California Department of Food and Agriculture PD/GWSS
Progress Report
March 2015

Report Title: Renewal Progress Report for CDFA Agreement Number 12-0117-SA

Project Title: Breeding Pierce's disease resistant winegrapes.

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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 southwestern *Vitis* species and southeastern grape hybrids, to allow the introduction of diverse and 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. These progeny have undergone greenhouse testing to verify their resistance and those with the highest levels of resistance have had wines made for multiple years. Over the past 6 years we have made wine from vines that are at the 94% *to 97% vinifera* level from the same resistance background. They have been very good and don't have the hybrid flaws (blue purple color and strong 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* denoted *Pdr1b* and *PdR1a* respectively. These selections have been introgressed into a wide range of winegrape backgrounds over multiple generations. Based on preliminary DNA sequence data and PD phenotypic symptom differences with *PdR1b* containing genotypes, we have renewed efforts in the b40-14 line, which is the source of *PdR1c* also on LG14. We are now integrating this resistance source with PdR1a and PdR1b. Accurate markers will be critical to confirm the stacking of multiple PD resistance genes in our next round of crosses. 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 shows the seedling populations produced from F1 crosses made in 2014 to 7 new parental sources of PD resistance from the southwestern US and northern Mexico. These populations are being considered for possible genetic mapping so that genetic markers can be developed to expedite breeding. The resistant parental accessions were chosen based on their low ELISA values, minimal expression of PD symptoms in the greenhouse screen and their diverse geographic origins and genetic profiles. Multiple copies of each genotype have been made and greenhouse testing is scheduled to begin in March to characterize the inheritance of PD resistance. Resistance sources with cpDNA haplotype noted as 'unique' indicate that they differ from the 12 southwestern US resistance sources used in previous crosses in the Walker Lab breeding program.

Table 1. Seedlings produced from F1 crosses made in 2014 to develop genetic maps in new accessions from the southwestern US and Mexico. F2-35 is a female flowered progeny of Carignane and Cabernet Sauvignon and 100% *vinifera*.

Resistance Source	**Pure *vinifera* Types Used in 2013 Crosses**	**Geographic Origin**	**Progeny DNA Tested for Trueness to Type**	**4" Pot Mother Vines Established**	**cpDNA Haplotype Similar to:**	**LG14 *PdR1* Region Similar to:**
A14	French Colombard	Nogales, AZ	53	40	Unique	Similar to b43-17
A28	Rosa Minna	Wilcox, AZ	42	42	Unique	Shares 1 allele with ANU67
ANU67	F2-35	Mohave, AZ	31	31	Similar to ANU71	Shares 1 allele with A28
ANU71	Grenache blanc	Mohave, AZ	38	38	Similar to ANU67	Unique
C23-94	Nero d' Avola	Sedona, AZ	64	50	Unique	Shares 1 allele with ANU67
DVIT2236.2	F2-35	Veracruz, Mexico	90	40	Unique	Shares 1 allele with SAZ7
SAZ7	F2-35	San Rafael Valley, AZ	52	52	Unique	Shares 1 allele with DVIT2236.1

Table 2 shows the BC1 crosses made in 2014 to promising new PD resistant lines and being advanced to early greenhouse testing. The 2012 cross to b46-43, a glabrous *V. arizonica* collected in Big Bend Park about 250km west of San Antonio, Texas has the most unique PD resistance of any of the many F1 crosses to wild species we have tested. All progeny were highly resistant by both ELISA and phenotype. These BC1 crosses, and a much larger population from the cross of 12305-55 to F2-35, will be used for mapping and should provide insight into the inheritance of PD resistance in this promising new source. The BC1 progeny of b40-29, a brushy *V. arizonica* sourced by Professor Olmo 80km north of Chihuahua, Mexico, are the foundation of new breeding lines to broaden our sources of PD resistance for future source gene stacking. Two of the most resistant genotypes from an additional 2012 cross (12-314) based on ANU5, a *V. girdiana*-like accession from Littlefield, AZ were used to make BC1 crosses in 2014. Selections from these progeny will be greenhouse screened for PD resistance in the fall of 2015.

Table 2. Seedlings produced from BC1 crosses made in 2014 to develop breeding lines from new accessions from the southwestern US and Mexico. F2-35 is a female flowered progeny of Carignane and Cabernet Sauvignon and 100% *vinifera*.

Resistance Source	**Resistant Parent**	***vinifera* Parent(s)**	**Progeny DNA Tested for Trueness to Type**	**Genotypes Advanced to GH Testing**
b46-43	12305-55	Rosa Minna	47	38
b46-43	12305-56	F2-35, Rosa Minna	62	44
b46-43	12305-83	F2-35	32	32
b40-29	12340-13	F2-35	50	25
b40-29	12340-14	F2-35	35	24

In addition to the 596 genotypes marker tested for trueness to type in Tables 1 and 2 above, an additional 1,599 genotypes are being MAS tested this year (Table 3). In Table 3a we are testing for the presence of the *PdR1b* locus in 9 different cross combinations of our 3 most PD resistant b42-26 *V. arizonica/candicans* line genotypes at the BC2 88% *vinifera* level crossed with 3 of our most resistant *PdR1b* 97% *vinifera* line selections. These crosses result in progeny enriched in PD resistance from two seemingly different and robust PD resistance sources at the 92% *vinifera* level. These could be stand-alone crosses for release or more likely the most resistant will be selfed and then, after MAS to select those homozygous for *PdR1b* and rigorous greenhouse screen selection, crossed one last time to elite *vinifera* wine cultivars where all progeny would carry *PdR1b* and be enhanced in b42-26 line resistance. The Walker Lab also has diverse sources of powdery mildew (PM) resistance and incorporating powdery mildew resistance into novel PD resistant varieties would add substantial value. Powdery mildew resistance from *Ren1* and *Ren4* were combined with PD resistance from *PdR1b* and b42-26 in a 4-way stack at the 90% *vinifera* level. Similarly in Table 3c a 3-way PDR-PMR stack was created using the *Ren4* PM resistant locus. We continue to broaden the diversity at and advance the backcross level of the *PDR1c* line from the b40-14 *V. arizonica* background (Table 3d). Our most promising mapping and breeding crosses to novel new PD resistant sources are being tested for trueness to type (Table 3e).

Table 3. 2014 PDR crosses marker aided testing plan.

Group Name	**% *vinifera***	**MAS Test For**	**# Crosses to Test**	**# to MAS EST**
3a. *PdR1b* x b42-26 Stacked	92%	*PdR1b*, no PMR Loci	9	892
3b. PM with most R *PdR1b* x b42-26	90%	*PdR1b*, LG13,LG18	2	137
3c. PM with most R *PdR1b* x b42-26	89%	*PdR1b*, LG18	1	80
Total *PdR1b*		*PdR1b*	12	1109
3d. BC2 & BC3 b40-14 line	88%, 94%	*PdR1c*, no PMR loci	3	220
3e. b46-43 F1 map, BC1 ANU5 line	50%, 75%	Veracity, no PMR loci	5	270

Table 4 provides a list of the PD greenhouse screens analyzed, initiated and/or completed over the last 6 months. In group A we tested individuals from the *PdR1b* x b42-26 stacked line (84% *vinifera* level) as well as 25 PD resistant rootstock genotypes from 4 crosses made in 2011 – advanced *PdR1b* rootstocks were crossed to Ramsey, 420A or Schwarzmann. Based on phenotype and ELISA results, 3 selections involving *PdR1b* x Ramsey look particularly promising. These also have broad nematode resistance and are undergoing additional greenhouse and horticultural testing to confirm these results. In group B we tested 23 BC2 individuals in the b42-26 multigenic resistance source and identified three particularly promising genotypes for further advancement and stacking with *PDR1b* (Table 3a). New southwestern US species were tested in groups A, B, E, and F to facilitate PD resistance gene discovery work being done in our companion PD resistance mapping project. To date we have tested a total of 296 different accessions, the most resistant among them multiple times. BC2 progeny in the b40-14 line from crosses made in 2012 were tested in group D and based on encouraging results additional progeny are being tested in group G. In Group E, 27 BD5-117 x Haines City intercross genotypes were tested and only two were identified as promising. This low resistance rate at only a 75% *vinifera* level again demonstrates the challenges of working with resistant species from the southeastern US. I n this same group we tested 13 selections with *PDR1a* resistance with one identified as outstanding. Should this result be confirmed and pending further horticultural evaluations, this accession could be advanced to multiple vine trials. We also initiated test group G to confirm previous results in the b40-14 line and are testing multiple backcross levels in the same trial to insure we capture any PD resistance contribution from minor resistance loci. ELISA results are still pending on groups F and G.

Table 4. 2014-15 Greenhouse testing.

Group	Genotypes	No. of Genotypes	Inoculation Date	ELISA Sample Date	Resistance Source(s)
A	*PdR1b* x b42-26 stacked, new PD rootstocks, *PdR1a* advanced, southwestern US species	165	3/20/2014	6/19/14	F8909-08, *PdR1a*, b42-26, Ramsey, V. species
B	b42-26 BC2 & southwestern US Species	48	4/8/2014	7/8/14	b42-26, V. species
C	2013 southwestern US F1 Cross Seedlings	192	4/24/2014	7/29/14	b41-13, b43-57, SC36, T03-16
D	88% b40-14, Additional 2013 southwestern US Cross Seedlings	145	5/15/2015	8/14/14	b40-14, b43-57, b47-32
E	southeastern US crosses, southwestern US Species, *PdR1a*, b42-26 BC2	115	9/11/14	12/11/14	b40-14, Haines City BD5-117
F	SWUS Species & Promising Genotypes	229	10/2/14	12/18/14	V. species, F8909-08, Ramsey
G	b40-14 F1, BC1, BC2; 2014 Cross Parents	170	9/23/14	1/8/15	F8909-08, b40-14, b42-26

Table 5 presents 3 promising PD resistant genotypes being sent this spring to Foundation Plant Services for certification and possible release. These will join the 13 scion selections sent to FPS in the previous two springs. We test selections with the potential for release multiple times in the greenhouse screen to ensure that only selections with the highest levels of resistance are considered for release. These selections have much better resistance than two cultivars with long histories of field survival in the southern US – Blanc du Bois and Lenoir. We want to avoid having any selections we release be tolerant to *Xf* and therefore act as hosts and facilitate disease spread within a vineyard. This process involves passing our severe greenhouse screen multiple times. To make this list, selections must also possess desirable horticultural traits and have potential for high quality wine production. Producing small lot wines from multiple vine field trials in Davis and in PD hot spots around the State complete the evaluation process. PD resistant scions may need PD resistant rootstocks in case low levels of the bacteria work their way into a susceptible rootstock. Three such rootstock selections were sent to FPS in spring 2013 and another 25 genotypes were in our greenhouse test (Table 4a) with 3 of the latter being promising as discussed above.

Table 5. The 97% *vinifera* level *PdR1b* selections being advanced to Foundation Plant Services (FPS) in spring 2015.

Genotype	Parentage	Color	Berry Wt (g)	Cluster Wt (g)	Season	Flavor
09314-102	07370-028 x Cabernet Sauvignon	W	1.3	325	Mid	Pear, yellow apple, melon; can be like Sauv blanc

09330-07	07370-039 x Zinfandel	B	1.4	275	Mid	Berry, red apple, cassis
09338-016	07371-20 x Cabernet Sauvignon	W	1.3	250	Mid	Green apple, spicy, can be like Sauv blanc

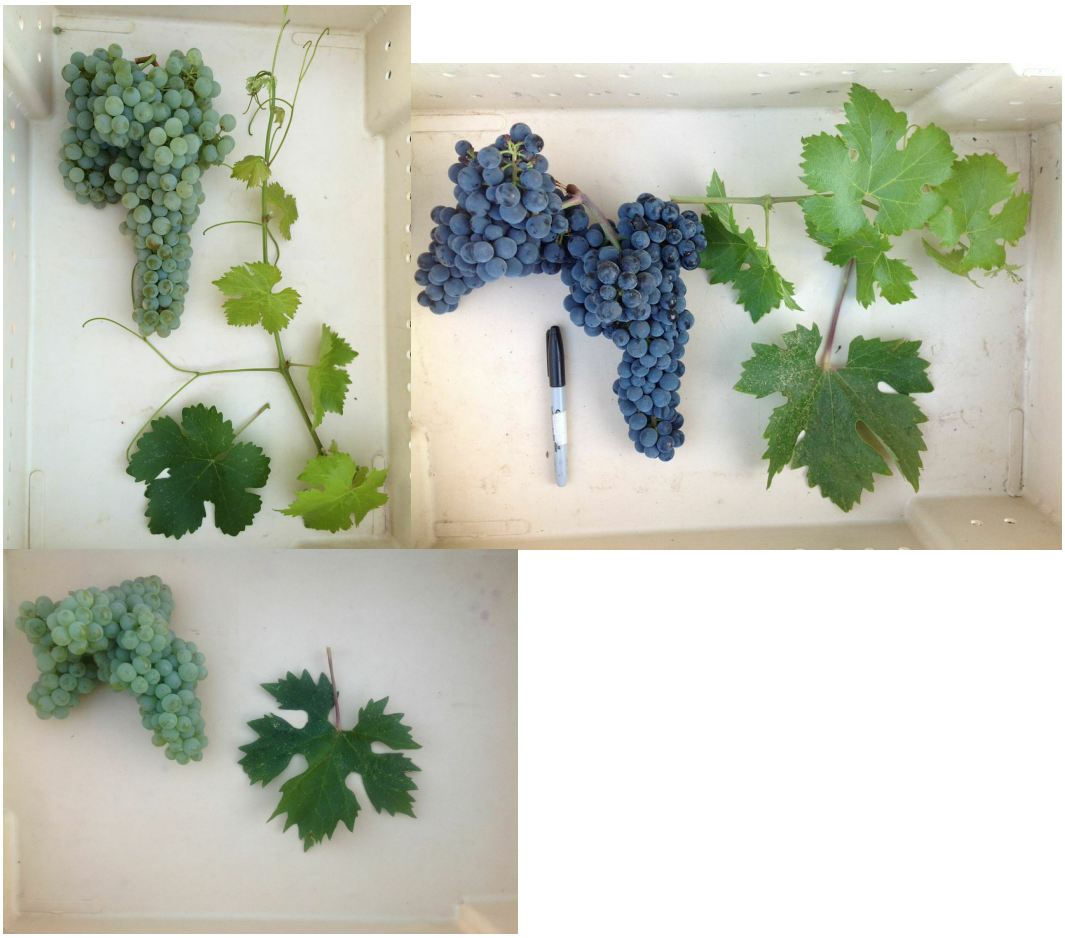

Figure 1. Detached cluster images of 09314-102, 09330-07 and 09338-016 (clockwise from upper left), the three selections being sent to FPS this Spring.

Figure 2. Vineyard shot of 09314-102 and 09330-07 being sent to FPS this Spring.

On 3/12/2015, 15 tasters comprised of UC Davis faculty, staff, and students as well as industry professionals tasted the white and red wines made from the 2014 vintage. The tasting included wines from *PdR1* genotypes at the 94% and 97% *vinifera* level, pure *vinifera* controls, and Lenoir and Blanc du Bois as historic PD resistant controls. Grapes were sourced from our Beringer field trial in Napa and from the UC Davis vineyards. Responses were very positive and the results will be presented in our next report.

CONCLUSIONS AND LAYPERSON SUMMARY

We continue to make strong 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 reports from our companion project) 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. We have screened through about 1,000 progeny from the 2009 and 2010 crosses that are 97% *vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to fruit and those with high quality fruit are advanced to greenhouse testing, where only those with the highest resistance to *Xf*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and at PD hot spots in California. The best of these will be advanced to 25 vine commercial wine testing. We have now sent 13 scion and 3 rootstock advanced selections to FPS over the last two seasons to be certified and begin the release process with another 3 scion selections being sent this year. PD resistance from *V. shuttleworthii* and BD5-117 are also being pursued, but progress is limited by the multigenic nature of their resistance. Other forms of *V. arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine/stack multiple resistance sources and ensure durable resistance. Very small-scale wines from 94% and 97% *vinifera PdR1b* selections have been very good, and have been received well at tastings in the campus winery and at pubic tastings in Davis, Sacramento, Healdsburg (twice), Napa, Fresno and Temecula (CAWG) and Santa Rosa (Sonoma Winegrape Commission).

PUBLICATIONS AND PRESENTATIONS

Talks at Grower Meetings (Extension/Outreach)

Industry show and tell tasting of PD resistant selections at the UCD vineyard. August 28, 2014

Walker grape breeding program. Department Seminar October 3, 2014

Brazil grape breeding PD and PM. November 4, 2014

Not only do they resist PD, they look like winegrapes, taste like winegrapes, make very good wines, and they are getting ready for release" FPS Annual Meeting November 20, 2014

Not only do they resist PD, they look like winegrapes, taste like winegrapes, make very goo wines , and getting ready for release" Current Issues in Vineyard Health, UC Davis, December 2, 2014

Walker breeding program. Gallo Lab Tech Team, UC Davis, December 5, 2014

PD resistant wine grapes nearing release. PD/GWSS Annual Meeting, Sacramento, CA December 16, 2014

Walker, A. 2015. Molecular genetics ready to launch a golden age of winegrape breeding. The Conversation. January 7, 2015. (https://theconversation.com/molecular-genetics-ready-to-launch-a-golden-age-of-winegrape-breeding-35464)

Walker grape breeding program. Flash talk for NGWI, UC Davis, January 26, 2015

Releasing PD resistant winegrapes. Viticulture and Enology Research Conference, UC /Davis February 9

Breeding for powdery mildew resistance using lessons from PD breeding. Napa Valley Grape Growers, March 4, 2015

Tasting of of 2014 Vintage PD resistant wines, UC Davis, March 12, 2015

Presentations at Scientific Meetings

Agüero, C. B., S. Riaz, A. Tenscher, X. Xie and M. A. Walker. 2014. Functional analysis of Pierce's disease resistance genes from *Vitis arizonica*. 65th ASEV National Meeting, Austin, TX

Riaz, S., R. Hu, A. Tenscher and M. A. Walker. 2014. Comparative sequence analysis of the Pierce's disease resistance locus *PdR1.* 65th ASEV National Meeting, Austin, TX

Walker, A., A. Tenscher and S. Riaz. 2014. Breeding Pierce's disease resistant winegrapes.

Proceedings of the 2014 Pierce's Disease Research Symposium, Sacramento, Dec. 15-17 pp 220-226

Walker, A., S. Riaz, and C. Agüero. 2014. Map-based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce's disease resistance in grape. Proceedings of the 2014 Pierce's Disease Research Symposium, Sacramento, Dec. 15-17 pp 227-239.

Walker, A., C. Agüero, and S. Riaz. 2014. Molecular and functional characterization of the putative X*ylella fastidiosa* resistance gene(s) from b43-17 (*Vitis arizonica*). Proceedings of the 2014 Pierce's Disease Research Symposium, Sacramento, Dec. 15-17 pp 240-248.

Walker, A., D. Cantu and S. Riaz. 2014. Molecular breeding support for the development of Pierce's disease resistant winegrapes. Proceedings of the 2014 Pierce's Disease Research Symposium, Sacramento, Dec. 15-17 pp 249 – 255

RESEARCH RELEVANCE

The goal of this research is two-fold: to produce PD resistant wine grapes that can be used in PD hot spot in California and across the southern US; and to provide breeding, maintenance and screening support for our gene characterization and genetic mapping efforts. We now have hundreds of selections at the 97% *vinifera* level and have begun the process of determining which are most resistant and most suitable for release. Thirteen winegrape selections were sent to FPS last Winter to be certified and prepared for release; three more were added this Winter.

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

INTELLECTUAL PROPERTY: PD resistant varieties will be released through the Office of Technology Transfer (Patent Office) of the University of California, Davis.