CDFA PD/GWSS Progress Report
July 2010

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

Reporting period: April 2010 to July 2010

II. Principal Investigators and Cooperator:
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 2 years (seed to seed) with aggressive vineyard training and with marker-assisted selection (MAS) within the *V. arizonica/candicans* b43-17 *PdR1* resistance sources and their progeny. Our goal at this point is to introgress *PdR1* and other PD resistance sources into a large number of *V. vinifera* winegrape backgrounds. We have reached the backcross 4 (BC4) generation with 96.8% *V. vinifera* and *PdR1* resistance. We have focused our efforts on growing out larger numbers of progeny from a variety of crosses. Table 1 presents the crosses made in 2010 with an estimate of the number of seeds expected. The goals of the 2010 crosses were:

1) Use the *PdR1b* allele from the F8909-08 to increase our populations of *vinifera* winegrape lines at the 96.8% *vinifera* level. These populations will add to those we planted in the vineyard this Spring (Table 2). Seedlings planted this Spring will begin fruiting in 2011 and seeds from the 2010 crosses will be harvested this Fall and planted Spring 2011. Five of the crosses this Spring used the *V. vinifera* cultivar as the maternal parent (last 5 crosses in Table 1a). This process requires emasculation of the flowers on these vines to prevent selfing – a laborious process. We made these crosses to introgress in unusual characters (aromas from Viognier, muscat flavor from Muscat blanc and red juice color from Alicante Bouschet) and to determine the impact of using pure *vinifera* as the maternal parent. Because these hermaphroditic *vinifera* cultivars need to be emasculated before they can be crossed, we have only used *vinifera* cultivars to pollinate female flowered PD resistant selections, and reaped the benefits of more rapid progress. This also yields lots of resistant female flowered vines each generation, but also many hermaphrodites.

2) To continue to advance *PdR1a* into 93.75% *V. vinifera* populations while avoiding *V. rupestris*. *Vitis rupestris* has very dark black purple juice color. Although it imparts very strong color to wines, the juice also has diglucoside anthocyanins, which are used to identify and

restrict the sales of hybrid wines. Juice from the early generation hybrids with *PdR1* from F8909-08 (*V. rupestris* x *V. arizonica/candicans*) often had intense color and peppery herbaceous characters from *V. rupestris*. Thus we are advancing backcross generations using *PdR1* from the *V. arizonica* parent, b43-17, to avoid these characters.

Since March 2010 five groups of plants have been evaluated in the greenhouse for PD resistance or have had testing initiated (Table 3). Groups K and M are being examined to test the possible role of different levels of susceptibility from the *V. vinifera* parent. These differing roles were also being examined in the Group J plants. These results are currently being compared with a similar earlier test group (Group E), which is being retested.

Group L plants are being examined to test the impact of different *X. fastidiosa* strains on the *PdR1* resistance source. We used a strain from Beringer's Yountville vineyard, one from Stag's Leap, another from the Mounts' Vineyard in Dry Creek Valley and one from Temecula. The test plants include U0505-01 (a highly resistant 88% *vinifera PdR1* selection), U0505-22, Blanc du Bois and Roucaneuf as resistant but relatively good supporters of *X. fastidiosa* populations, and the highly susceptible Chardonnay.

Group N was a retest of the 35 rootstock selections made with crosses of F8909-08 and commercial rootstocks. We will be selecting those with the best rooting, lowest *X. fastidiosa* levels and resistance to nematodes prior to field evaluation with 94 and 97% *vinifera PdR1* selections.

Table 3a presents the list of seedlings that were screened for resistance with *PdR1* markers and then planted in the vineyard. These plants will be further screened for viticultural attributes and the best will be greenhouse screened for PD resistance. The best of these will be expanded to 8 to 10 vine replicates in preparation for small scale wine evaluations and advancement to field trials.

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 with other than *PdR1*-based resistance. In order to better understand the limits of other PD resistance sources the following resistance sources are being studied:

***V. arizonica* b40-14 –** This pure *V. arizonica* from Chihuahua. Mexico has strong PD resistance. Mapping efforts to date put its locus for resistance in the same region as *PdR1*, although the plants are quite different morphologically and are from a very different region of Mexico. We have termed this resistance source *PdR1c* to distinguish it from the *PdR1* alleles "a" and "b" from b43-17. We are planning on using MAS to insure we pyramid this resistance source with *PdR1a* or *PdR1b* and both if we are able. Table 3b notes the number of seedlings that were maker tested and the 26 75% *V. vinifera PdR1c* progeny that went to the field. These populations derive their resistance from R8918-05 (*V. rupestris* Wichita Refuge x *V. arizonica* b40-14).

***V. arizonica/girdiana* b42-26** – Eleven sets of crosses were made this Spring to produce populations that are 75% *V. vinifera* and 25% PD resistant from the non-*PdR1* b42-26 (*V.*

arizonica/girdiana from Loreto, BC Mexico) source (Table 1c). We know from past research that b42-26's resistance is controlled by multiple genes and that it functions well under greenhouse testing. The long-term goal is to introgress this resistance source into advanced *PdR1* resistant selections to broaden PD resistance. The choice of parents for these crosses was speculative this year since we had not completed the greenhouse testing of the 07344 selections for PD resistance. These tests will be complete this Winter and will allow us to focus our choices of which populations should be maintained.

We also made crosses to expand an additional mapping population for the b42-26 resistance source (Table 1d). The current 05347 mapping population is an F1 cross between *V. vinifera* and b42-26. Preliminary test results of the progeny found that their susceptibility level was not as high as we expected, which might impact the mapping effort. So we are creating an alternative BC1 population with 05347-02 (F2-35 x b42-26) x Grenache that We are expecting wider segregation in this new population. We marker tested 100 additional seedlings of the 05347 population and planted 100 in the field (Table 3c).

Rootstocks – 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 if the rootstock become infected by downward movement of *X. fastidiosa*. We have tested the PD resistance of a population of 101-14 Mgt x F8909-08 (*V. rupestris* x *V. arizonica* b43-17) and have 8 promising candidates (reported on earlier). These are currently undergoing greenhouse screening for nematode resistance. We have used some of these rootstock selections for the PD field trials planted this year.

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 occurred in May 2010. Selections from the 87% and 75% vinifera PdR1 populations continue to be inoculated by needle and natural sharpshooter feeding at the Yountville plot.

We planted a field trial at the Mounts Vineyard in Healdsburg in June 2010. Table 4 lists the four PD resistant selections grafted on 3 resistant rootstocks. These vines were planted with varying numbers of 5 vine replicates. 07329-37, 07355-75 and 07713-51 are 94% *vinifera* with *PdR1*, and U0502-20 is 87% *vinifera* with *PdR1*. The site is surrounded by PD habitat on two sides and is chronically and severely infected. We will also hand inoculate these vines starting next year. A planting of 9 advanced 94% vinifera selections totaling approximately 125 vines has been grafted to two of our PD resistant rootstock selections for planting at the Beringer Vineyard later this Summer.

We sent 87% *vinifera PdR1* to Dr. Elina Coneva at Auburn University in Alabama (501-12 (50% Syrah) 30 plants, 502-01 (50% Chardonnay) 32 plants and 502-10 (50% Chardonnay) 34 plants.

All were grafted on Dog Ridge rootstock due to its PD resistance. They were repotted there and will be planted out in Spring 2011. We also sent cuttings of five 87% *vinifera PdR1* selections to Jim Kamas in Fredericksburg, TX for a trial there (U0502-10, U0502-20, U0502-26, U0502-38 and U0505-35). A trial with most of these is underway in Galveston, TX in collaboration with Lisa Morano.

Wine Making – Wines from 88% and 94% *vinifera PdR1* selections were made in Fall 2009 and will be made again Fall 2010. 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. These results (Table 5) were reported in our October, 2009 progress report and are included again here as a reference for selections reported on in this report. Despite the challenging Spring weather this year, we expect that several additional 94% *vinifera* level genotypes will have enough fruit for small lot winemaking. Following veraison later this Summer, the actual number of lots will be further refined.

V. Publications or Reports from this Project

Cheng, D.W., H. Lin, Y. Takahachi, M.A. Walker, E.L. Civerolo and D.C. Stenger. 2010. Transcriptional regulation of the grape cytochrome P450 monooxygenase gene CYP736B expression in response to *Xylella fastidiosa* infection. BMC Plant Biology 10:135 doi:10.1186/1471-2229-10-135.

Riaz, S. and M.A. Walker. Development and characterization of 71 new SSR markers for grape. American Journal of Viticulture and Enology (submitted).

Pio Viana, A., S. Riaz and M.A. Walker. Evaluating genetic diversity and optimizing parental selections in a segregating table grape population. American Journal of Viticulture and Enology (submitted).

VI. Presentations on PD Research

Breeding PD resistant winegrapes. Texas Pierce's Disease Symposium, Marble Falls, TX, Mar. 2, 2010.

Pest and disease issues for grapevines. UC Davis Wine Executive Program, Davis, CA, Mar. 23, 2010.

UCD grape breeding program. Monterey Cooperative Extension Meeting, Salinas, CA, Apr. 13, 2010.

Sustainable viticulture. UC Berkeley Executive Education Program, Clos Pegas Winery, Calistoga, CA, Apr. 17, 2010.

Rootstock and PD breeding at UC Davis. Beringer Viticulture Staff, St. Francis Winery, Santa Rosa, CA, Apr. 23, 2010.

Grape breeding at UC Davis. Croatian Wine Association. UC Davis, CA, Jul. 13, 2010.

Abstracts

Walker, M.A., S. Riaz and A. Tenscher. Optimizing the breeding of Pierce's disease resistant winegrapes with marker-assisted selection. International Grape Breeding and Genetics Conference. Geneva, NY, August 2, 2010.

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 marker-assisted selection. We have planted 97% *vinifera PdR1* containing seedlings, which will be evaluated for release as winegrapes. We have also developed genetic markers from the morphologically and genetically different source of PD resistance *V. arizonica* b40-14 and have expanded crosses with this source to broaden the base of PD resistance. Populations with *X. fastidiosa* resistance from other sources are being maintained and expanded, but progress is slower with these sources since genetic markers do not exist for them. 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. This Fall wines will be made for the second time from PD resistant selections at the 93.8% *vinifera* level.

VIII. Lay Summary

Progress continues in the breeding PD resistant winegrapes and has been greatly accelerated by the incorporation of marker-assisted selection (MAS) for the PD resistance gene, *PdR1* (see companion report). The use of MAS and our acceleration of the seed to seed breeding cycle to two years by aggressive viticultural training practices has allowed very rapid progress towards PD resistant winegrapes. Large numbers of progeny from the 97% *V. vinifera* level were marker tested this Spring and over 1,800 had the *PdR1* resistance locus and were planted in the vineyard. These will fruit for the first time in Fall 2011 and selections for commercial release will be chosen from them. Two PD hot-spot plots in California are now planted with 94% *vinifera* PD resistant selections and 87% *vinifera* resistant selections have been sent to Alabama and Texas for field testing. These vines will be mechanically inoculated each Spring and small scale wines will be made from them. Small-scale wines have been made from 87% and 94% *vinifera* selections and there was a large improvement between these generations. The 94% have good commercial quality, which heightens the promise of the current 97% *vinifera* populations.

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 and with regard to their patent process.

Table 1. Crosses made in 2010.

Resistant Type	*Vinifera* Parent\Grandparent of Resistant Type	*Vinifera* Types used in 2010 crosses	Estimated # of Seed
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 96.875% *V. vinifera* parentage. F2-35 is 100% *vinifera* cross of Cabernet Sauvignon x Carignane.			
07355-020	Petite Sirah\Cabernet Sauvignon	Barbera	85
07370-028	F2-35\Chardonnay	Chardonnay, Riesling	750
07371-020	F2-35\Chardonnay	Barbera	350
07355-075	Petite Sirah\Cabernet Sauvignon	Muscat blanc (Maternal Parent)	500
07329-037	Chardonnay	Muscat blanc (Maternal Parent)	75
07370-084	F2-35\Chardonnay	Viognier (Maternal Parent)	150
07713-051	F2-35\Chardonnay	Viognier (Maternal Parent)	150
07355-075	Petite Sirah\Cabernet Sauvignon	Alicante Bouschet (Maternal Parent)	75
1b. b43-17 *V. arizonica/candicans PdR1a* resistance source avoiding *V. rupestris* from F8909-08 to produce progeny with 93.75% *V. vinifera* parentage.			
08329-035	Tannat, Chenin blanc	Cabernet Sauvignon	85
08329-074	Tannat, Chenin blanc	Cabernet Sauvignon, Carignane	900
08329-095	Tannat, Chenin blanc	Cabernet Sauvignon	240
1c. Crosses to the b42-26 *V. arizonica/girdiana* resistance source to produce progeny that are 75% *vinifera* and 25% resistant.			
07344A-09	Grenache	Carignane	225
07344A-11	Grenache	Carignane, Cabernet Sauvignon, Chardonnay	315
07344A-12	Grenache	Carignane	180
07344A-15	Grenache	Carignane	360
07344A-25	Grenache	Carignane	360
07344A-32	Grenache	Carignane	180
07344A-33	Grenache	Carignane, Cabernet Sauvignon	180
07344A-51	Grenache	Carignane	225
07344A-54	Grenache	Carignane, Cabernet Sauvignon	270
07344A-56	Grenache	Carignane, Cabernet Sauvignon	270
07344A-61	Grenache	Carignane, Cabernet Sauvignon	360
1d. Cross to increase the 07344 b42-26 75% *vinifera* for a possible mapping population.			
05347-02	F2-35	Grenache	760

Table 2. Seedling populations that were marker tested and planted in May 2010.

Resistant Type	*Vinifera* Parent\grandparent of Resistant Type	*Vinifera* Types used in 2009 crosses	No. Seedlings Produced	No. Seedlings MAS Tested	No. Seedlings to Field
2a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 96.875% *V. vinifera* parentage. F2-35 is a 100% *vinifera* cross of Cabernet Sauvignon x Carignane.					
07354-50	Merlot\Cabernet Sauvignon	Cabernet Sauvignon, Chardonnay	116	65	22
07355-020	Petite Sirah\Cabernet Sauvignon	Cabernet Sauvignon, Chardonnay, Chenin blanc, Zinfandel	1555	1110	450
07370-028	F2-35\Chardonnay	Cabernet Sauvignon, Chenin blanc, Pinot noir, Zinfandel	757	390	202
07370-039	F2-35\Chardonnay	Cabernet Sauvignon, Chenin blanc, Riesling, Sylvaner, Zinfandel	806	400	134
07370-097	F2-35\Chardonnay	Cabernet Sauvignon, Chardonnay, Chenin blanc, Pinot noir	408	235	92
07371-19	F2-35\Chardonnay	Cabernet Sauvignon, Chardonnay, Chenin blanc, Sylvaner	947	760	320
07371-20	F2-35\Chardonnay	Cabernet Sauvignon, Chenin blanc, Pinot noir, Sylvaner	2256	1453	627
07371-36	F2-35\Chardonnay	Cabernet Sauvignon, Chardonnay, Chenin blanc, Mourvedre, Riesling, Zinfandel	1443	50	21
2b. Crosses to the b40-14 *V. arizonica* resistance source to produce progeny that are 75% *vinifera*, 12.5% b40-14 ad 12.5% *V. rupestris*.					
07744-038, -120	Airen	Cabernet Sauvignon, F2-35, Malaga Rosada	698	50	26
2c. Cross to increase the b42-26 *V. arizonica* x *vinifera* mapping population.					
b42-26	F2-35 (Cab. Sauv. x Carignane)	F2-35	120	100	100

Table 3. Groups of plants greenhouse screened for *X. fastidiosa* resistance.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Date	Resistance Source(s)
A	07744 Mapping population	39	12/16/09	4/2/09	b40-14

Table 3. Cont'd.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Date	Resistance Source(s)
B	AW spacing trial	10	1/15/09	5/21/09	F8909-08
C	07386 Mapping population	40	2/3/09	5/21/09	b40-14
D	07744 mapping population	64	2/12/09	5/21/09	b40-14
E	2007 cross families #1	67	4/21/09	8/13/09	F8909-08
F	9621 recomb, retest and untested	72	4/21/09	8/13/09	b34-17, b42-26
G	04191 mapping population #2	123	10/13/09	1/21/10	F8909-17
H	04191 mapping population #1	51	11/24/09	2/25/10	F8909-17
I	PD Rootstocks	23	11/24/09	2/25/10	b43-17
J	2009 PDR & Vinifera	50	11/24/09	2/25/10	F8909-08
K	2007 Cross Families #2	68	12/8/09	3/9/10	F8909-08
L	Xf Strain Trial	6	3/30/10	7/6/10	F8909-08
M	2007 Cross Families #3	145	4/13/10	7/22/10	F8909-08
N	PD Rootstocks Retest	35	6/8/10	9/7/10	F8909-08
O	08 PD Stocks & Recombinants	22	7/15/10	10/14/10	F8909-08

Table 4. Field trial at the Mounts' Vineyard in Healdsburg. Planted June 18, 2010. Table lists the number of replicates of 5 vine sets planted in a randomized design. The 07 series are all 94% *V. vinifera* and the U0502-20 is 84% *V. vinifera*

Genotype	No. Reps
07329-37/J09-24	3
07329-37/J08-48	3
07355-75/J09-24	5
07713-51/J09-24	6
U0502-20/J09-24	1
U0502-20/J08-48	2
U0502-20/Dogridge	2

Table 5. Results of a blind tasting of 2008 & 2009 vintage wines tasted 1/26/10 by 6 tasters comprised of faculty and staff in the Department of V&E, UCD. The wines were rated on a hedonic quality scale from 1 = poor to 5 = v. good. Note that the 94% *vinifera* PD resistant hybrids faired better this year that the classic *vinifera* varieties.

Wine Name	% *vinifera*	Total	Max	Min	12/09/08 Consensus Descriptors: color; aroma; flavor-texture
2009 Vintage White Wines					
07713-51	94%	20.5	4.5	2	straw yellow; honey dew, blossom, Viognier; warm, full, round, touch phenolic

Table 5. Cont'd.

Wine Name	% *vinifera*	Total	Max	Min	12/09/08 Consensus Descriptors: color; aroma; flavor-texture
Blanc du Bois	~66%	18.0	4	2	straw yellow; aromatic, floral; simple, tart, green
U0502-20	88%	16.5	4.5	2	pale yellow; gooseberry, grassy; tart, slightly thin
Chardonnay	100%	11.0	3	1	med yellow; vinous; buttery, spice, course, lacks balance
2009 Vintage Red Wines					
U0502-10	88%	21.5	5	2	dark red-purple; blackberry, current, licorice; cola, low tannin
07355-75	94%	20.3	4	2.75	dark inky; stewed dark fruit, chocolate; tannic but lacks body
U0505-35	88%	18.8	4	2	dark inky; blackberry, black fruit; grapy, tannic
U0502-26	88%	18.0	4	2	dark purple; grapy, ripe fruit, black olive; black fruit, current; structured, good weight
07355-12	94%	18.0	4	2	dark almost black; dark fruit, current, ripe; black pepper, slightly hard tannins
U0501-12	88%	15.8	5	1	medium purple; fruit leather, strawberry; red fruit, slightly thin
Cabernet Sauvignon	100%	13.0	3.5	1	med red-purple; veggie, cooked; cooked veggie, thin
Lenoir	unknown	12.0	3	1	med-dark w\ brown edge; porty, jam, pepper; simple, lacks tannin
Barbara	100%	11.5	3.5	1	light-med; strawberry jam, candied; overripe, lacks weight, slightly bitter
2008 Red Wines (bottled under plastic screw caps)					
U0502-10	88%	18.8	4	1	med-dark w\brown edge; plum, mature; ripe, good tannin structure and mouth feel
Syrah	100%	14.8	3.75	1	dark w\ brown edge; coffee, chocolate; viscous, moderate length,
U0501-12	88%	11.0	3	1	dark w\ brown edge; porty, leather; mature, slightly green tannins
U0502-01	88%	9.5	3.5	1	light-med brown; oxidized, tired; tired, tea, simple