well as fruit evaluation and must analysis from numerous other genotypes from crosses involving elite wine cultivars were reported in our last two progress reports. These wines were also evaluated at the UCD Viticulture and Enology alumni gathering on May 15, 2009 and at the North American Grape Breeders Conference in Tallahassee, Florida on August 7, 2009 with similar results. In 2008, at least six vines of four other particularly promising 87.5% *vinifera PdR1* selections, siblings of the 2006 plantings, were planted. Concurrently we planted at least six vines of eight 93.75% *vinifera PdR1* selections (50% Petite Syrah, Chardonnay or F2-35 from the last cross). This fall, 12 fermentations were made: three (two red, one white) at the 94% *vinifera* level; five (four red, one white) at the 87.5% *vinifera* level; and four (two red, two white) *vinifera* and PD controls. Vine, fruit and juice analyses are presented in **Tables 4a-c** and images of the vines, leaves and fruit are in **Figures 1 and 2**. 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.

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 to allow the characterization of the *PdR1* resistance locus. The first testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2007, then again in 2008 and they scored remarkably well. Small-scale wine lots from advanced selections with 93.75% *vinifera* were made in Fall 2009 and appear even more promising.

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Table 1. 2009 Wine and mapping crosses with estimated number of seeds produced.

Resistant Type	*Vinifera* Parent of Resistant Type	*Vinifera* Parents used in 2009 Crosses	Est. No. Seeds
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 96.875% *V. vinifera* parentage.			
07354-50	Merlot	Cab. Sauv., Chard.	125
07355-020	Petite Syrah	Cab. Sauv., Chard., Chenin blanc, Zinfandel	1750
07370-039	F2-35 (Cab. Sauv. x Carignane)	Cab. Sauv., Chenin blanc, Riesling, Sylvaner, Zinfandel	1450
07370-097	F2-35	Cab. Sauv., Chard., Chenin blanc, Pinot noir	650
07370-28	F2-35	Cab. Sauv., Chenin blanc, Pinot noir, Zinfandel	950
07371-19	F2-35	Cab. Sauv., Chard., Chenin blanc, Sylvaner	375
07371-20	F2-35	Cab. Sauv., Chenin blanc, Pinot noir, Sylvaner	925
07371-36	F2-35	Cab. Sauv., Chard., Chenin blanc, Mourvedre, Riesling, Zinfandel	800
1b. *Vitis arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.			
07307-10	Zinfandel	Cab. Sauv., Chenin blanc	115
1c. Crosses to the b40-14 *V. arizonica* resistance source to produce progeny that are 75% vinifera and 12.5% the resistance source.			
07744-038,-120	Airen	Cab. Sauv., F2-35, Malaga Rosada	790
1d. Cross to increase the b42-26 *V. arizonica* x *vinifera* mapping population.			
b42-26	F2-35 (Cab. Sauv. x Carig.)	F2-35	200

Table 2. PD resistant winegrape progeny completed or currently in greenhouse screening for PD resistance.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Date	Resistance Source(s)
A	2008 *PdR1* Parents, mini-mapping, e6-23 VR series	47	11/25/2008	2/26/2009	b43-17 (both alleles), VR
B	07744 mapping population	37	12/16/2008	4/2/2009	b40-14
C	Greenhouse spacing trial	NR	1/15/2009	5/21/2009	F8909-08
D	07386 Mapping population	45	2/3/2009	5/21/2009	b40-14
E	07744 mapping populations	70	2/12/2009	5/21/2009	b40-14
F	9621 recombinants, 2007 crosses of interest	122	4/21/2009	8/13/2009	F8909-08
G	04191 mapping population, PD Rootstocks	82	6/23/2009	9/22/2009	F8909-08
H	04191 mapping pop (MPP)	130	10/8/2009	1/7/2010	F8909-08

Table 3. Greenhouse screen results for the 07744 cross (R8918-05 x Airen).

Resistance Class	Resistance Class Parameters	No. in Class	% Total
R	mean cfu/ml ~<100k	26	24%
R?	mean cfu/ml >~100k and max cfu/ml <1M cfu/ml	9	8%
S?	mean cfu/ml >~300k and <~3M cfu/ml	18	17%
S	mean cfu/ml >~1M and max cfu/ml >3.5M	54	50%
Total		107	100%

Table 4a. Phenotypic observations of reference varieties and select progeny with the *PdR1* resistance source used for small lot winemaking in 2009.

Genotype	Parentage	Percent *vinifera*	2009 Bloom Date	Berry Color	Berry Size (g)	Ave Cluster Wt. (g)	Ripening Season	Prod 1=v low, 9=v high
Barbara	Historic	100%	05/09/09	B	2.4	290	late	6
Chardonnay	Gouais blanc x Pinot noir	Historic	05/14/09	W	1.0	190	early	5
07355-12	U0505-01 x Petite Sirah	93.75%	05/10/09	B	1.0	137	early-mid	6
07355-75	U0505-01 x Petite Sirah	93.75%	05/07/09	B	1.3	234	early	8
07713-51	F2-35 x U0502-48	93.75%	05/07/09	W	1.4	210	early	8
U0501-12	A81-138 x Syrah	87.50%	05/18/09	B	1.1	194	late	4
U0502-10	A81-138 x Chardonnay	87.50%	05/07/09	B	1.4	198	early	7
U0502-20	A81-138 x Chardonnay	87.50%	05/14/09	W	1.7	313	late	8
U0502-26	A81-138 x Chardonnay	87.50%	05/10/09	B	1.6	375	mid	7
U0505-35	A81-138 x Cab. Sauvignon	87.50%	05/10/09	B	1.1	158	early	6
Blanc du Bois	Fla D6-148 x Cardinal	~66%	05/14/09	W	1.2	125	mid-late	7
Lenoir	*V. aestivalis* hybrid	<50%	05/20/09	B	0.8	201	late	6

Table 4b. Analytical evaluation of advanced selections with the *PdR1* resistance source used for small lot winemaking in 2009. 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)
07355-12	2.79	26.8	2050	3.42	0.78	275	127	585	2178
07355-75	2.88	28.2	2180	3.49	0.74	217	5	680	1941
07713-51	1.31	23.4	1700	3.56	0.49	146	-	-	-
U0501-12	2.11	21.8	1610	3.46	0.58	263	49	555	1026
U0502-10	3.97	24.9	2170	3.60	0.73	362	48	1006	1162
U0502-20	4.18	23.3	2230	3.51	0.76	383	-	-	-
U0502-26	2.24	24.0	1900	3.40	0.73	237	67	411	947
U0505-35	4.03	28.7	2450	3.66	0.81	476	47	886	1446

Table 4c. Sensory evaluation of reference varieties and advanced selections with the *PdR1* resistance source used for small scale winemaking in 2009.

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)
Barbara	pink-brown	low	neutral, acidic	jam, berry	2	4	nutty, spicy	3
Chardonnay	green-gold	medium	apple, pear	sl fruity	1	4	nutty	4
07355-12	red	med-dark	red fruit	plum, berry	3	3.5	woody, spicy	1
07355-75	red	medium	plum, fig	jam,prune	2	3	hot, woody	2
07713-51	green-gold	medium	apple, pear	neutral	2	3.5	woody, spicy	3
U0501-12	red	med-dark	fruity	fruit jam	2	4	neutral	2
U0502-10	pk-red-orng	med-dark	slight vegetal	sl fruity	1	4	nutty, spicy	1
U0502-20	green	medium	neutral, fruity	grass	1	4	spicy, bitter	1
U0502-26	pink	medium	bright, spicy	fruity	2	4	nutty	3
U0505-35	red	medium	CS-veg, berry	sl CS-veg	2	4	spicy	2
Blanc du Bois	gold	med-dark	floral, vegetal	sl vegetal	1	4	spicy, bitter	4
Lenoir	red	dark	mildly fruity	fruity	1	4	nutty	4

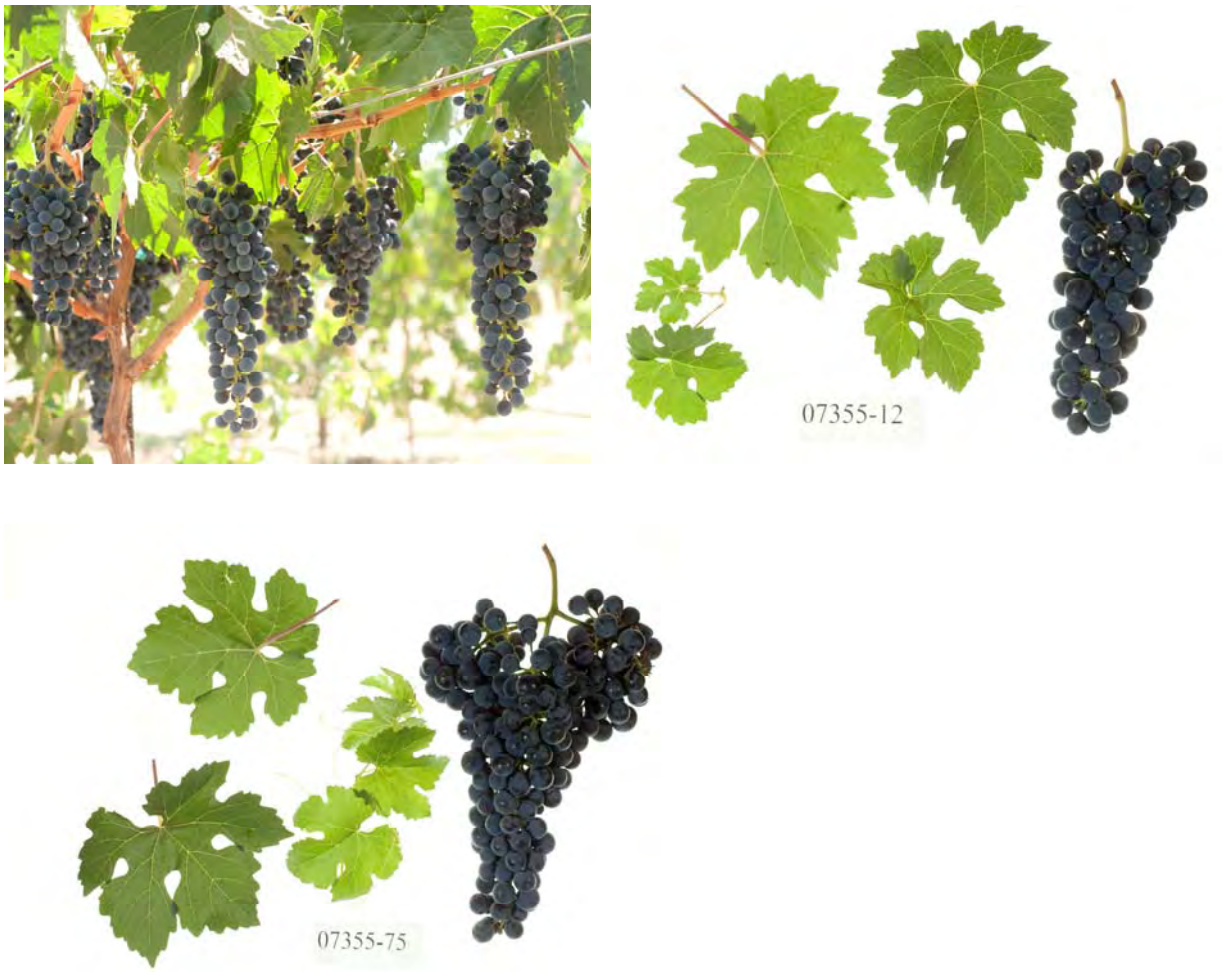

Figure 1. Images of the two red 94% *vinifera* PD resistant winegrape selections (U0505-01 x Petite Sirah) used for small-scale winemaking at UCD in Fall 2009. 07355-12 is above and 07355-75 is below.

Figure 2. Images of the white 94% *vinifera* PD resistant wine grape selection (F2-35 (Cabernet Sauvignon x Carignane) x U0502-48) used for small-scale winemaking at UCD in Fall 2009.