CDFA PD/GWSS Annual Progress Report
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I. Project Title: Breeding Pierce's Disease Resistant Winegrapes.

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III. List of Objectives and Description of Activities
Objective 1. Breed PD resistant winegrapes through backcross techniques using high quality *Vitis 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

The breeding cycle for the development of PD resistant grapes has been reduced to 2 years (seed to seed) using marker-assisted selection (MAS) with the b43-17 *PdR1* 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% *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. In 2010, we planted about 2,000 97% *vinifera* seedlings all of which had passed MAS and have *PdR1*. These seedlings were aggressively trained and many will fruit this year, allowing the first selection for fruit quality and propagation of the best for wine quality testing. The training of these plants involves bi-weekly attention amounting to about 1,000 hours of training. The best of these seedlings will also be sub-propagated for greenhouse testing to ensure we advance only the most resistant selections.

2010 Crosses

Table 1 presents the crosses made in 2010 with numbers of seeds and seedlings produced along with the number of progeny that are scheduled for MAS testing in Spring 2011. The following were the goals for the 2010 crosses:

1) Use the *PdR1b* allele from the F8909-08 to advance *vinifera* winegrape lines to the 97% *vinifera* level. We have about 2,000 seedlings that were made in 2009 and were planted in the vineyard and trained during 2010. All of these had *PdR1* after MAS, and they will begin fruiting this year. 2010 crosses with *PdR1* should add about 500 97% *vinifera PdR1* resistant progeny and they will be planted this Spring. At each generation the progeny are 50% their last *vinifera* parent. The 2010 crosses at this level were directed at increasing acid balance in red wines and emphasizing floral characters in white wines.

2) Advance the *PdR1b* line, without the presence of *V. rupestris*, to the 94% *vinifera* level. Because we first used F8909-08 (*V. rupestris* x *V. arizonica/candicans* b43-17*)* in our efforts to backcross *PdR1* into *vinifera* we brought along *V. rupestris*. Although this species adds intense coloration to hybrids, it also adds an intensely peppery and herbaceous character

to wines. Thus, we have been backcrossing b43-17 into *vinifera* parents. We should add about 150 94% *vinifera PdR1* progeny from these crosses to the vineyard this Spring.

3) Advance resistance from *V. arizonica/girdiana* b42-26 to the 75% *vinifera* level (BC2). b42-26's strong resistance is controlled by multiple genes, as opposed to the single gene resistance from b43-17. We have been genetically mapping this resistance and are getting closer to establishing effective markers (see companion report). The breeding program maintains and evaluates the populations being used in the mapping studies. In 2010 we generated over 3,000 seeds in 11 populations, utilizing parents from the 07344A population (b42-26 resistance). These parents had been greenhouse tested and the pistillate individuals with the lowest *X. fastidiosa* populations were selected as parents. The resistance in the 07344A population came from *V. vinifera* F2-35 x *V. arizonica/girdiana* b42-26 and therefore lacks *V. rupestris*, which should improve wine quality. If we can develop useful markers to this quantitative resistance it will greatly improve our ability to combine this resistance with *PdR1*.

4) Increasing the *V. arizonica/girdiana* b42-26 mapping population. We are expanding the 07344A population (05347 (F2-35 x b42-26) x Grenache) and would like to have about 500 individuals for more precise mapping of this quantitative trait. Over 800 seeds were produced and are currently being germinated. We will use a limited mapping strategy to map the quantitative trait loci associated with b42-26's resistance. We developed this approach in our recent publication on powdery mildew resistance mapping (Theoretical and Applied Genetics 122:1059-1073.

Greenhouse Testing

Table 2 presents the number of individuals that were tested for *X. fastidiosa* resistance since March 2010 and why they were tested; 727 genotypes were tested with four replicates of each. Bio-control standards are used with each set so that data can be compared over time and across resistance sources.

The Group A (Table 2) tests from the loss of several key PD source plants in the greenhouse and having to depend on lab cultures of *X. fastidiosa*. We normally maintain our *X. fastidiosa* inoculum in greenhouse grown Chardonnay, but through a miscommunication we lost a few plants at a key point for a pending inoculation. We inoculated with a lab colony, but the resulting infections were much less severe than we had expected. Thus, we needed to find a more virulent strain for testing. We tested four strains: a newly extracted strain from Yountville (Beringer); the Temecula strain from Bruce Kirkpatrick; our lab Stag's Leap strain (from cultures but not greenhouse plants); and a newly extracted strain from a Dry Creek PD hot spot (Mounts) where we are field testing advanced selections. We used these strains in our greenhouse screen and tested Chardonnay (highly susceptible) and U0505-01 (strongly resistant) in a new long greenhouse with a relatively large temperature gradation from end to end. The results found large differences in the strains (Table 3) with the new Beringer strain resulting in the highest *X. fastidiosa* populations in Chardonnay (12.6 – mean log transformed cfu/ml). All four strains were separable with a t-test with Beringer having the highest *X. fastidiosa* levels, followed by Temecula (11.9), Stags Leap (11.1) and Mounts (8.8). The Mounts strain was judged to be avirulent, which is unusual given the very severe PD symptoms in that area. We are now using the Beringer strain in our inoculations.

We tested these plants across benches that we judged to be very warm, warm and cool based on mean recorded temperatures. This temperature differential was a result of proximity

to the cooling pads on the north end of the greenhouse. The warm and very warm benches were statistically the same, but Chardonnay on the cool bench had a lower *X. fastidiosa* level. We are no longer using the cool end of this greenhouse.

Groups C, D and E include tests of PD resistant rootstocks, retests of recombinants from our genetic mapping efforts, and tests of the 97% *vinifera* selections with *PdR1*. This last group consists of selections that had been marker tested and grew well. We replicated these and put 6 or 7 vines of each on normal vineyard spacings to more rapidly generate 97% vinifera PD resistant fruit for wine evaluation. Once we have the greenhouse screen data we can focus on the most resistant of these.

Table 4 presents greenhouse screen data from twenty-eight 97% *vinifera PdR1* selections chosen at random from the 2,000 produced. These plants were sub-propagated before planting in the field and 4 to 5 replicate plants were greenhouse tested. The data show that some individuals at this BC4 generation possess *PdR1* but are not highly resistant and have *X. fastidiosa* levels well above b43-17 or U0505-01 (<50,000 cfu/ml), but lower than the field resistant Blanc du Bois (1,000,000 cfu/ml) and much lower than the highly susceptible Chardonnay (>3,000,000 cfu/ml). These test results are evidence that repeated generations of backcrossing might be diluting *PdR1* resistance. However, the majority of the tested 97% *vinifera PdR1* genotypes were resistant and many had *X. fastidiosa* levels equivalent to b43-17. These results suggest that linkages to minor genes that help regulate *X. fastidiosa* resistance might be disrupted as a result of repeated backcrossing. They also emphasize how important the careful testing is, so that only those selections with the highest resistance are advanced.

Group H tests are underway with results expected in April/May. These crosses were made to combine both alleles of PdR1 in one background. Our SSR marker testing allows us to distinguish the two alleles and these tests will help determine whether having both alleles confers higher levels of resistance or more consistent resistance as in b43-17.

The greenhouse data on Groups F and G is being processed. Group F is a cross of *V. vinifera* F2-35 x b42-26 and will add supply the phenotypic data we need for a developing map of b42-26's multigenic resistance at the F1 level, which will compliment the BC1 07344A mapping population. Group G greenhouse screen data is also being processed and these populations also have resistance from b42-26. Preliminary information suggests that PD resistance may be linked to sex expression in populations originating from b42-26 and these populations will help determine if this is true.

Group I tests are underway with results expected in June/July. This group tests resistance from *V. shuttleworthii* 'Haines City' at the BC1 75% *vinifera* level. We previously tested the F1 generation and although data for the bio-controls indicated that the test was only moderately severe, all of the tested genotypes were resistant. The results of this test will help us determine whether we should pursue this resistance source and develop a genetic map. Haines City has strong resistance to *X. fastidiosa* and may be quite different from *V. arizonica* sources.

Field Testing

Testing of advanced selections continues at the Beringer vineyard in Yountville, CA. In addition to the natural PD pressure at this Napa Valley hot spot, we needle inoculate each Spring. Eleven selections from the BC3, 94% *vinifera* crosses, grafted on our PD resistant rootstock selections, were planted at Beringer in July 2010. They are listed followed with their last *V. vinifera* parent: 07329-01, 07329-037 (Chardonnay), 07355-042, 07355-048, 07355-

057, 07355-075 (Petite Sirah), 07370-128, 07371-025, 07371-027 and 07713-051 (Carignane x Cabernet Sauvignon). We also planted a field trial at the Mounts Vineyard in Healdsburg in June 2010 with 07329-37, 07355-75 and 07713-51 (all three 94% *vinifera* with *PdR1*), and U0502-20 (87% *vinifera* with *PdR1*). These vines were planted with varying numbers of 5 vine replicates. The site is surrounded by PD habitat on two sides and is chronically and severely infected, they will also be needle inoculated. 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. They were repotted there and will be planted out in Spring 2011. We also sent cuttings in Winter 2010 and 2011 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

We began making selections for wine quality from *PdR1* resistant populations at the 87% and 94% *vinifera* and have made wines for the few years. Results of these tastings have shown that white wines are easier to make at micro-scale than red wines, and that we are getting better at making these wines. Although these wines are very good, these efforts are primarily in support of our ability to select the best progeny at the 97% level. These selections are all grown in the UC Davis vineyard with up to 8 vines of each on a "Y" trellis with 6 feet between vines. Many of these are also planted with multi-vine replicates at our Beringer (Yountville) and Mounts (Healdsburg) field plots and we hope to have fruit for wine making from the Beringer plot this Fall.

Table 5 presents summary data from a recent tasting of wines we made in Fall 2010. The new campus winery and attention from the Department winemaker, Chik Brenneman, has improved our winemaking. We included Chardonnay and Cabernet Sauvignon as pure *vinifera* controls and the two best PD resistant cultivars currently available Blanc du Bois (a white grape with about 66% *vinifera*) and Lenoir (aka Jacquez and Black Spanish, a red wine grape with 50% *vinifera*). All made with the same amounts of fruit as the *PdR1* selections. We had 16 experienced tasters composed of faculty, staff and students from the Viticulture and Enology Department. When the data was analyzed to distinguish the PD resistant selections with *PdR1* from pure *vinifera*, and from the PD resistant southeastern US cultivars, it showed a significant preference for the *PdR1* selections, and the ranking within the *PdR1* selections was also significant although weakly ($p = 0.0766$). The tasters significantly preferred the whites to the reds and the 94% *vinifera* over the 87% *vinifera* confirming that progress is being made each generation. The general comments by the panel made after the tasting about improvements in wine quality were consistent with these statistically supported findings.

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

Yang, L., H. Lin, Takahashi, Y., Chen, F., Walker, M.A. and Civerolo, E. 2011. Proteomic analysis of grapevine stem in response to *Xylella fastidiosa* inoculation. Physiological and Molecular Plant Pathology (doi:10.1016/j.pmpp.2010.11.002)

Riaz, S., A.C. Tenscher, D.W. Ramming and M.A. Walker. 2011. Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use in marker-assisted breeding. Theoretical and Applied Genetics 122:1059-1073

VI. Presentations on PD Research

California viticulture and problems. John Deere Corp. UC Davis visit, April 1, 2010.

Beneficial outcomes of the UCD grape breeding program. Monterey County Grape Day, Salinas, CA, Apr. 13, 2010.

Sustainable agriculture in the vineyard. UC Berkeley Haas Business School Course, Calistoga, CA Apr. 17, 2010.

UCD grape breeding program (rootstocks, PD and powdery mildew). Fosters/Beringer Grower Representatives and Technical Staff, Santa Rosa, CA Apr. 23, 2010.

UC Davis grape breeding program. Grape Growers and Wine Makers from Croatia, UC Davis, July 13, 2010.

UC Davis grape breeding program. University of Florida Graduate Students / ASHS visit, UC Davis, CA Aug. 10, 2010.

Grape growing in California. South African Grape Growers and Winemakers, UC Davis, CA Aug 23, 2010.

Breeding PD resistant grapevines. Annual Pierce's Disease Research Symposium, San Diego, CA, Dec. 16, 2010.

Optimizing the breeding of wine grapes for resistance to powdery mildew. Current Wine and Wine Grape Research 2011, UC Davis, Feb. 24, 2011.

Abstracts

Walker, M.A., S. Riaz and A. Tenscher. 2010. Optimizing the breeding of Pierce's disease resistant winegrapes with marker-assisted selection. 10th International Conference on Grapevine Breeding and Genetics, Geneva, NY, Aug. 1-5, 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. In Fall 2011, we will begin to evaluate fruit from 2,000 97% *vinifera PdR1* containing genotypes, 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. Mapping in the b42-26 background is also underway and markers will greatly facilitate the use of this quantitative resistance source. 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 testing of small-scale wine from advanced selections with 87.5% and 94% *vinifera* level demonstrated that we can select and make high quality wines from these generations and it will be exciting to make wine from the BC4 97% *vinifera* selections that we hope to release as PD resistant winegrape cultivars.

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 two years have allowed very rapid progress towards PD resistant winegrapes. In the Spring 2010, we planted 2,000 97% *vinifera* seedlings all of which that contain PdR1 resistance. They will begin fruiting in Fall 2011 and we will begin selecting individuals for multiplication and wine testing. We will release PD resistant cultivars from this generation. We planted a new plot in Healdsburg with 94% *vinifera PdR1* selections and added new materials to our plot in Yountville, and have distributed cuttings of 87% *vinifera PdR1* resistant selections to Texas for a field plot near Fredericksburg. This plot augments trials with the same selections in Auburn, AL, and Galveston, TX. Small scale wines were made from 94% *vinifera PdR1* selections grown in the UCD vineyards and they were better than Chardonnay and Cabernet Sauvignon, and the PD resistant cultivars Lenoir and Blanc du Bois made with the same quantities of fruit.

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

The release of new selections will be through UC Davis.

Table 1. 2010 wine and rootstock type crosses, numbers of seeds & seedlings produced with number marker tested.

Resistant Type	Parent\grandparent of Resistant Type	*Vinifera* Types used in 2010 Crosses	# Seeds Produced	#Seedlings Produced	#Seedlings to MAS
1a. *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 97% *vinifera* parentage. F2-35 is 100% *vinifera* cross of Cabernet Sauvignon x Carignane.					
07355-020	Petite Sirah\Cab S.	Barbera	303	237	160
07370-028	F2-35\Chardonnay	Chardonnay, Riesling	1464	1269	555
07329-355	Chard, Petite\Cab S.	Muscat Blanc	460	123	75
07370-713	F2-35\Chardonnay	Viognier	53	34	10
07371-20	F2-35\Chardonnay	Barbera	1076	830	350
1b. *V. arizonica/candicans PdR1* resistance source without *V. rupestris* to produce progeny with 94% *V. vinifera* parentage.					
08329-035	Tannat\Chenin blanc	Cab.Sav.	366	175	100
08329-074	Tannat\Chenin blanc	Cab.Sav., Carignane	1018	427	250
08329-095	Tannat\Chenin blanc	Cab.Sav.	170	15	0
1c. b42-26 *V. arizonica* resistance crosses to produce progeny that are 75% *vinifera*.					
07344A-09	Grenache	Carignane	158		
07344A-11	Grenache	Carignane, Cab.Sav., Chardonnay	633		

07344A-12	Grenache	Carignane	52		
07344A-15	Grenache	Carignane	524		
07344A-25	Grenache	Carignane	273		
07344A-32	Grenache	Carignane	180		
07344A-33	Grenache	Carignane, Cab.Sav.	243		
07344A-51	Grenache	Carignane	478		
07344A-54	Grenache	Carignane, Cab.Sav.	242		
07344A-56	Grenache	Carignane, Cab.Sav.	288		
07344A-61	Grenache	Carignane, Cab.Sav.	83		
1d. Cross to increase the 07344A b42-26 75% *vinifera* mapping population.					
05347-02	F2-35	Grenache	728		

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	Xf Strain Trial	6	3/30/10	7/6/10	F8909-08
B	2007 Cross Families #3	145	4/13/10	7/22/10	F8909-08
C	PD Rootstocks Retest	35	6/8/10	9/30/10	F8909-08
D	08 PD Stocks & Recombinants	22	7/15/10	10/14/10	F8909-08
E	97% *vinifera*, Y-trellis	23	7/26/10	11/23/10	F8909-08
F	05347 Mapping	122	9/23/10	1/6/11	b42-26
G	07344A, 07744RT, 2010 Parents	79	11/9/10	2/10/11	b42-26, b40-14, b43-17
H	Combining *PdR1a* & *PdR1b*	122	1/13/11	4/14/11	b43-17
I	Haines City & Supplemental	173	3/24/11	6/23/11	*shuttleworthii*

Table 3. *Xylella fastidiosa* strains tested for pathogenicity on Chardonnay

Xf Strain	t-test				Least Sq Mean	Comment
Beringer	A				12.6	virulent
Temecula		B			11.9	mod virulent
Stags Leap			C		11.1	mod virulent
Mounts				D	8.8	avirulent

Table 4. Greenhouse screen results from 97% *vinifera PdR1* selections.

Cross ID	R-parent	S-parent	#R	#S	%Reduced Resistance
09314	07370-028	Cabernet Sauvignon	5	3	38%
09331	07355-020	Zinfandel	3	2	40%
09332	07355-020	Chenin blanc	3	1	25%
09333	07355-020	Chardonnay	4	3	43%
09340	07355-020	Cabernet Sauvignon	4	0	0%

Table 5. Results of a blind tasting of 2010 vintage wines tasted 3/3/11 by 16 tasters comprised of faculty, staff and students 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 PdR1* genotypes faired better than both their pure *vinifera* and SEUS counterparts.

Wine Name	% *vinifera*	Total	Max	Min	12/09/08 Consensus Descriptors: color; aroma; flavor-texture
2010 Vintage White Wines					
07713-55	94%	60.0	5	2	pale yellow; blossom, fermentation bouquet, exotic; fruity-pineapple, crisp
07713-51	94%	55.5	5	2	pale yellow; aromatic, tropical; citrus, grassy, tart, green
U0502-20	88%	51.5	4	2	pale yellow; gooseberry, grassy; apple, tart, slightly tannic
Blanc du Bois	66%	50.3	5	1	quite pale; strongly floral, peach; alcoholic, lean or thin
Chard.	100%	44.0	4	1	med yellow; vinous, apple, melon, tropical; buttery, slightly sweet
2010 Vintage Red Wines					
07355-75	94%	53.5	5	2	dark red-purple; blackberry, black pepper; dark fruits, balanced acidity and tannin
07355-42	94%	50.0	4.5	1	dark red-purple; current, cassis, pepper spice; slightly thin, short, tart
U0502-10	88%	48.0	5	1	med-dark; brambles, strawberry, herbal; slightly dry tannic finish
07355-12	94%	47.5	4.5	1	dark almost black with purple edge; plum, spice, some herbs; structured but less full
U0505-35	88%	46.0	4	1	dark red-purple; black cherry, current; tart, short
U0502-26B	88%	39.0	4	1	Light red; light fruity, Bing cherry; red fruit, slightly thin
Cab. Sav.	100%	38.3	4	1.5	med red; veggie, green bean; earthy, bitter, thin
Lenoir	50%	36.5	4	0.5	med-dark w\ brown edge; porty, jam, chemical; old, oxidized, lacks tannin