Progress report for CDFA Contract 03-0293
August 2009

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

Reporting period: March 2009 to August 2009

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 *Xylella fastidiosa* resistant selections and sources characterized from our previous efforts.
Objective 2. Continue the characterization of *X. fastidiosa* 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.

IV. Summary of Research Accomplishments
Objective 1. 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 *V. arizonica/V. candicans* 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* winegrape 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. Using MAS and the 3-year seed-to-seed cycle we will plant BC4 progeny in 2010. Table 1 presents the crosses made in 2008 with numbers of seedlings produced, the number of progeny that were MAS tested and then the number planted to the field in Spring 2009. Table 2 shows the crosses made in 2009. The goals of the 2009 crosses were: 1) Use the *PdR1* allele from F8909-08 to advance the *vinifera* winegrape line to the 96.9% *vinifera* level; 2) broaden the *PdR1* F8909-08 allele by *vinifera* winegrape lines at the 93.75% *vinifera* level; 3) Use the F1 progeny of the heterozygous R89 x vinifera with PD resistance from b40-14 *V. arizonica* to produce a breeding population that is 75% *vinifera* and 12.5% the resistance source; and 4) increase the b42-26 mapping population by remaking the F2-35 x b42-26 cross.

During this period, eight groups of plants were tested in the greenhouse for *X. fastidiosa* resistance (Table 3). Group A tests confirmed the resistance of the parents used in the 2008 crosses, 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 Dr. Olmo's breeding program. However, 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 PD resistance of the 50% *vinifera*, 25% b40-14 *V. arizonica* resistance source 07744 and 07386 populations. Table 4 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 the 75% *vinifera* crosses in 2009 and advance this promising new PD resistance line by another generation (Table 2c). 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 (2008), we found that tighter spacing increased the mean ELISA values relative to our standard spacing in both susceptible and resistant selections. Group F consisted of additional 9621 population recombinants that were tested to aid in fine scale mapping of *PdR1*. We also tested additional 2007 crosses to continue the exploration of *PdR1* resistance initiated in Group A. Groups G & H focus on the F8909-17 allele of *PdR1* to elaborate any differences in resistance behavior of this allele compared to the F8909-08 allele. We included testing of our new PD resistant rootstocks 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* 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. 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 less complex 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 the encouraging greenhouse screen results for this resistance source, in 2008 we made the BC1 (75% *vinifera*) and BC2 (87.5% *vinifera*) using a BC1 from our earlier table grape work that was very resistant and had reasonable wine grape characteristics (Table 1c). If we can develop reliable genetic markers for *Xf* resistance from this resistance source, 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 7 years we have greenhouse tested 45 F1 progeny of PD susceptible *V. rupestris* Wichita Refuge crossed with PD resistant *V. arizonica* b40-14 (the R89 series). Forty-two were highly resistant and three had intermediate reactions (data not shown). In 2006, we made the 06339 population (*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 series genotypes and planted a total of 163 progeny in Spring 2008. One of these is the 07744 population (F1 50% *vinifera*, 25% b40-14) – see Tables 2c, 3 and 4. Preliminary mapping of this population places PD resistance from b40-14 on LG14 but in a different location than *PdR1*. To date we have completed greenhouse testing of seven 06339 genotypes (F1 50% *vinifera*, 25% b40-14); they all lack PD symptoms and have low ELISA values. We are planning on using the progeny of the 06339 x *V. vinifera* crosses made in 2008 (Table 1c.) for further mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance.

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 if *X. fastidiosa* moved into the rootstock. We have tested their rooting and grafting ability (with two scion varieties) and replicated many for greenhouse testing of their *X. fastidiosa* resistance (Table 5). Greenhouse screening for phylloxera and nematode resistance will be done on PD resistant selections followed by field testing.

Beringer Field Testing

We continue to use the Beringer vineyard in Yountville, CA to test the PD resistance of advanced selections under field conditions. We do not depend on natural sharpshooter vectoring, but instead needle inoculate each plant with *X. fastidiosa*. Selections from the BC3, 94% *vinifera* crosses from the 07-355 (U0505-01 x Petite Sirah) and 07-370 (*vinifera* F2-35 x U0502-38) populations were grafted onto Dog Ridge (currently the only certified virus-free PD resistant rootstock) on February 17, 2009 and planted at Beringer on June 18, 2009. These genotypes have been marker tested and their PD resistance status will be confirmed by greenhouse testing in the coming months. Inoculation in the field will take place in Spring 2010. 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 on June 3, 2009. This June we also planted the seven most promising 87.5% *vinifera PdR1* wine types (2 white and 5 red; 6 reps each) grafted onto Dog Ridge for small-scale winemaking trials. Genotypes planted were 06325-42, 06325-43, U0502-01, U0502-10, U0502-35, U0502-38, U0502-41.

Wine Making

In 2007, we planted at least six vines of eight 87.5% *vinifera PdR1* selections (50% Syrah or Chardonnay from the last cross) to prepare for the making of small-scale wine making tests in Fall 2008. Four of these selections set enough fruit to make small scale wine lots. Sensory evaluation of these wines 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. After early August 2009 crop estimation, 12 fermentations are planned this fall: 3 (2 red, 1 white) at the 94% *vinifera* level (Figure 1); 5 (4 red, 1 white) at the 87.5% *vinifera* level and 4 (2 red, 2 white) *vinifera* and PD controls.

V. Publications or Reports from this Project

Baumgartel, J.E. 2009. Optimizing screening technology for breeding Pierce's disease resistant *Vitis*. M.S. Thesis. University of California, Davis.

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek, D.W. Ramming and M.A. Walker. 2009. Using marker-assisted selection to breed Pierce's disease resistant grapes. Amer J Enol Viticult 60:199-207,

Vezzulli, S., D. Micheletti, S. Riaz, M. Pindo, R. Viola, P. This, M.A. Walker, M. Troggio and R. Velasco. 2009. A SNP transferability survey within the genus *Vitis*. BMC Plant Biol 8:128

Cheng, D.W., H. Lin, M.A. Walker, E.L. Civerolo and D. Stenger. 2009. Transcriptional regulation of the grape cytochrome P450 monooxygenase gene, CYP736B expression in response to *Xylella fastidiosa* infection. DOI 10.1007/s10658-009-9473-8

VI. Presentations on PD Research

"Creating *Xylella* resistant grapevines by conventional breeding", CDFA Pierce's Disease Symposium, San Diego, CA, December 17

"Grape breeding with an emphasis on flavor" Recent Advances in Viticulture and Enology, UC Davis, March 19

"Lessons learned about plant materials & PD resistant winegrapes coming soon", Temecula Grape Day, Temecula, CA, April 2

"Grape breeding at UC Davis" American Vineyard Foundation Board Meeting, Modesto, CA, April 25.

"20 years of grape breeding at UC Davis" Honorary Research at ASEV meeting, Napa, June 24

"Breeding winegrapes with resistance to Pierce's disease" ASEV Annual Meeting, Napa, June 25

"Walker grape breeding program" North American Grape Breeder's meeting, FAMU, Tallahassee, FL, August 6

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 second testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2008, and they scored remarkably well.

VIII. Lay Summary

Progress continues on breeding 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 2008 crosses were screened with MAS for PD and were planted in the field this Spring. Results from needle inoculated 75% and 87.5% *vinifera PdR1* containing selections planted in field trials at Beringer in Napa County found that these selections have very strong resistance to PD in the greenhouse and field. Finally, small scale wine lots were made from four 87.5% *vinifera PdR1* containing wine grape selections for the second year, and they scored remarkably well. Fruit evaluation and must analysis were

performed on numerous other promising progeny of 87.5% *vinifera PdR1* lines; fruit from 94% *vinifera PdR1* selections will be fermented this Fall.

IX. Status of Funds

These funds are scheduled to be spent by the end of the grant.

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. 2008 wine and rootstock type crosses, numbers of seeds & seedlings produced with number marker tested.

Resistant Type	Vinifera Parent of Resistant Type	Vinifera Types used in 2008 crosses	# Seedlings Produced	# Marker Tested	# Planted to Field
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.					
U0502	Chardonnay	F2-7	114	50	22
U0505	Cabernet Sauvignon	Tannat	151	100	32
1b. 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, Malaga Rosada, Tannat	402	250	137
06372	Malaga Rosada	Clairette blanche, Tannat	716	455	177
06381	F2-7 (Cab x Carignane)	Tannat	113	75	44
1c. Other PD resistance sources: b40-14 *V. arizonica* (06339) progeny are 87.5% *V. vinifera.* The *V. shuttleworthii* PD resistance sources 0098-03 progeny are 87.5% *V. vinifera* and 04394 progeny are 75% *V. vinifera*					
06339	F2-35 (CS x Carig)	Tannat	251	NR	212
9985-03	Ruby Seedless	Cabernet Sauvignon, Chardonnay	109	NR	40
04394	Flame Seedless	Cabernet Sauvignon, Clairette blanche, F2-35, Tannat	338	NR	150
1d. Rootstock crosses to combine PD and nematode resistance.					
03300-048	101-14 mgt	06301,Wyoming Riparia, Riparia Gloire, 44-53 Maleque	691	MAS Test after hort evaluation	330

Table 2. 2009 Wine and mapping crosses with estimated number of seeds produced.

Resistant Type	Vinifera Parent of Resistant Type	Vinifera Types used in 2009 crosses	Estimated # of Seeds
2a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 96.875% *V. vinifera* parentage.			
07354-50	Merlot	Cabernet Sauvignon, Chardonnay	125
07355-020	Petite Syrah	Cabernet Sauvignon, Chardonnay, Chenin blanc, Zinfandel	1750
07370-039	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chenin blanc, Riesling, Sylvaner, Zinfandel	1450
07370-097	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chardonnay, Chenin blanc, Pinot noir	650
07370-28	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chenin blanc, Pinot noir, Zinfandel	950
07371-19	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chardonnay, Chenin blanc, Sylvaner	375
07371-20	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chenin blanc, Pinot noir, Sylvaner	925
07371-36	F2-35 (Cab. Sauv. x Carignane)	Cabernet Sauvignon, Chardonnay, Chenin blanc, Mourvedre, Riesling, Zinfandel	800
2b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *V. vinifera* parentage.			
07307-10	Zinfandel	Cabernet Sauvignon, Chenin blanc	115
2c. 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	Cabernet Sauvignon, F2-35, Malaga Rosada	790
2d. Cross to increase the b42-26 *V. arizonica* x *vinifera* mapping population.			
b42-26	F2-35 (Cab. Sauv. x Carignane)	F2-35	200

Table 3. PD resistant winegrape progeny just 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 4. Greenhouse screen results for the 07744 cross (R8918-05 x Airen).

Resistance Class	Resistance Class Parameters	# 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 5. New PD resistant rootstocks grafted to resistant and/or susceptible scion currently in GH testing as part of Group G in Table 3 above. Stock parentage is 101-14 Mgt x F8909-08 except for 06304-26 which is 03300-088 (101-14 Mgt x F8909-08) x 9621-152

Rootstock Genotype	Sex	Vigor of stock	2008 GH PD Screen mean cfu/ml (rootstock)	% successful grafting & rooting to Chardonnay	# advanced to GH test	% successful grafting & rooting to A81-17	# advanced to GH test
03300-048	F	high	44,234	88.9%	6	88.9%	6
03300-082	M?	high	29,001	66.7%	6	100.0%	6
03300-072	M	high	94,820	88.9%	6	66.7%	6
03305-18	F	high	30,220	12.5%	0	66.7%	5
03300-099	F	mod-high	102,813	22.2%	2	50.0%	4
03305-12	F	h-v high	32,632	50.0%	4	22.2%	2
06304-26	F	mod-high	60,062	33.3%	3	33.3%	3
03300-047	M	h-v high	7,585	11.1%	0	44.4%	4
101-14 Mgt	F	mod-high	858,055	55.6%	5	50.0%	4

Figure 1. Images of the three 94% *vinifera* PD resistant wine grape selections (U0505-01 x Petite Sirah) to be used for small-scale winemaking at UCD in Fall 2009.

Figure 1. Images of the three 94% *vinifera* PD resistant wine grape selections (F2-35 (CabSav x Carig) x U0502-48) to be used for small-scale winemaking at UCD in Fall 2009.