California Department of Food and Agriculture PD/GWSS
Progress Report
March 2012

Report title: Renewal Progress Report for CDFA Agreement Number 03-0293 Amend 4

Project Title: Breeding Pierce's disease resistant winegrapes

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SUMMARY

We continue to make rapid progress breeding Pierce's disease (PD) resistant winegrapes. Aggressive vine training and selection for precocious flowering has allowed us to reduce the seed-to-seed cycle to 2 years. We are also using marker-assisted selection (MAS) for the PD resistance gene, *PdR1* (see companion report) to select resistant progeny as soon as seeds germinate. These two practices have greatly accelerated the breeding program and allowed us to produce four backcross generations with elite *V. vinifera* wine grape cultivars in 10 years. In Spring 2010, we planted about 2,000 97% *vinifera* seedlings with *PdR1*. We finished evaluating the fruit quality of over 1,200 of these in September 2011, and produced a small-scale wine of one, 09333-178. We are preparing to greenhouse test the best of these to verify which have the highest level of resistance to PD prior to multiplication and grafting for larger scale field trials. We plan to release commercially useful varieties from populations at this 97% *vinifera* level. The resistance above is based on 8909-08, which has one of the two alleles, *PdR1b*, from the *Vitis arizonica/candicans* b43-17 resistance source. The other resistance allele, *PdR1a*, is in 8909-17 and we have advanced this resistance to the 94% *vinifera* level and have combined it with the *PdR1b* allele to determine whether resistance with both alleles is stronger. There is also strong resistance in b42-26 a form of *V. arizonica/girdiana* form Baja California. b42-26's resistance is controlled by multiple genes, as opposed to the single gene resistance found in b43-17. We made crosses this year to advance the b42-26 resistance to the 87% *vinifera* level and have been surprised no only by the strength of resistance but also by the relatively large number of resistant progeny each generation. We are now re-evaluating its resistance markers to verify that it is not another form of *PdR1*. Finally, we evaluated the first set of about 50 accessions collected across the southwestern US for PD resistance. There were many with very strong resistance and these will be tested to verify that their resistance is different from b43-17's. This year's wine making also included wines made as blends with elite vinifera winegrapes, a likely use of our eventual releases. These selections could be used in severe PD hot spots and the fruit could be blended into the rest of the vineyard in a 25/75% ratio. We used Napa Valley (Oakville Station) Sauvignon blanc and Merlot with 07713-051 and 07355-075, respectively. We also made wine at the same scale with the Napa Sauvignon blanc and Merlot, and Davis Sauvignon blanc and Merlot (as growing region controls).

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 having 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. We have selected progeny with *PdR1* from the b43-17 *V. arizonica*/*candicans* resistance source for fruit quality at the backcross 4 (BC4), 97% *vinifera* level. They are also undergoing greenhouse testing to verify their resistance and those with the highest levels of resistance will be prepared for small-scale winemaking this winter by grafting them onto PD resistant rootstocks and planting 6 to 8 vines sets on commercial spacing and trellising. We have made wine from vines that are 94% *vinifera* level from the same resistance background for two years. They have been very good and do have the hybrid flaws (blue purple color and herbaceous aromas and taste) that were prevalent in wines from the 87% *vinifera* level. There are two forms of *PdR1*, 8909-08 and 8909-17 – sibling progeny of b43-17 and they have different alleles of

PdR1. 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 yet been developed to expedite breeding.

OBJECTIVES

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.
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

Table 1 details the actual number of seeds produced in our 2011 PD crosses, the number of seedlings produced and the planned number of these seedlings to be MAS tested.

Table 1. Pierce's disease resistant crosses made in 2011. Values in italics are estimates.

Resistant Type	*Vinifera* Parent\grandparent of resistant type	*Vinifera* types used in 2011 crosses	# Seeds Produced	# Seedlings Produced	# Seedlings to MAS Testing
1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny between 96.9% and 98.4% *V. vinifera* parentage. F2-35 is Cabernet Sauvignon x Carignane.					
07355-020	Petite Sirah\Cabernet Sauvignon	Nero d'Avola	323	277	210
07370-039	F2-35\Chardonnay	Nero d'Avola	408	303	150
09-331	Zinfandel\Petite Sirah	Nero d'Avola, Pinot blanc	332	223	140
1b. Monterrey V. *arizonica/candicans* resistance source (b43-17) to produce progeny with 75% *V. vinifera* parentage for minor PdR gene discovery.					
04373-02	F2-35	Pinot blanc, Zinfandel	657	*100*	*50*
04373-22	F2-35	Pinot blanc, Zinfandel	1121	*100*	*50*
1c. Crosses to the b42-26 *V. arizonica* resistance source to produce progeny that are 87.5% *vinifera* and 12.5% the resistance source.					
07344A-10	Grenache	F2-35	357	-	-
07344A-11	Grenache	Zinfandel	261	-	-
07344A-24	Grenache	F2-35	201	-	-
07344A-35	Grenache	F2-35	673	*50*	-
1d. Cross made to pyramid *PdR1b* Monterrey *V. arizonica/candicans* and b42-26 *V. arizonica* resistance lines to produce progeny between 84.3% and 85.9% *vinifera*.					
09-331	Zinfandel\Petite Syrah	Grenache\F2-35	702	*170*	-
07-355	Petite Sirah\Cabernet Sauvignon	Grenache\F2-35	1076	-	-
07370-039	F2-35\Chardonnay	Grenache\F2-35	147	-	-

The 2011 crosses were made to: 1. Broaden the wine quality backgrounds of the most advanced lines by crossing to Nero d'Avola and Pinot blanc – two varieties that we had not been used previously (Table 1a). These offspring will add to the 583 seedlings from the 2010 crosses that were marker-tested (Barbera 263, Chardonnay 67, Muscat Blanc 31, and Riesling 222) and the 2009 crosses that were planted in 2010. The range of *vinifera* parents (these seedlings are 50% of the last *vinifera* parent used)

at this 97 to 98% vinifera level now number 11 and include Barbera, Cabernet Sauvignon, Chardonnay, Chenin blanc, Muscat blanc, Nero d'Avola, Pinot blanc, Pinot noir, Riesling, Sylvaner and Zinfandel.

We find that one of our most resistant genotypes, in terms of very low *X. fastidiosa* levels, is *V. arizonica/candicans* b43-17, and better than any of the later generation backcrosses with b43-17 resistance. In an effort to determine if this resistance is the result of minor genes contributing to *PdR1*'s effect we have been making generational crosses that trace back to b43-17 and not 8909-08 or 8909-17. Table 1b presents the populations created to examine these minor genes.

Crosses continue to be made with resistance from *V. arizonica/girdiana* b42-26. The 75% *vinifera* level has very good resistance in the greenhouse screen last year, and we broadened the wine quality backgrounds by using F2-35 and Zinfandel to bring these populations to the 88% *vinifera* level. We made the first crosses to combine *PdR1* with the b42-26 resistance this year to produce offspring with about 85% *vinifera* (Table 1d).

Table 2. PD greenhouse screens initiated or completed over the last year.

Group	Genotypes	# Genotypes	Inoculation Date	ELISA Sample Date	Resistance Source(s)
A	*PdR1a* & *PdR1b* together	122	1/13/2011	4/14/2011	b43-17
B	Haines City BC1 & BC2 Progeny	173	3/31/2011	7/12/2011	*V. shuttleworthii*
C	New *V. arizonica* Sources	54	5/12/2011	8/11/2011	*V. arizonica*
D	PD Rootstocks	15	6/14/2011	9/15/2011	F8909-08
E	*Rotundifolia* and VR Hybrids	94	11/3/2011	2/2/2012	*M. rotundifolia*
F	94% *PdR1b* & BD5-117 Source Selections	109	12/15/2012	3/16/2012	F8909-08, BD5-117
G	97% *PdR1b* Elite Selections	77	3/10/2012	6/9/2012	F8909-08

Table 2 lists the PD greenhouse screens initiated or completed over the last year. Group A tested genotypes in which *PdR1a* and *PdR1b* were re-established in one background to determine if both resistance alleles will provide better resistance. The greenhouse screen is complete, but MAS results are pending. Group B tested BC1 and BC2 progeny and their parents in the Haines City *V. shuttleworthii* line. Although this resistance source is promising in terms of our phenotypic scoring system, the ELISA results presented in Table 3a indicate that the level of *X. fastidiosa* suppression is not very strong compared to PdR1 resistance. The most resistant individuals (geometric mean of ln cfu/ml <300k) are shown in Table 3b. There was a broad range of responses, and a high degree of variability also indicating a multigenic resistance.

Table 3a. Erosion of resistance with successive backcross generation in the *V. shuttleworthii* 'Haines City' line. In this trial, the F1 generation only had the two parents of the BC1 generation, but the whole generation was resistant.

Back Cross Level	t-test			Mean ln cuf/ml	# Geno-types tested	% Resistant
F1	A			11.1	2	100%
BC1		B		13.8	98	53%
BC2			C	14.4	22	27%

Table 3b. Range of values based on a 75% *vinifera PdR1* control (Mean ln cfu/ml <300) of *V. shuttleworthii* 'Hanes City' progeny

GH Screen Result (ref U0505-01)	Geometric mean (cfu/ml)	Mean (ln cfu/ml)	Std Dev (ln cfu/ml)	Reps
R	59,190	11.0	1.9	9
R	75,917	11.2	1.4	10
R	60,458	11.0	1.3	5
R	216,122	12.3	2.0	5
R	13,950	9.5	0.7	5
R	167,009	12.0	2.0	4
R	49,050	10.8	1.9	4
R	278,507	12.5	2.1	5
R	95,235	11.5	2.6	4
R	91,409	11.4	2.1	5
R	275,240	12.5	1.0	4
R	123,834	11.7	2.3	5
R	193,842	12.2	1.0	4
R	14,907	9.6	1.0	19
R	11,052	9.3	0.3	26
R	142,943	11.9	1.9	10

Of the 54 newly collected or evaluated accessions tested in the *V. arizonica* Group C (Table 2, Group C), 47 were resistant when compared to our b43-17 *V. arizonica/candicans* standard, and 25 were still resistant when compared to another *V. arizonica* accession with even greater levels of *X. fastidiosa* suppression. Based on the combination of extremely low ELISA readings and leaf and cane scores after greenhouse testing, and diverse geographic origins, at least 6 of these accessions are very promising candidates for breeding, marker development and gene discovery.

We have been introgressing PD resistance into rootstocks for use against nematodes some of these rootstock selections are being tested in our Yountville field trial. Two selections, 08314-15 and 08314-46, have excellent PD resistance and root-knot nematode resistance and salt and drought tolerance. These plants will be used when we advance selections to field trials.

Muscadinia rotundifolia is also resistant to PD, and we have been testing various *vinifera*/*rotundifolia* (VR) hybrid selections to determine their value in PD breeding. When compared to b43-17, *M. rotundifolia* 'Trayshed', one of our top rotundifolia selections for rootstock breeding and fanleaf virus tolerance, isn't as resistant; nor is it as resistant when compared to other *rotundifolia*s in this trial (Table 4a). We also tested progeny from the 07190 (Fry x Trayshed) population – a pure *rotundifolia* cross. The results found variation in their level of resistance, but all had relatively very low ELISA readings. We also examined parental, F1 and BC1 and BC2 genotypes and although they had relatively low ELISA readings they did express leaf and cane symptoms. Based on our past experience genotypes with leaf and cane symptoms equivalent to those seen in this VR hybrid BC2 generation are not likely to survive under prolonged exposure. Although not conclusive, given the very difficult nature of working in a VR background, it appears that the *rotundifolia* line may not be an accessible and productive avenue to pursue.

Table 4a. Segregation for PD resistance in the pure *rotundifolia* 07190 (Fry x Trayshed) population.

Genotype	GH Screen Result (ref b43-17)	Geometric mean (cfu/ml)	Mean (ln cfu/ml)	Std Dev (ln cfu/ml)	Reps
07190-002	R	36,607	10.5	1.3	4
07190-004	R	84,356	11.3	0.8	4
07190-009	R	21,371	10.0	1.1	5
07190-010	R	22,727	10.0	1.1	5
07190-013	R	56,145	10.9	1.0	5
07190-015	R	15,378	9.6	1.0	5
07190-016	R	22,480	10.0	0.8	5
07190-027	R	10,000	9.2	0.0	4
b43-17	R	10,994	9.3	0.3	7
Blanc du Bois	S	1,911,885	14.5	1.0	7
Chard uninoc	R	10,450	9.3	0.1	14
Chardonnay	S	6,499,917	15.7	0.0	7
Fry	R	10,000	9.2	0.0	8
Magnolia	R	17,007	9.7	0.8	8
Male	R	10,000	9.2	0.0	8
Roucaneuf	S	1,374,499	14.1	1.3	7
Trayshed	R	61,537	11.0	1.3	7
U0505-01	S	223,284	12.3	1.4	7
U0505-22	S	6,276,355	15.7	0.1	7
U0505-35	S	4,050,294	15.2	0.7	7

Table 4b. ELISA greenhouse results for parental, F1, BC1 and BC2 VR hybrids genotypes.

Genotype	Parentage	Back Cross Level	GH Screen Result (ref U0505-01)	Geometric mean (cfu/ml)	Mean (ln cfu/ml)	Std Dev (ln cfu/ml)
Trayshed	pure *rotundifolia*	R-Parent	R	61,537	11.0	1.3
T6-42	F2-35 x Trayshed	F1	R	540,689	13.2	1.4
T6-38	F2-35 x Trayshed	F1	S	1,141,553	13.9	1.0
b59-45	T6-42 x Scolokertek Kiralynoje	BC1	R	545,577	13.2	0.9
05389-01	F2-35 x b59-45	BC2	R	919,238	13.7	0.5
05389-02	F2-35 x b59-45	BC2	S	4,867,544	15.4	0.4
U0505-01	PdR1b R reference	BC3	R	223,284	12.3	1.4

Table 2 Group F lists the last of our elite 94% *vinifera PdR1b* selections and the phenotypically most promising selections of wine types from backcrosses within the BD5-117 resistance source. BD5-117 is a southeastern US hybrid with good resistance, but quantitative inheritance that limits breeding progress. The 94% *vinifera PdR1b* selections have been surpassed by the next backcross generation, but these selections have excellent fruit and are being evaluated to see if any others warrant field-testing and larger scale wine making. Group G is a test of our most advanced material at the 97% *vinifera* level and with PdR1b. These selections were made from the first set of offspring that fruited in Fall 2011. We are now testing to verify which have the lowest *X. fastidiosa* levels after greenhouse screening. The best of these selections are destined for large-scale winemaking leading to commercial release.

Table 5 presents the results of the 2011 small-scale wine making. When all wines tasted were included in the analysis, the tasters preferred the whites to the reds and the pure *vinifera* to the PD resistant 94% *vinifera* wines. We re-evaluated the statistical analysis after removing the Lenoir and the only example of a 97% *vinifera* wine (09331-178, which was made from only a few pounds of fruit). When this was done, color was no longer a significant preference and the two blended wines were preferred equally to the pure *vinifera* wines. This was a key result given that the most likely use of PD resistant varieties in California will be planting in "hot spots" followed by blending with pure *vinifera* varieties. When only the unblended varieties were considered, the tasters preferred the pure *vinifera* with the white wine tasting, but preferred one of the 94% *vinifera* wines (07355-75) to the pure Oakville Merlot. The results of this tasting ranked these 94% selections in a similar way to the wines made during the 2010 vintage.

Table 5. Results of a blind tasting of 2011 vintage wines tasted 2/16/12 by 14 tasters comprised of faculty, staff and students in the department of V&E, UCD.

Wine	% *vinifera*	Color	Total	Max	Min	Consensus Descriptors: color; aroma; flavor-texture
Sauvignon blanc (Napa)	100%	White	49.5	5	3	pale; lemon-lime, grassy, clover, estery; tart
SB/07713-51 blend	98%	White	46.0	5	2	pale straw; fruity, lemon, peach, tropical; medium body, round, balanced
U0502-20	88%	White	42.0	4.5	2	yellow-gold, brilliant; ripe apple, tropical, white peach; structured, balanced, petillant
07713-51	94%	White	29.5	4	1	medium yellow, touch cloudy; melon, apple, macaroon, even nutty; round, oily, minerally
Merlot/07355-075 Blend	98%	Red	48.5	5	3	bright purple-red, med-dk; spice, cherry, pepper, some pyrazine; light-medium body, low astringency, some bitterness
07355-75	94%	Red	45.3	5	2	red-purple, med-dk; plum, current, strawberry, bk pepper, some CS veg; medium body, structured; pleasant sweet finish, EtOH
Merlot (Oakville)	100%	Red	40.1	4	2	irridescent purple, med-dk; grapy, vegetal, candy, rose petal; round, light tannins, sl. bitter
07355-048	94%	Red	37.0	5	1	purple-garnet, dark; rose, plum, cassis, stemmy, earthy, sl reduction; medium body, soft-med tannins, bitter
09331-178	97%	Red	36.5	5	1	red-garnet, med; simple red fruit, mildly herbal; light-med body, sl astringent
U0502-38	88%	Red	35.0	5	1	purple w\ violet edges, dark; medicinal, candy, cassis, tar, rose petal; med body, tart and tannic
07329-31	94%	Red	32.8	5	1	red w\ purple edge, med; green herbs, vegetal, berry, chemical; med body and astringency, medicinal
Lenoir	50%	Red	23.5	5	0	brown-red, dark; tea, foxy, odd medicinal, bitters, oxidized; salty, acrid, flabby

We continue to use the Beringer Yountville vineyard for field-testing advanced selections and are planning 25 - 100 vine plantings of 97% *vinifera* selections after they pass 6 to 8 vine tests at UC Davis. We inoculate test plants there each year and evaluate symptom expression and ELISA readings each Fall. Last summer's cool weather decreased the expression of symptoms and ELISA readings on inoculated plants and but younger vines of vinifera controls F2-35 and Petite Sirah continue to die with strong PD symptoms. The PdR1 containing selections continue to perform well, those without have much higher ELISA readings express severe symptoms. A second field plot has been established with 87 and 94% vinifera PdR1 selections in Dry Creek/Healdsburg. We will be inoculating it for the first time in May.

PUBLICATIONS AND PRESENTATIONS
No publications for this year.

Presentations
UC Davis grape breeding program, Croatian Grape Growers and Wine Makers Group, UC Davis, July 13
UC Davis grape breeding program, University of Florida Horticulture Graduate Students Association visit, UC Davis, August 10
Using marker-assisted selection to optimize breeding for resistance to powdery mildew, American Vineyard Foundation Research Forum, UC Davis, February 24
Grape growing and breeding at UCD, Culinary Institute of America Foodies Tour, UC Davis, March 11
Grape research and careers, Early Academic Outreach Program, UC Davis, March 15
UCD grape breeding program, Lodi/Woodbridge Grape Growers Meeting, Lodi, CA, March 18
Sustainable winegrape growing, UC Berkeley Haas Business School Top Tech Program, Mondavi Winery, Oakville, CA, April 9
Grape breeding at UC Davis BOKU : University of Natural Resources and Life Sciences, Vienna, Austria, June 13
PD resistant winegrapes are approaching wine quality and field testing, 62nd Annual Meeting of the American Society of Enology and Viticulture, Monterey, CA, June 22
Grape breeding at UCD. Hopland Growers Meeting, Hopland, CA November 21
Grape breeding progress. Daniel Roberts Growers Meeting, Santa Rosa, CA December 5
Resistance Round Table: Breeding for resistance to grape pests and diseases. Annual Meeting of the Association of Applied IPM Ecologists, Napa, CA, December 16
Grape breeding progress update. Wilbur Ellis Viticulture Team, Santa Rosa, CA, February 9
Breeding for resistance to grape diseases. Ag Unlimited Annual Meeting, Napa, CA, March 1

STATUS OF FUNDS: These funds are scheduled to be spent by the end of the grant.

INTELLECTUAL PROPERTY: The resistance genes identified in this research will be handled by PIPRA, UC Davis.