BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

Principal Investigator:
Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Co-Principal Investigator:
Alan Tenscher
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
actenscher@ucdavis.edu

Reporting Period: The results reported here are from work conducted October 2007 through September 2008.

ABSTRACT
The use of marker-assisted selection (MAS; see our companion report) and our acceleration of the seed to seed breeding cycle to three years have allowed very rapid progress towards our goal of Pierce's disease (PD) resistant winegrapes. Populations from the 2007 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 2008 crosses were made to: 1) Use the *PdR1* allele from 8909-08 to broaden the *vinifera* winegrape lines at the 93.75% *vinifera* level; 2) Combine *PdR1* with the powdery mildew resistance gene *Run1* at the 90.6% *vinifera* level; 3) Combine *PdR1* with the LG13 powdery mildew resistance gene *REN1* at the 87.5% *vinifera* level; 4) Use 8909-17 based resistance with diverse *vinifera* winegrapes to produce resistant progeny at the 87.5% *vinifera* level; 5) Use the F1 progeny of the homozygous PD resistant b40-14 *V. arizonica* to produce a breeding and mapping population that is 75% *vinifera*; 6) use elite winegrapes to broaden and expand the *V. shuttleworthii* breeding lines producing progeny that are 75% and 87.5% *vinifera;* and 7) Produce rootstocks with *PdR1* and broad-based nematode resistance. Inoculations were made to selections with *PdR1* and either 87.5% and 75% *vinifera* at our Beringer, Napa County trial. Finally, small-scale wine lots were made from five 87.5% *vinifera PdR1* selections from wine grape backgrounds. 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 *Xylella fastidiosa* (*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 *Xf* resistance into commercial grapes. They have produced plants that are 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 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 marker-assisted selection (MAS) with the b43-17 resistance sources and their progeny. 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 3-year seed-to-seed cycle, we will plant BC4 progeny in 2010. **Table 1** presents the crosses made in Spring 2008 with the numbers of seeds produced. The goals of this years crosses were: 1) Use the *PdR1* allele from 8909-08 to broaden the *vinifera* winegrape lines at the 93.75% *vinifera* level; 2) Combine *PdR1* with the powdery mildew resistance gene *Run1* at the 90.6% *vinifera* level; 3) Combine *PdR1* with the LG13 powdery mildew resistance gene *REN1* at the 87.5% *vinifera* level; 4) Use 8909-17 based resistance with diverse *vinifera* winegrapes to produce resistant progeny at the 87.5% *vinifera* level; 5) Use the F1 progeny of the homozygous PD resistant b40-14 *V. arizonica* to produce a breeding and mapping population that is 75% *vinifera*; 6) Use elite winegrapes to broaden and expand the *V. shuttleworthii* breeding lines producing progeny that are 75% and 87.5% *vinifera*; and 7) Produce rootstocks with *PdR1* and broad-based nematode resistance.

To date, three groups of plants have been greenhouse screened for *Xf* resistance in 2008 (**Table 2**). Group A tests were done to verify the expression of *PdR1* from b43-17 in the 04190 (*V. vinifera* F2-7 x 8909-08) population. This group also tested advanced 87.5% *V. vinifera PdR1* carrying parents, which were used in the 2007 crosses to create 94% *V. vinifera* progeny with *PdR1*. This group also included the parents of new mapping populations: one based on single gene resistance from *V.*

arizonica b40-14 (R89); and the other based on multigenic resistance from *V. arizonica/girdiana* b42-26 (05347). The Group B tests 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 use of BD5-117 produced seven resistant plants in population of 18 and none in an additional population of eight. The use of Midsouth and a *V. smalliana* x *vinifera* parent both resulted in one resistant plant of six progeny. All 13 progeny from a cross using Haines City had lower ELISA values than the known resistant Blanc du Bois in the greenhouse screen. Two of these progeny were used as parents in the 2008 crosses (**Table 1e**) and greenhouse tests found them to be as resistant as parents carrying *PdR1*, although Haines City does not contain *PdR1*. Eight promising rootstocks based on *PdR1* were also tested in this group and all were resistant. Group C tests focused on recombinants from our 2006 breeding populations to aid fine-scale *PdR1* mapping efforts and on the F1 progeny of b40-14 crossed to *V. vinifera* discussed below.

<u>*Objective 2*</u> – 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 *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 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. Greenhouse testing of 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*) was completed last year. In 2007, crosses using elite *V. vinifera* wine type pollen were made to a number of females in this population and 140 genotypes were planted this spring for future evaluation. This spring the cross 05347 was repeated to expand this mapping population (**Table 1e**).

V. arizonica b40-14 – Over the last seven years, we have greenhouse tested 45 F1 progeny of PD susceptible *V. rupestris* Wichita Refuge crossed with PD resistant b40-14 (R89 series). Only one genotype has failed to test resistant over that time period (data not shown). In 2006, we crossed *V. vinifera* F2-35 x b40-14 and established 198 seedlings for testing. In 2007, we crossed the *V. vinifera* variety Airen onto two of the PD resistant R89 genotypes and planted a total of 163 genotypes in Spring 2008. We have initiated greenhouse screening of these two populations for initial mapping of a new *PdR* locus. From our previous R89 testing, we expect the F1 progeny of b40-14 crossed to *V. vinifera* to all be PD resistant. To date, we have completed greenhouse testing of seven genotypes. Lack of PD phenotypic symptoms on all seven and very low mean cfu/ml ELISA values for the first three give some credibility to that expectation. We are planning on using the progeny of the 06339 crosses made this year (**Table 1e**) for further mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance.

V. shuttleworthii Haines City – Based on the encouraging greenhouse screen results for this resistance source as reported above, in 2008 we made the BC1 (75% *vinifera*) and BC2 (88% *vinifera*) using a BC1 from our earlier table grape work that tested particularly well and had reasonable wine grape characteristics (**Table 1e**).

Given that low levels of *Xf* exist in resistant plants, it will be important to also have PD resistant rootstocks to graft with resistant scions and prevent them from dying on susceptible rootstocks. We completed screening of eight promising progeny from crosses of 101-14 x F8909-08. Evaluation for grafting ability and testing against phylloxera and nematodes and finally field testing will follow. In 2008, we made additional PD resistant rootstock crosses resulting in 1397 seeds (**Table 1f**).

<u>*Field and Wine Evaluations*</u> – The A81 series (BC1, 75% *vinifera*) 8909-08 allele type of *PdR1* is in its third 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*) were grafted onto Dog Ridge (currently the only certified PD resistant rootstock) and were planted at Yountville in Spring 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). These were needle inoculated for the first time on May 22, 2008. The A81 series was inoculated at the same time for the second time. Both groups will be sampled for ELISA testing this fall.

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 in 2006 and wines made again this fall. Between four and 20 liters of wine from each were produced along with similar amounts of Barbara and Zinfandel as *V. vinifera* controls and Lenoir as the standard PD resistant control to standardize these very small-scale fermentations. Two additional wines were made for the first time this year from siblings of the above crosses: U0502-20 (white) and U0502-26 (red). All 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 3a** and **3b**, 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 (<u>www.etslabs.com</u>) 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 were established in our Davis vineyard this spring so that we can get small-scale wine lots made for evaluation in 2009. 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.

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. The 2008 season field evaluations of the 2006 crosses show the markers correlating perfectly with field resistance to powdery mildew on the leaves, canes, rachis and fruit. The goal with these individuals is to cross our advanced PD resistant selections with selections from these powdery mildew resistant progeny (**Table 1b**). This spring 537 plants of crosses between genotypes with *PdR1* and other types with *Run1* were planted on 1' x 1' spacing in a nursery to screen for powdery mildew resistance. This allowed the elimination of weak plants and reduces the cost of MAS screening where we continue to see segregation distortion against the *Run1* locus in some lines. We tested 136 plants in the nursery screen for powdery mildew resistance celll of which are preparing for marker testing for both *Run1* and *PdR1* to verify the utility of MAS for the combined traits. Plants with both loci will go to the field for evaluation of fruit and horticultural characteristics. In 2008 we also made crosses to examine powdery mildew in two other backgrounds: a source of the *REN1* locus (a separate powdery mildew resistance locus on chromosome 13, from the *V. vinifera* table grape Karadzhandal (**Table 1c**) and the Chinese species *V. romanetii*. We produced 564 seeds using Karadzhandal, clusters from crosses with *V. romanetii* are being processed.

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 locus. The first testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2007, and they scored remarkably well. Evaluation of the 2008 wines is pending.

REFERENCES CITED

Buzkan, N., A.F. Krivanek, A. Eskalen and M.A. Walker. 2003. Improvements in sample preparation and polymerase chain reaction detection techniques for *Xylella fastidiosa* in grapevine tissue. American Journal of Enology & Viticulture. 54:307-312.

Buzkan, N. L. Kocsis and M.A. Walker. 2005. Detection of *Xylella fastidiosa* from resistant and susceptible grapevine by tissue sectioning and membrane entrapment immunofluorescence. Microbiol. Res. 160:225-231.

Krivanek, A.F., J.F. Stevenson and M.A. Walker. 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology 95:36-43.

Krivanek, A.F. and M.A. Walker. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44-52.

Krivanek, A.F., T.R. Famula, A. Tenscher and M.A. Walker. 2005. Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* x *Vitis arizonica* hybrid population. Theor, Appl. Genet. 111:110-119.

Krivanek, A.F., S. Riaz and M.A. Walker. 2006. The identification of *PdR1*, a primary resistance gene to Pierce's disease in *Vitis*. Theor. Appl. Genet. 112:1125-1131.

Mortensen, J.A. 1968. The inheritance of resistance to Pierce's disease in *Vitis*. J. Am. Soc. Hort. Sci. 92-331-337.

Riaz S, A.F. Krivanek, K. Xu and M.A. Walker. 2006. Refined mapping of the Pierce's disease resistance locus, *PdR1*, and Sex on an extended genetic map of *Vitis rupestris* x *V. arizonica*. Theor. Appl. Genet. 113:1317-1329.

Ruel, J.J. and M.A. Walker. 2006. Resistance to Pierce's disease in *Muscadinia rotundifolia* and other native grape species. American Journal of Enology & Viticulture. 57:158-165.

Riaz, S., S. Vezzulli, E.S. Harbertson, and M.A. Walker. 2007. Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's disease. Amer. J. Enol. Vitic. 58:494-498.

Riaz, S., A.C. Tenscher, J. Rubin, R. Graziani, S.S. Pao and M.A, Walker. 2008. Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theor. Appl. Genet. 117:671-681.

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.

Table 1. 2008 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.			
U0502	Chardonnay	F2-7 (Cab x Carignane)	262
U0505	Cabernet Sauvignon	Tannat	694
1b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Run1* powdery mildew resistance to produce progeny with 90.6% *vinifera* parentage.			
U0502	Chardonnay	06353, e78 allele pattern	82
U0505	Cabernet Sauvignon	06717, e78 allele pattern	138
1c. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and a *vinifera* PM resistance source to produce progeny with 87.5% *vinifera* parentage.			
A81-17	A38-7	Karadzhandal	564
1d. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 87.5% *V. vinifera* parentage.			
06324	Chenin blanc	Airen, Cabernet Sauvignon, Chardonnay, Tannat	484
06372	Malaga Rosada	Clairette blanche, Tannat	946
06381	F2-7 (Cab x Carignane)	Tannat	151
1e. Other PD resistance sources: b40-14 *V. arizonica* (06339) progeny are 87.5% *vinifera.* The *V. shuttleworthii* PD resistance sources 0098-03 progeny are 87.5% *vinifera* and 04394 progeny are 75% *vinifera*			
06339	F2-35 (Cab x Carignane)	Malaga Rosada, Tannat	325
0098-03	NR	Cabernet Sauvignon, Chardonnay	324
04394	NR	Cabernet Sauvignon, Clairette blanche, F2-35, Tannat	1,130
b42-26	NR	F2-35	827
1f. Rootstock crosses to combine PD and nematode resistance.			
03300-048	06301,Wyoming Riparia, Riparia Gloire, 44-53 mgt		1,397

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

Group	Genotypes	N	Inoculation Date	ELISA Date	Resistance Source(s)
A	04190, 9621, 2007 parents	150	10/18/2007	1/31/2008	b43-17 (both alleles)
B	D89, R89, 9621, 03300/5 (PD rootstocks) , 03182, 03187, 04183, 04394, 2007 parents retest	157	3/20/2008	6/26/2008	b43-17, BD5-117, Midsouth, Haines City
C	2006 recombinants, 06339	29	5/20/2008	8/21 & 9/25/08	b43-17, b40-14

Table 3a. Phenotypic observations of reference varieties and select progeny with the *PdR1* resistance source.

Genotype	Parentage	Percent *vinifera*	2008 Bloom Date	Berry Color	Berry Size (g)	Avg Cluster Wt. (g)	Ripening Season	Prod 1= v low 9 = v high
Barbara	Historic	100%	5/5/08	B	2.4	290	Late	6
Zinfandel	Historic	100%	5/5/08	B	2.6	405	Mid	7
U0501-12	A81-138 x Syrah	87.5%	5/11/08	B	1.0	160	late	4
U0502-01	A81-138 x Chardonnay	87.5%	5/5/08	B	2.0	210	mid-late	4
U0502-10	A81-138 x Chardonnay	87.5%	5/5/08	B	1.7	275	very early	8
U0502-20	A81-138 x Chardonnay	87.5%	5/10/08	W	2.0	201	Late	8
U0502-26	A81-138 x Chardonnay	87.5%	5/9/08	B	2.1	375	mid-late	6
Lenoir	*V. aestivalis* hybrid	<50%	5/16/08	B	0.8	201	Late	6

Table 3b. Analytical evaluation of reference varieties and advanced selections with the *PdR1* resistance source. 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)
Barbara	2.83	25.0	2170	3.36	0.87	431	31	201	300
Zinfandel	2.43	23.5	1870	3.55	0.62	191	34	322	386
U0501-12	3.22	27.2	2020	3.51	0.74	3.98	48	781	1161
U0502-01	7.36	23.3	3240	3.70	0.96	567	81	364	530
U0502-10	4.36	22.3	1800	3.47	0.82	305	73	565	828
U0502-20	4.94	24.0	2600	3.62	0.90	544	-	-	-
U0502-26	5.55	24.3	2420	3.64	0.91	699	65	225	811
Lenoir	5.54	28.7	3050	3.63	0.83	230	160	405	2396

Table 3c. 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)
Barbara	pink-brown	low	neutral, acidic	jam, berry	2	4	nutty,spicy	3
Zinfandel	orng-brown	medium	jam, hay	fruity	2	4	nutty,bitter	1
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
U0502-20	green	medium	neutral, fruity	fruity	1	4	spicy,bitter	1
U0502-26	pink	medium	bright, spicy	fruity	2	4	nutty	3
Lenoir	red	dark	mildly fruity	fruity	1	4	nutty	4

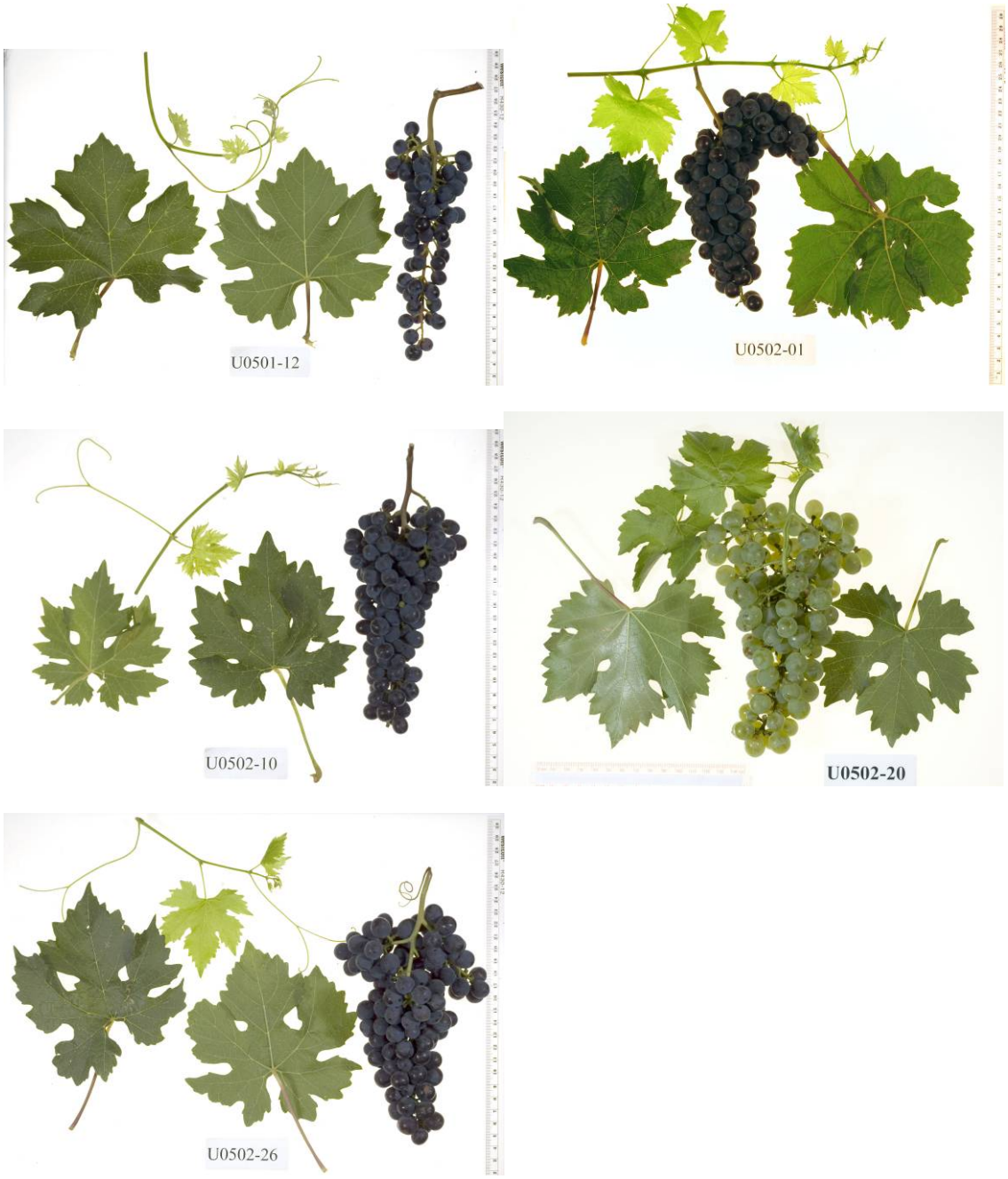

Figure 1. Pictures of the 87.5% *vinifera* PD resistant wine grape selections used for small-scale winemaking at UCD in 2008.

Section 6: Economics