CDFA PD/GWSS Progress Report
March 2010

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

Reporting period: October 2009 to March 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 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 *PdR1* and other PD resistance sources into a large number of *V. vinifera* winegrape backgrounds. We now have reached the backcross 4 (BC4) generation with 96.8% *V. vinifera* and PD resistance from *V. arizonica* b43-17, and have focused our efforts on growing out larger numbers of progeny from a variety of crosses.

Table 1 presents the crosses made in 2009 with numbers of seeds and seedlings produced along with the number of progeny that are scheduled for MAS testing in 2010. The goals of the 2009 crosses were: 1) Use the *PdR1b* allele from the F8909-08 to advance the *vinifera* winegrape lines to the 96.8% *vinifera* level; 2) Advance the *PdR1b* line absent *V. rupestris* to the 93.75% vinifera level; 3) Use the BC1 progeny of the of the PD resistant b40-14 *V. arizonica* to produce a breeding and mapping population that is 75% *vinifera* and 12.5% the resistance source; and 4) increase the b42-26 *V. arizonica* mapping population.

During the last year, 11 groups of plants were greenhouse tested or are in testing for *X. fastidiosa* resistance (**Table 2**). Groups A, C and D tested the PD resistance from b40-14 *V. arizonica* and facilitated the mapping of this resistance to chromosome 14. In 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 A & D allowed us to make the 75% *vinifera* crosses in 2009 and advance this promising new PD resistance line by another generation (**Table 1c**). Group B tests were done to further streamline the greenhouse screening process and decrease greenhouse space requirements. Consistent with Baumgartel (2009), we found that tighter spacing increased the mean ELISA values relative to our standard spacing in both susceptible and resistant selections. Groups E and K test relatively large numbers of genotypes within the same cross to explore any contribution toward PD resistance from the *vinifera* parent. Results from the first

test seem to indicate a relationship that is being confirmed in the second test. The relative PD resistance of various *vinifera* cultivars including those used in the past as parents in our crosses is being explored independently in Group J along with the 2009 *PdR1* 94% *vinifera* parents to confirm the continued correlation of *PdR1* marker results and *Xf* titers in plants. Tests were also conducted on recombinants in the 9621 population to aid in fine-scale mapping of the *PdR1* locus. 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 I. 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. ELISA results are pending.

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 –** In years past we have greenhouse tested 45 F1 progeny of PD susceptible *V. rupestris* Wichita Refuge crossed with PD resistant *V. arizonica* b40-14 (R89 series). Forty-two were highly resistant and three had intermediate reactions (data not shown). In 2007, we crossed the *V. vinifera* variety Airen onto two of the PD resistant R89 series genotypes and planted a total of 163 genotypes in Spring 2008. We completed greenhouse screening of these two populations in 2009. Preliminary mapping of the larger population (n=110) places PD resistance from b40-14 on chromosome 14, but in a different location than *PdR1*. Table 1c shows the 2009 crosses made to advance this very strong and morphologically and genetically different source of PD resistance.

***V. arizonica* b42-26 –** *Xylella fastidiosa* 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). Testing of the first 63 genotypes of 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*) was completed in 2008. In Spring 2009 we added 100 genotypes to this population from seed produced in 2008 and remade the cross producing 467 seeds to further expand this F1 mapping population.

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. Greenhouse screening results and horticultural evaluation of 8 promising progeny from crosses involving the rootstock 101-14 Mgt x F8909-08 (*V. rupestris* x *V. arizonica* b43-17) are shown in **Table 3**. Results from their evaluation for rooting and grafting ability are shown and ELISA results from tests for PD resistance in the greenhouse are pending. 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 (**Table 3**). 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 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). In Fall 2009, 12 fermentations were made: 3 (2 red, 1 white) at the 94% *vinifera* level; 5 (4 red, 1 white) at the 87.5% *vinifera* level; and 4 (2 red, 2 white) *vinifera* and PD controls. Sensory evaluation of the wines made in 2009 is summarized in Table 5. 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. These results were reported in our October, 2009 progress report.

V. Publications or Reports from this Project

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

Cheng, D.W., H. Lin, M.A. Walker, D.C. Stenger, and E.L. Civerolo. 2009. Effects of grape xylem sap and cell wall constituents on in vitro growth, biofilm formation, and cellular aggregation of *Xylella fastidiosa*. European J Plant Path. 125:213-222.

VI. Presentations on PD Research

Impact of invasive species: breeding for resistance to PD. CSREES Review, UC Davis, Jan. 13, 2009.

Current issues in grapevine pests and diseases. UCD Wine Executive Short Course, Mar. 10, 2009.

Crape breeding with an emphasis on flavor. Recent Advances in Viticulture and Enology, Mar. 19, 2009.

PD resistant winegrapes coming soon. Temecula Grape Day,Temecula CA, April 2, 2009.

Grape breeding at UCD. International Grape Research Coordination Network for Grape Functional Genomics, Granlibaken, Lake Tahoe, CA, May 16, 2009.

Twenty years of grape breeding at UC Davis. Honorary Research Lecture, ASEV 60th Annual Meeting, Napa, CA June 24, 2009.

Breeding PD resistant winegrapes. National Grape Breeders Conference, Tallahassee, FL, Aug. 6, 2009.

Breeding grapes with resistance to Pierce's disease. Current Issues in Plant Health, FPS/UCD Extension, Davis, CA, Nov. 19, 2009.

Will you be ready for PD resistant wine grapes? Dept. Viticulture and Enology Seminar, UC Davis, CA, Nov. 20, 2009.

Breeding PD resistant grapevines. CDFA PD/GWSS Meeting, Sacramento, CA, Dec. 10, 2009.

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

Abstracts

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. Phytopathology 98:537.

Riaz, S., A.C. Tenscher, R. Graziani and M.A. Walker. 2009. Breeding winegrapes with resistance to Pierce's disease. 60th Annual Meeting, American Society for Enology and Viticulture, Napa, CA, Technical Abstracts.

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 are now germinating 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 from *V. arizonica* b40-14 and expanding crosses with this source to broaden the base of PD resistance. Populations with Xf 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. The third testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2009 as well as the first testing of wines at the 93.8% vinifera level. 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. Large numbers of the 2009 crosses are currently being screened by MAS and will be planted in the field this Spring. Results from needle inoculation with *X. fastidiosa* of both the 75% and 87.5% *vinifera PdR1* scions 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 five 87.5% *vinifera* and three 93.8% vinifera *PdR1* containing wine grape selections were evaluated and they scored remarkably well. Fruit evaluation and must analysis were performed on numerous other promising progeny of 93.8% *vinifera PdR1* lines.

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. 2009 wine and rootstock type crosses, numbers of seeds & seedlings produced with number marker tested. Planned or projections numbers are in *italics*.

Resistant Type	*Vinifera* Parent\grandparent of Resistant Type	*Vinifera* Types used in 2009 crosses	# of Seeds Produced	# of Seedlings Produced	# of Seedlings MAS Tested
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.					
07354-50	Merlot\Cabernet Sauvignon	Cab Sauv, Chardonnay	144	116	65
07355-020	Petite Sirah\Cabernet Sauvignon	Cab Sauv, Chardonnay, Chenin blanc, Zinfandel	1808	1555	1110
07370-039	F2-35\Chardonnay	Cab Sauv, Chenin blanc, Riesling, Sylvaner, Zinfandel	859	806	400
07370-097	F2-35\Chardonnay	Cab Sauv, Chardonnay, Chenin blanc, Pinot noir	1202	408	235
07370-028	F2-35\Chardonnay	Cab Sauv, Chenin blanc, Pinot noir, Zinfandel	1597	*335*	*230*
07371-19	F2-35\Chardonnay	Cab Sauv, Chardonnay, Chenin blanc, Sylvaner	1120	947	760
07371-20	F2-35\Chardonnay	Cab Sauv, Chenin blanc, Pinot noir, Sylvaner	2897	*1411*	*1165*
07371-36	F2-35\Chardonnay	Cab Sauv, Chardonnay, Chenin blanc, Mouvedre, Riesling, Zinfandel	1970	*662*	*525*

1b. Monterrey *V. arizonica/candicans* resistance source absent *V. rupestris* to produce progeny with 93.75% *V. vinifera* parentage.					
07307-10	Zinfandel	Cabernet Sauvignon, Chenin blanc	207	*83*	*70*
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	Cabernet Sauvignon, F2-35, Malaga Rosada	1653	*605*	*500*
1d. Cross to increase the b42-26 *V. arizonica* x *vinifera* mapping population .					
b42-26	F2-35 (Cab. Sauv. x Carignane)	F2-35	467	*175*	*150*

Table 2. 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	07744 Mapping population	39	12/16/2009	4/2/2009	b40-14
B	AW spacing trial	10	1/15/2009	5/21/2009	F8909-08
C	07386 Mapping population	40	2/3/2009	5/21/2009	b40-14
D	07744 mapping population	64	2/12/2009	5/21/2009	b40-14
E	2007 cross families #1	67	4/21/2009	8/13/2009	F8909-08
F	9621 recombinants, retest and untested	72	4/21/2009	8/13/2009	b34-17,b42-26
G	04191 mapping population #2	123	10/13/2009	1/21/2010	F8909-17
H	04191 mapping population #1	51	11/24/2009	2/25/2010	F8909-17
I	PD Rootstocks	23	11/24/2009	2/25/2010	b43-17
J	2009 PDR & Vinifera	50	11/24/2009	2/25/2010	F8909-08
K	2007 Cross Families #2	68	12/8/2009	3/9/2010	F8909-08

Table 3. Promising PD resistant rootstocks with rootability and greenhouse PD screen for the ungrafted stock graft success to two different scions.

Rootstock Genotype	Rootstock phenotype 1=v. good, 5=discard	Sex	Vigor of stock	Ungrafted Rooting %	GH PD Screen mean cfu/ml (rootstock)	% successful grafting & rooting to Chardonnay	% successful grafting & rooting to A81-17
03300-047	1.8	M	high	50%	7,585	11%	44%
03300-048	1.5	F	high	100%	44,234	89%	89%
03300-072	2	M	high	30%	94,820	89%	67%
03300-082	1.9	M	high	100%	29,001	67%	100%
03300-099	1	F	mod-high	100%	102,813	22%	50%

Rootstock Genotype	Rootstock phenotype 1=v. good, 5=discard	Sex	Vigor of stock	Ungrafted Rooting %	GH PD Screen mean cfu/ml (rootstock)	% successful grafting & rooting to Chardonnay	% successful grafting & rooting to A81-17
03305-12	2	F	high	100%	32,632	50%	22%
03305-18	2.5	F	high	100%	30,220	13%	67%
06304-26	2	F	mod-high	90%	60,062	33%	33%
101-14 Mgt	1	F	mod-high	100%	858,055	56%	50%

Table 4. Summary of greenhouse (GH) and several years field ELISA means for genotypes with (R) and without (S) the *PdR1* marker.

Group	% Vinifera	N (geno-types)	PdR1 Marker Score or GH score	2009 mean ELISA cfu/ml (field)	2008 mean ELISA cfu/ml (field)	2007 mean ELISA cfu/ml (field)	mean ELISA cfu/ml (Green-house)
04-5554	88%	12	R	45,479	52,374	-	157,037
04-5554	88%	6	S	1,463,536	4,634,182	-	6,356,825
A81	75%	15	R	14,314	46,685	93,362	94,875
A81	75%	5	S	377,622	2,550,697	2,680,311	2,895,812
Roucaneuf	56%	1	R	402,069	415,864	235,573	681,000
Blanc du Bois	66%	1	R	328,644	467,756	-	681,000
Chardonnay	100%	1	S	2,876,401	3,807,851	1,561,321	6,500,000

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
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

Wine Name	% *vinifera*	Total	Max	Min	12/09/08 Consensus Descriptors: color; aroma; flavor-texture
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