CDFA PD/GWSS Progress Report
February 2008

I. Project Title: Breeding Pierce's Disease Resistant Winegrapes.

II. Principal Investigators and Cooperators:
Andrew Walker and Alan Tenscher, Dept. of Viticulture and Enology, University of California, Davis, CA 95616-8749; awalker@ucdavis.edu

III. List of objectives and description of activities

Objective 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.

The breeding cycle for the development of PD resistant grapes has been reduced to 3 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, seed produced and those seed populations that have sprouted by February 2008. 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 (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, and confirm *PdR1* in parents used in 2006. 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 progeny of Haines City 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 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 included additional 04190 progeny and

remnants from the 9621 (8909-15 x 8909-17) population that had not been tested. 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 resistance from *V. arizonica* b40-14 (R89); and the other based on multigenic resistance from *V. arizonica/girdiana* b42-26 (05347).

Objective 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.

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).

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, 4 were recombinants (1 resistant and 3 susceptible in the greenhouse test). They will be inoculated in Spring 2008.

Four of eight advanced red wine selections (U0501-12, U0502-01, -07 and -10) containing *PdR1* that are 87.5% *vinifera* from crosses with Syrah and Chardonnay were replicated for ultra small-scale fermentation this Fall. About 4 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 3a, 3b and 3C, and images of the leaves and fruit are in Figure 1. The tasting notes for these wines are included in Table 4.

Numerous other genotypes from crosses involving elite *vinifera* wine cultivars were examined for fruit evaluation and must analysis. ETS Laboratories 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.

Given that low levels of *X. fastidiosa* 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. In 2006 we tested all of our PD resistant rootstock crosses as seedlings for *PdR1* using SSR markers before planting in the field. From these seedling populations we selected 9 progeny that have *PdR1*, root well, and have good cane morphology (long internodes, few laterals, good vigor) (Table 5). Greenhouse screening for PD resistance, evaluation for grafting ability, testing against phylloxera and nematodes and finally field testing will follow.

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.

V. Publications or Reports from this Project

Riaz, S, A.C. Tenscher, B.P. Smith, D.A. Ng and M.A. Walker. 2008. Use of SSR markers to assess identity, pedigree, and diversity of cultivated *Muscadinia rotundifolia*. J. Amer. Soc. Hort. Sci. (accepted final revision)

Lowe, K.M., S. Riaz and M.A. Walker. 2008. Variation in recombination rates across *Vitis* species. Tree Genomics Genet. (In second review)

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. (submitted).

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. Viticult. 58:494-498.

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.

Ruel, J.J. and M.A. Walker. 2006. Resistance to Pierce's Disease in *Muscadinia rotundifolia* and other native grape species. Amer. J. Enol. Viticult. 57:158-165.

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 linkage map of *Vitis rupestris* x *V. arizonica*. Theor. Appl. Genet. 113:1317-1329.

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.

Ruel, J.J. and M.A. Walker. 2006. Resistance to Pierce's Disease in *Muscadinia rotundifolia* and other native grape species. Amer. J. Enol. Viticult. 57:158-165.

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 linkage map of *Vitis rupestris* x *V. arizonica*. Theor. Appl. Genet. 113:1317-1329.

VI. Presentations on PD Research

A. Walker. Genetic diversity in grapes. Wine from Sunny Places Seminar, UCD Extension, Davis, CA, April 21, 2007.

A. Walker. Grape breeding and phylloxera research at UC Davis. EMBRAPA, Bento Goncalves, Brazil, April 24, 2007.

J. Baumgartel and M. A. Walker. Optimizing greenhouse evaluations of Pierce's disease resistance. American Society for Enology and Viticulture Annual Meeting, Reno, NV, June 21, 2007.

J. R. Rubin and M. A. Walker. Allelic diversity of *PdR1* in *Vitis arizonica/candicans* selections from Monterey, Mexico. American Society for Enology and Viticulture Annual Meeting, Reno, NV, June 21, 2007.

A. Walker. Marker-assisted selection for Pierce's disease resistance. Applied Grape Genomics Meeting, UC Davis, July 16, 2007

A. Walker. Will there be GMOs in California vineyards – what are the issues and what can we expect. Lodi Woodbridge Grape Growers Meeting, Lodi, CA, July 17, 2007.

S. Riaz, A. Tenscher, and M. A. Walker. Molecular breeding: marker-assisted selection for Pierce's disease and powdery mildew resistance in grapevine. National Viticulture Research Conference, UC Davis, July 20, 2007

A. Walker. Grape breeding at UC Davis. North American Grape Breeder's Meeting, UC Davis, August 22, 2007

A. Walker. Breeding as an essential part of sustainable viticulture. Master's of Wine Course, Oakville, CA, October 22, 2007.

A. Walker. GMOs in viticulture – pollen movement and implications. Napa Farm Bureau, Napa, CA, November 19, 2007.

A. Walker. Classical and molecular breeding to combat PD. CDFA PD/GWSS Annual Meeting, San Diego, CA, December 14, 2007.

VII. Research Relevance Statement

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. 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.

VIII. Lay Summary

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 has 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 4 of 8 selected 87.5% *vinifera PdR1* containing wine grape types. Fruit evaluation and must analysis were performed on numerous other promising progeny at this level.

IX. Status of Funds

All funds are scheduled to be spent by July 2009.

X. Summary and Status of Intellectual Property Produced

Thus far no selections have been released from this breeding program. When they are they will be released through UC Davis.

Table 1. 2007 PD resistant wine grape crosses, final numbers of seed produced and number of seedlings to be marker tested for *PdR1*.

Resistant Type	*Vinifera* Parent of Resistant Type	*Vinifera* Types used in 2007 crosses	Total Seeds	# Being Germinated	# To be Marker Tested
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.					
U0501	Syrah	F2-7, F2-35	1055	645	150
U0502	Chardonnay	F2-7, F2-35	2769	1310	200
U0503	Sauvignon blanc	Chardonnay, Palomino, Semillon	126	126	50
U0505	Cabernet Sauvignon	Chardonnay, F2-7, LCC, Merlot, Palomino, Petite Sirah	3229	1578	300
1b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 87.5% *V. vinifera* parentage					
05310	Alicante Bouschet	Burger, Carignane, LCC	1666	498	75
05312	Cabernet Franc	Zinfandel	194	100	25
05317	Tempranillo	Burger, LCC	371	209	75
05319	Zinfandel	Cabernet Franc, LCC	144	144	50
A81-17	A38-7	Carignane, Grenache noir, LCC	705	287	75
1c. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Run1* powdery mildew resistance to produce progeny with 87.5% *V. vinifera* parentage except for the A81-17 group which is 81.5% *V. vinifera*					
Resistant Type	*Vinifera* Parent of Resistant Type	*Vinifera* Types used in 2007 crosses	Total Seeds	# Being Germinated	# To be Marker Tested
U0501, U0504	Syrah	e-series, e78 and e88 allele	499	499	50
U0502	Chardonnay	e-series, e78 and e88 allele	837	837	100
U0505	Cabernet Sauvi.	e-series, e78 and e88 allele	642	542	100
A81-17	A38-7	e-series, e78 allele	603	256	50

1d. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 75% *V. vinifera* parentage.

04373-02 12K	F2-35 (Cab x Carignane)	Alicante Bouschet, Aligote, Carignane, Chardonnay, Zinfandel	597	597	200

1e. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 75% *V. vinifera* (Germination of seeds in progress).

04373-02	F2-35 (Cab x Carig)	Alicante Bouschet, Aligote, Carignane, Chardonnay, Zinfandel	597
04373-08	F2-35 (Cab x Carig)	Aligote, Cabernet franc, Carig	938
04373-64	F2-35 (Cab x Carig)	Grenache noir	293

1f. Other resistance sources. R89 is 50% *vinifera*, 25% resistance source. 05347 is 75% *vinifera* and 25% resistance source (Germination of seeds in progress).

R89(b40-14)	NR	Airen	238
05347 (b42-26)	F2-35	Aligote, Chard, Grenache, Zin	1,877

1g. Rootstock crosses to combine PD and nematode resistance (Germination of seeds in progress).

9621-257	9365-85		653
9365-43	9621-161		112

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

	Genotypes	N	Inoculation Date	ELISA Date	Resistance Source(s)
A	04190, parents	160	1/18/2007	4/19/2007	b43-17 (F8909-08)
B	SEUS and *PdR1* parents	76	2/22/2007	5/24/2007	BD5-117, Midsouth, Haines City
C	*arizonica* as rootstock	4	4/5/2007	6/26/2007	b43-17 (both alleles)
D	04190, 04191, 05347	120	6/14/2007	9/18/2007	b43-17 (both alleles), b42-26
E	04190, 9621, 2007 parents	150	10/18/2007	1/31/2008	b43-17 (both alleles)

Table 3a. 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 3b. 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 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)
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

Table 4. Tasting notes from ultra small-scale wines at the 87.5% *V. vinifera* level and containing *PdR1*. The wines were described on 11/1/07 by a campus tasting panel. Walker, Tenscher and V&E winemaker Brenneman described the wines on 1/3/08. Cab Sauv and Pinot noir were made in the same way as comparisons as were PD resistant Lenoir and Midsouth.

Wine name	Group Total	Low score	High Score	11/1/07 Consensus Descriptors: color; aroma; flavor-texture	1/3/08 Post Bottling Descriptors: color; aroma; flavor-texture
U0501-12	33.5	2	4.5	dark purple; grapy, smoky, blueberry; warm, chocolate, rich, balanced, structured	very dark purple-red; ripe, fruity with hint of reduction; , rich, structured tannins
U0502-07	32	2.5	4	dark purple; grapy, earthy-smoky; rich, good structure & balance	Dark purple-red; complex, spicy, slightly porty; good structure but a bit low in acid
Cab. Sauv	27	2	5	red with hint of brown; herbal, weedy, bell pepper; warm, flat, with good tannin structure	fair color, red with brown edges; improved but still vegetal, hot region red fruit; good structure
U0502-10	27	1	5	dark purple; bright red fruit, odd herbal-plastic note; non-vinifera flavor, okay structure	Dark purple-red; canned asparagus over bright red plum fruit; okay acid.
Lenoir	26	2	4.5	dark purple-black; blackberry, dried fig, slightly weedy, odd non-vinifera herbalness; lacks structure	very dark purple unlike vinifera; fruity sweet spice; no structure.
U0502-01	24	1.5	4	light-medium red with hint of brown; candy, red fruit; moderate body, slightly earthy, oxidized	medium red with slight brown edges; good fruit, better than PN; medium body
Pinot noir	20	1	3.5	light pink-red; simple red fruits; odd vegetal, cherry, light	light red; improved but warm region simple red fruit, candied; light, simple
Midsouth	18.5	1	3	red with slight brown edge; vegetal, oxidized, simple	red with brown margins; slightly herbal, mature; simple lacking structure

Table 5. PD resistant rootstock crosses evaluated in fall 2007 in the field for rootstock quality traits before advancing to the GH PD screen.

Cross ID	Cross	PD resistance source	Rootstock Traits		Rooting/Prop.	
			# evaluated	# passed	# evaluated	# passed
03301	D8909-15 x Riparia Gloire	b42-26	56	9	9	5
03306	D8909-15 x 1616C	b42-26	56	7	7	3
03300/5	101-14 mgt x F8909-08	PdR1	172	26	9	6
06301	03300-018 x 9365-85	PdR1	32	12	5	2
06304	03300-088 x 9621-152	PdR1	25	9	2	1
06305	03300-088 x 9621-161	PdR1	21	4	2	0
06307	Florilush x 9621-161	PdR1	24	9	3	0
06308	Florilush x 9621-244	PdR1	23	7	3	0

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