California Department of Food and Agriculture PD/GWSS
Interim Progress Report – February 2016

REPORT TITLE: Renewal Progress Report for CDFA Agreement Number 15-0425-SA

PROJECT TITLE: Breeding Pierce's disease resistant winegrapes.

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INTRODUCTION

We continue to make rapid progress breeding Pierce's disease resistant winegrapes. Our main focus to date has been on the *PdR1* locus from the b43-17 *V. arizonica*/*candicans* resistance source. 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. Marker-assisted selection (MAS) is used to select candidate resistant progeny as soon as seeds germinate which combine with aggressive vine training and selection for precocious flowering have allowed us to reduce the seed-to-seed cycle to two years. PD resistance is confirmed in the greenhouse using rapid screening techniques for *Xylella fastidiosa* (*Xf)* resistance developed in the Walker Lab (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005). New varieties 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 this same *PdR1* resistance background. So far 16 scion selections and 3 PD resistant rootstocks have been advanced to Foundation Plant Services (FPS) to begin the certification and release process. Other forms of *V. arizonica* and other southwestern US (SWUS) species are being studied (see companion report) and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Stacking of *PdR1b* with b42-26 (*V. arizonica-girdiana*) Pierce's disease resistance began in 2011 and last year was advanced to the 92% *V. vinifera* level using MAS to confirm the presence of *PdR1b* and greenhouse screening to verify higher than usual levels of Pierce's disease resistance. Resistance from southeastern United States (SEUS) species is being advanced more slowly since resistance in these latter lines is complex and markers have not yet been developed to expedite breeding.

OBJECTIVES

1. Identify additional unique sources of *Xf* resistance; develop breeding populations and phenotype them with our greenhouse screen to characterize their inheritance of resistance.
2. Develop ~97% *vinifera*-based PD resistant lines of winegrapes utilizing diverse sources of resistance to *Xf*, and conduct fruit and wine evaluations.
3. Utilize marker-assisted selection to allow stacking of resistance loci, screen for resistant genotypes, and develop backcross generations by crossing resistant selections to elite *vinifera* varieties in order to produce high quality and PD resistant winegrapes.
4. Develop and maintain new and existing genetic mapping populations to assist companion mapping/genetics project; begin the mapping of fruit quality traits such as color, tannin content, flavor, and productivity in PD resistant backgrounds.

RESULTS AND DISCUSSION

We have largely completed greenhouse testing and field evaluations of the promising 97% *vinifera PdR1* genotypes from crosses made in the 2009-11 period. We have selected for release four 97% and one 94% *vinifera* PD resistant scion genotypes with *PdR1b* resistance from those currently at FPS. Table 1 details their elite *vinifera* heritage. 07355-075 is a black-fruited variety at the 94% *vinifera* level.

The 97% *vinifera* reds selected for release are 09330-07 and 09331-047. The whites being released at this time are 09314-102 and 09338-016. Descriptions of their wine characteristics are detailed in Table 2. Also shown in Table 1 are the elite *vinifera* parentage of three additional new 97% *vinifera* selections that are being sent to FPS this Spring to begin the certification process. Fruit characteristics on these are introduced in Table 3.

Table 1.Elite *vinifera* wine-type heritage of the 5 scion varieties selected for release (first 4 columns) and the 3 selections being advanced to FPS for certification this spring (last two columns).

Elite *vinifera* parentage - Genotype	07355-075	09314-102, 09338-016	09330-07	09331-047	09333-370	10302-178, 10302-293
Cab. Sauvignon	25.0%	62.5%	12.5%	12.5%	12.5%	12.5%
Carignane		12.5%	12.5%			12.5%
Chardonnay		12.5%	12.5%		50.0%	12.5%
Petite Sirah	50.0%			25.0%	25.0%	
Riesling						50.0%
Zinfandel			50.0%	50.0%		
Other *vinifera*	18.8%	9.4%	9.4%	9.4%	9.4%	9.4%
V. arizonica	3.1%	1.6%	1.6%	1.6%	1.6%	1.6%
V. rupestris	3.1%	1.6%	1.6%	1.6%	1.6%	1.6%

The 5 selections advanced to release this reporting period were tasted 1/21/2016 by 18 tasters comprised of winemakers, viticulturists and wine writers held at the UC Oakville Field Station in the Napa Valley of California. The wines were rated on a hedonic quality scale from 1 = poor to 5 = very good. Results are summarized in Table 2. It can be seen from the range of quality scores for each of the wines that the tasters didn't assess the wines uniformly. However no taster rated every wine as poor and every wine was considered "very good" by at least one taster. With a mean score of 3.17 and a standard deviation of 0.95 for all wines considered together, they were perceived as being of average quality. This is significant praise from a group of professionals familiar with evaluating some of the finest *vinifera* wines in the world especially considering that the wines were produced from grapes grown in Davis, were less than 6 months old, had no oak elaboration and had been in bottle less than a month at the time of tasting.

Table 2. Results of a tasting of 2015 vintage wines tasted.

Wine Name	% *vinifera*	Color	Average Score	Max Score	Min Score	1/21/16 Consensus Descriptors: color; aroma; flavor-texture
09314-102	97%	White	3.3	4.5	2	Pale straw; tropical, floral, with grassy notes, like SB; citrus, herbal, light-mod body, crisp but balanced acidity.
09338-016	97%	White	3.4	5	2	Lt straw; mod intensity, apple, peach, hints of floral, like CH; pear, touch honey, full, moderate acidity.
07355-075	94%	Red	3.4	5	2	Med-dark red w\purple edge; berry, coffee, plum, white pepper; brambly, spice, potpourri, mod body & tannin.
09330-07	97%	Red	2.9	5	1	Dark inky red-purple; grapy, blueberry, plum, herbal, like Syrah-PS; cola, plum, mint, round, juicy, sl low acid, mod tannins.
09331-047	97%	Red	2.9	4.5	1	Med+ red-purple; dark fruit, black olive, bell pepper, like CS; berry, spice, slightly low acidity, grippy tannins.

Table 3 presents the fruit characteristics for the three new additions to our PD resistant scion selections at FPS. 09333-370 was selected for the lighter bodied wine it makes, which reminds some tasters of Pinot noir. With 50% chardonnay in its background this isn't totally unexpected. It was also selected as being one of the most highly resistant genotypes in our greenhouse tests. In the *PdR1b* line we have been challenged to select good quality and highly resistant white selections. These two new white additions, 10302-178 and 10302-293 should provide some more floral Riesling-like options to the two white PDR releases 09314-102 which some characterize as Sauvignon blanc-like and the 09338-016 which has been described as similar to Chardonnay.

Table 3. Fruit characteristics of three new 97% *vinifera* scion selections destined for FPS this spring.

Genotype	Parentage	Color	Berry Wt (g)	Cluster Wt (g)	Season	Consensus Descriptors: Color; Aroma; Flavor Texture
09333-370	07355-020 x Chardonnay	B	1.3	300	Mid	med red-purple; light strawberry, candied red fruit; spicy, dried herbs; soft, mod weight
10302-178	07370-028 x Riesling	W	1.1	110	Early	pale gold; floral-honey, touch petrol; citrus peel, spice, dough; rich, hint of acidity
10302-293	07370-028 x Riesling	W	1.1	130	Mid	Green-gold; Pear, melon, floral, slight spice; lychee, light, balanced finish

Figure 1. Pictures of 3 selections being sent to FPS this spring. Left to right : 09333-370, 10302-178, 10302-293

Having nearly completed our evaluations of all the various 97% *vinifera PdR1b* cross progeny and with five of the best *PdR1b*-based selections soon to be available to the industry, we have turned our breeding focus to stacking *PdR1*-based PD resistance with that of the quantitatively resistant b42-26 line to create PD resistant scion varieties capable of resisting more aggressive mutant forms of *X. fastidiosa*. Table 4a presents the number of progeny in each pairing of a 3 x 3 crossing of the *PdR1b* x b42-26 lines at the 92% *vinifera* level in a recently completed greenhouse screen. Table 4b presents the population mean cane maturity index (CMI) results that quantify the severity of Pierce's disease symptoms of the genotypes in each population. Most importantly, Table 4c shows the ratio of progeny where all reps show no cane symptoms to the number of genotypes tested. Bacteria levels typically correlate fairly well with CMI scores, and the CMNI scores are important considerations in the selection of resistant genotypes. ELISA testing of these selections is underway. Thus far, with 25% of the samples completed the values track closely with the relative population mean CMI scores. Numerous individuals have non-detectable *X. fastidiosa* levels as well as having no CMI symptoms of PD. The next step in our stacking, which will be completed this Spring, is to intercross a number of the most resistant individuals descending from different parent combinations identified from this group to create breeding genotypes homozygeous at *PdR1b*, enriched in b42-26 QTLs, and showing no *Xf* titer by ELISA and no CMI symptoms. A final step would then be to cross the most promising and resistant of these to any number of elite *vinifera* varieties to create populations that are 96% *vinifera* in which all progeny have *PdR1b* and all should be highly PD resistant. The most promising selections would then be advanced to FPS for certification and release as the next iteration of our PD resistant scion breeding efforts.

Table 4. PD Cane Maturity Index (CMI) symptoms ratings on progeny from nine *PdR1b* x b42-26-line crosses 13 weeks after *X. fastidiosa* inoculation in our greenhouse screen: # tested, progeny means, and ratio # rated zero to # tested. "n – xx" denotes the 10-301 b42-26 pollen parent most recently descended from Chardonnay and the 09331-xxx identifies the *PdR1b* seed parent most recently related to Zinfandel.

PdR1b x b42-26 Parents	4a. # Progeny Tested			4b. Progeny mean CMI			4c. Ratio # 0.0 / # tested		
	n-14	n-20	n-47	n-14	n-20	n-47	n-14	n-20	n-47
09331-033	53	34	14	0.8	0.4	0.2	0.32	0.47	0.71
09331-122	27	38	17	0.4	0.5	0.4	0.37	0.42	0.47
09331-153	20	21	21	1.0	1.3	1.1	0.00	0.14	0.19

Table 5 details our MAS testing plan for the PD resistance crosses made in 2016. In addition to the PD resistance resources discussed above, 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. Since PM is a universal scourge to *vinifera* grape culture, the identification of resistance loci with published genetic markers is extensive. To enhance functionality of our advanced PDR lines we continue stack them with these various PM resistance loci. In Table 5a powdery mildew resistance from a *V. vinifera* and *V. romanetii* source (*Ren1* and *Ren4*) were combined with PD resistance from *PdR1b* and b42-26 in a 4-way stack at the 95% *vinifera* level. Similarly in Table 5b a 3-way PDR-PMR stack was created using the *Ren4* PM resistant locus and our two most advanced PD sources. In Table 5c we created 3-way stacks of *PdR1b* PD resistance with the *Ren1* and *Ren4* PM resistance sources while in Table 5d we use *Run1* (a *M. rotundifolia* resistance source) instead of *Ren1* in a similar 3-way stack. In Tables 5a-d the PD resistant parents are homozygeous at *PdR1b* so all progeny should carry this PD resistance. Our most promising mapping and breeding crosses to novel new PD resistant sources are being tested for trueness to type (Table 5e).

Table 5. 2015 PD resistance crosses scheduled for marker-assisted selection.

Group Name	% *vinifera*	MAS Test For	# Crosses to Test	# to MAS TEST
5a. PM with most R *PdR1b^2* x b42-26	95%	*Ren1, Ren4*	3	281
5b. PM with most R *PdR1b^2* x b42-26	*95%*	*Ren4*	4	176
5c. PM with most R *PdR1b^2*	96%	*Ren1, Ren4*	2	103
5d. PM with most R *PdR1b^2*	93%	*Run1, Ren4*	3	242
5e. *PdR1b*^2, b46-43 F1 map, BC1 SC36	75%-93%	Veracity, no PMR loci	10	415

Table 6 provides a list of the PD greenhouse screens analyzed, initiated and/or completed over the reporting period. In Group A we tested 6 BC1 (backcross 1) and 14 BC2 progeny in the b40-14 line. Only one at the BC1 level was considered exceptionally resistant and 4 at the BC2 level had good resistance. Six BD5-117 x Haines City intercross progeny were tested and only two were identified as of some interest. This absence of highly resistant progeny at only the 75% *vinifera* level (BC1) again demonstrates the challenges of working with resistant species from the southeastern US. These genotypes have good resistance, but it's controlled by multiple genes and is rapidly degraded when crossing with susceptible high quality *V. vinifera* parents. In this same group we also tested 7 BC3 and 11 BC4 selections with *PdR1a* resistance; these tests yielded 5 and 3 progeny with good resistance, respectively. One genotype was identified with outstanding resistance at the 97% *vinifera* (BC4) level. This result was confirmed in Group B testing, and pending further horticultural evaluations this selection could be advanced to multiple vine trials. Of the 46 *PdR1b* genotypes tested to confirm previous greenhouse screen results, five were classified as having good resistance and 8 were exceptionally resistant. Following further horticultural and wine quality evaluations, decisions will be made on the usefulness of advancing additional scion varieties in this resistance background. Also tested in Group A were 10 rootstock types from crosses of the PD resistant *V.* X. *champini*i 'Dog Ridge' to either 110R or 140 Ru. None were as resistant as our previously identified PDR rootstocks from our *PdR1b* x Ramsey crosses, and they will not tested further.

Another 22 progeny of the 13309 intercross of the two most highly resistant 07-344a BC1 genotypes in the b42-26 line were tested in Group B making a grand total of 48 genotypes tested. In total 45 were of intermediate resistance with 2 being susceptible and 1 being as resistant as the parent, b42-26. This confirms our assessment that some important resistance factors were left behind likely at the F1 level. Also in Group B we tested the first 23 genotypes in the 14399 b46-43 BC1 mapping population and found a resistant/susceptible ratio of 13:10. Additional genotypes are being tested in our companion mapping project to identify major and minor resistance loci. Fifteen genotypes at the 94% *vinifera* level in the *PdR1a* line were evaluated, but none were sufficiently resistant to advance. Seven genotypes in the *PdR1b* x b42-26 crosses detailed in Table 4 were tested with this group; all were resistant and 1 was exceptionally resistant. In addition, we tested 24 genotypes at the 89% *vinifera* level that are homozygeous at *PdR1b*, have some b42-26 resistance and also carry PM resistance from *Ren4*. All were resistant, 12 significantly so and 2 exceptionally so. This cross is the first instance where an elevated PD resistance has been observed at this advanced *vinifera* level and it warrants further investigation. Of interest in Group B is a test of 10 progeny from a cross at the 87% *vinifera* level involving *PdR1b* x b42-26 but

where the b42-26 side was emphasized by backcrossing a second time to another resistant b42-26 line genotype. All progeny were resistant, half significantly so and 2 exceptionally so. These results underscore the value of the combining the *PdR1b* and b42-26 resistance lines. Preliminary results from Group C are reported in Table 4 and its discussion above.

One hundred and twenty more progeny from the b42-26 background were tested in Group D in an effort to improve the genetic resistance map in this multigenic resistance background. In an attempt to identify missing resistance factors in the BC1 07344a b42-26 line, we also tested 25 genotypes in an alternate BC1 population derived from a different highly resistant F1 parent. We also tested 20 genotypes which have *PdR1b* and the *Ren1* and *Ren4* PM resistance loci.

We continue to refine our rapid GH screen with an experiment in Group E. We have observed that expression of PD symptoms increases when the test plants in a given trial become water stressed. In addition, in at least one trial symptoms were dramatically diminished when excess irrigation levels were maintained. Plant water status also appears to impact bacterial titer. In this experiment we will better define the water status impact on PD expression using our 3 *PdR1b* and 4 biocontrol genotypes that range in symptom levels and *X. fastidiosa* titers.

Table 6. Greenhouse PD screens analyzed, completed and/or initiated during the reporting period.

Group	Test Groups	No. of Progeny	Inoculation Date	ELISA Sample Date	PD Resistance Source(s)
A	b40-14, *PdR1a*, BD5-117xHC (HW)	119	8/25/2015	11/24/2015	b40-14, *PdR1a*, BD5-117xHC
B	b42-26^2 Inter,*PdR1*xb42-26xVRom Stack Promising, b46-43 BC1 map	168	9/17/2015	12/17/2015	*PdR1b*, b42-26
C	92% *PdR1b*xb42-26 Stack	274	10/27/2015	1/26/2016	*PdR1b*, b42-26
D	Addn b42-26 F1, Alt b42-26 BC1	172	*2/23/2016*	*5/25/2016*	*PdR1b*, b42-26
E	BC-UBC Irrigation Level Trial	7	*2/23/2016*	*5/25/2016*	*PdR1b* & biocontrols

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 have 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 *X. fastidiosa*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and at PD hot spots in California. We have now sent 16 scion and 3 rootstock advanced selections to FPS over the last three seasons to be certified and begin the release process with another 3 scion selections being sent this year. The first 5 advanced *PdR1b* varieties have been identified for release to the industry. Stacking of *PdR1b* resistance with resistance from the b42-26 *V. arizonica-girdiana* multigenic PD resistance source is advancing and promises enhanced levels of PD resistance and durability. 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 additional 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 public

tastings in Davis, Sacramento, Healdsburg, Napa, Fresno and Temecula (CAWG) and Santa Rosa (Sonoma Winegrape Commission).

PUBLICATIONS AND PRESENTATIONS

Xie, X., C.B. Agüero, Y. Wang and M.A. Walker. 2015. *In vitro* induction of tetraploids in *Vitis* X *Muscadinia* hybrids. Plant Cell, Tissue & Organ Culture DOI 10.1007/s11240-015-0801-8.

Xie, X., C.B. Agüero, Y. Wang and M.A. Walker. 2016. Genetic transformation of grape varieties and rootstocks via organogenesis. Plant Cell, Tissue and Organ Culture (submitted)

Talks at Grower Meetings (Extension/Outreach) 2015

Breeding for PD and PM resistance Napa Valley Grape Growers, Napa, CA March 4 2015
Vineyard of the Future Wine Executive Program lecture, UCD. Mar. 27
California viticulture. UC Berkeley DNV Business program Napa, CA. Apr. 18
Breeding for PD and PM resistance Diageo Winemakers, UC Davis, April 23, 2015
Breeding new winegrape varieties – PD, PM and beyond. Napa Marriot, May 6
A look to the future – what's in store for CA vineyards Anderson Valley Tech Conference, Philo, CA. May 15
Breeding PD and PM resistant winegrapes with tasting. Daniel Roberts Client Group, Santa Rosa, CA July 10
PD resistant wine grapes. Ventura Farm Press Interview, July 7
Breeding PD and PM resistant winegrapes. Sonoma County Winegrape Commission, Santa Rosa, CA July 31
PD resistant winegrapes – talk and tasting California Association of Family Farms, Valley Center, CA Aug 7
Grape breeding at UC Davis. Chilean Table Grape Association, UC Davis, Aug 25
Grape rootstock and scion breeding at UC Davis. North American Grape Breeders Association Meeting, Geneva, NY Aug 29.
PD Breeding Progress – report and tour. CDFA Administrators, UC Davis Oct 13
Grape breeding at UC Davis Interview for David Pelletier for International Wine Magazine, UC Davis Oct 13
Breeding PD resistant wine grapes – talk and tasting VEN on the Road, Santa Maria, CA Nov 5
UCD vineyard and winery tour, and PD wine tasting with Darrel Corti. Sacramento Private School support group and auction prize, UC Davis Nov 8
PD resistant winegrapes nearing release. FPS Annual Meeting, UC Davis Nov 10
Breeding PD resistant winegrapes. Napa Vit Tech Meeting, Napa, CA Nov 12
Grape breeding at UC Davis. Guest Lecturer at Chihuahua University, Chihuahua, MX Nov 25
Breeding PD resistant winegrapes. UCD Winegrape Day, UC Davis Dec 2
Walker grape breeding program. UC Cooperative Extension Grape Farm Advisor Meeting, UC Davis Dec 3
PD breeding update and tasting. Oak Knoll Growers Group, Napa, CA Jan 7, 2016
Walker grape breeding program update and tasting. Silverado SIMCO Growers Management Seminar, Napa, CA Jan 13, 2016
PD resistant winegrapes – update and tasting Napa/Sonoma growers meeting, Napa, CA Jan 21, 2016

Presentations at Scientific Meetings

Walker, A. , A. Tenscher and S. Riaz. 2015. Breeding Pierce's disease resistant winegrapes. Proceedings of the 2015 Pierce's Disease Research Symposium.

Walker, A., S. Riaz, C. Agüero and D. Cantu. 2015. Molecular breeding support for the development of Pierce's disease resistant winegrapes. Proceedings of the 2015 Pierce's Disease Research Symposium.

RESEARCH RELEVANCE

The goal of this research is two-fold: to produce PD resistant wine grapes that can be used in PD hot spots 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. Sixteen winegrape selections were sent to FPS last over the past 3 years to be certified and prepared for release; three were added this spring.

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.