FINAL REPORT FOR CDFA AGREEMENT NUMBER 15-0425-SA

PROJECT TITLE: Breeding Pierce's disease resistant winegrapes.

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REPORTING PERIOD: July 2015 to June 2018

ABSTRACT
Breeding Pierce's disease (PD) resistant winegrapes continues to advance accelerated by aggressive vine training and selection for precocious flowering resulting in a seed-to-seed cycle of two years. To further expedite breeding progress, we are using marker-assisted selection (MAS) for PD resistance genes to select resistant progeny as soon as seeds germinate. These two practices have allowed us to produce four backcross generations with elite *Vitis vinifera* winegrape cultivars in 10 years. We have screened through about 2,000 progeny from the 2009, 2010, and 2011 crosses that are 97% *V. vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. We select for fruit and vine quality and then move the best to greenhouse testing, where only those with the highest resistance to *Xylella fastidiosa*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and other test sites. The best of these have been advanced to field testing with commercial-scale wine production, the first of which was planted in Napa in June 2013. To date 20 scion and three PD resistant rootstocks have been advanced to FPS for certification. Five of these selections are now in pre-release to nurseries. Stacking of *PdR1b* with PD resistance from b42-26 (an alternative form of PD resistance controlled by multiple genes) has been advanced to the 96% *V. vinifera* level using MAS to confirm the presence of *PdR1* as well as the recently discovered (see companion report) PD resistance locus on chromosome (Ch) 8 from b42-26, *PdR2*. Other forms of *V. arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Pierce's disease resistance from *V. shuttleworthii* and BD5-117 are also being pursued but progress is limited by their multigenic resistance and the absence of associated genetic markers. Very small-scale wines from 94% and 97% *V. vinifera PdR1b* selections have been very good and have been received well at public tastings in Sacramento (California Association of Winegrape Growers; CAWG) and Santa Rosa (Sonoma Winegrape Commission), Napa Valley (Napa Valley Grape Growers and Winemakers Associations), Temecula (Temecula Valley Winegrape Growers and Vintners), Healdsburg (Dry Creek Valley and Sonoma Grape Growers and Winemakers) and UC Davis.

LAYPERSON SUMMARY
One of the most reliable and sustainable solutions to plant pathogen problems is to create resistant plants. We use a classical plant breeding technique called backcrossing to bring PD resistance from wild grape species into a diverse selection of high quality winegrapes. To date we have identified two different chromosome regions that house very strong sources of PD resistance from grape species native to Mexico and the southwestern United States (*V. arizonica*). Because we were able to locate these resistance genes/regions - *PdR1* (Krivanek et al., 2006), and *PdR2* (Riaz, et al., 2018) we have been able to use marker-assisted selection (MAS) to screen for DNA markers associated with both *PdR1* and *PdR2* allowing us to select resistant progeny shortly after seeds germinate. Marker-assisted selection and aggressive training of the selected seedling vines have allowed us to produce new PD resistant high quality winegrape selections that are more than 97% *V. vinifera* in only 10 years. We have evaluated thousands of resistant seedlings for horticultural traits and fruit quality. The best of these 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 around California. The best of these are advanced to field plots where commercial-scale wines can be produced. We have sent 20 advanced selections to Foundation Plant Services (FPS) over the past six winters to verify their virus-free status. Five of these selections are now in pre-release to nurseries. Three PD resistant rootstocks were also sent to FPS for certification. Other wild grape species are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable PD resistance. Very small-

scale wines made from our advanced *PdR1* selections have been very good and received well at professional tastings throughout California.

INTRODUCTION

We continue to make rapid 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 two years. To further expedite breeding progress we are using marker-assisted selection (MAS) for the PD resistance loci, *PdR1* and *PdR2* 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 Vitis *vinifera* winegrape cultivars in 10 years. We have screened through about 2,000 progeny from the 2009, 2010, and 2011 crosses that are 97% *V. vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to fruit and others are advancing to small scale wine trials. We select for fruit and vine quality and then move the best selections to greenhouse testing, where only those with the highest resistance to *Xylella fastidiosa* (*Xf*), after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and other test sites. The best of these have advanced to field testing with commercial-scale wine production, the first of which was planted in Napa in June 2013. To date 20 scion and three PD resistant rootstocks have been advanced to FPS for certification. Five of these have been pre-released to grapevine nurseries to build up the amounts available for grafting. Stacking of *PdR1b* with b42-26 Pierce's disease resistance has been advanced to the 96% *V. vinifera* level using MAS to confirm the presence of *PdR1* as well as the recently discovered (see companion report) PD resistance locus on LG8 from b42-26, *PdR2*. Initial selections for release will begin in 2018. Greenhouse screening is used to advance only genotypes with the highest possible levels of PD resistance. Other forms of *V. arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Pierce's disease resistance from *V. shuttleworthii* and BD5-117 are also being pursued but progress is limited by their multigenic resistance and the absence of associated genetic markers. Very small scale wines from 94% and 97% *V. vinifera PdR1b* selections have been very good and have been received well at public tastings in Sacramento (California Association of Winegrape Growers; CAWG) and Santa Rosa (Sonoma Winegrape Commission), Napa Valley (Napa Valley Grape Growers and Winemakers Associations), Temecula (Temecula Valley Winegrape Growers and Vintners), and Healdsburg (Dry Creek Valley and Sonoma Grape Growers and Winemakers).

The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xf* resistance (Buzkan et al., 2003; Buzkan et al., 2005; Krivanek et al., 2005a 2005b; Krivanek and Walker, 2005; Baumgartel, 2009), and having unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southwestern grape species, which allows the introduction of extremely high levels of *Xf* resistance into commercial grapes. We genetically mapped and identified what seems to be a single dominant gene for *Xf* resistance in *V. arizonica/candicans* b43- 17 and named it *PdR1*. This resistance has been backcrossed through four generations to elite *V. vinifera* cultivars (BC4) and we now have 97% *V. vinifera* PD resistant material to select from. Individuals with the best fruit and vine characteristics are then tested for resistance to *X. fastidiosa* under our greenhouse screen. Only those with the highest levels of resistance are advanced to small-scale winemaking trials by grafting them onto resistant rootstocks and planting six to eight vine sets on commercial spacing and trellising at Pierce's disease hot spots around California, where they continue to thrive. We have made wine from vines that are 94% *V. vinifera* level from the same resistance background for nine years and from the 97% *V. vinifera* level for six years. They have been very good and don't have typical hybrid flaws (blue purple color and herbaceous aromas and taste) that were prevalent in red wines from the 87% *V. vinifera* level. b43-17 is homozygous resistant to PD. We have named its resistance region/locus *PdR1* and the two forms/alleles of that locus *PdR1a* and *PdR1b*. Screening results reported previously showed no significant difference in resistance levels in genotypes with either one or both alleles. We have primarily used *PdR1b* in our breeding, but retain a number of selections at various backcross (BC) levels with *PdR1a* in the event that there is an as yet unknown *X. fastidiosa* strain-related resistance associated with the *PdR1* alleles. We also identified a PD resistance locus from *V. arizonica* b40-14 (*PdR1c*) that maps to the same region of Chromosome 14 as *PdR1* from b43-17. In the absence of an understanding of gene function and given the very disparate origins of the b43-17 and b40-14 resistance sources, differences in preliminary DNA sequence data between them, and differences in their PD symptom expressions, we have continued to advance the b40-14 (*PdR1c*) resistance line as a future breeding resource. Our companion research project is pursuing the genetic basis of these differences between *PdR1b* and *PdR1c*. In 2005, we started a PD resistant breeding line from another Mexican accession, b42-26. Markers linked to this resistance proved elusive but strong resistance was observable

in our greenhouse screens as we advanced through the backcross levels. In 2011, we started stacking resistance from *PdR1b* with that of b42-26 using marker-assisted selection (MAS) to select for *PdR1b* and a higher than usual resistance in our greenhouse screen to move the b42-26 resistance forward. Late in 2016 our companion project identified the location of a significant PD resistance locus from b42-26 on chromosome (Ch) 8, which we have called *PdR2*. In 2014, we advanced our *PdR1* x *PdR2* line to the 92% *vinifera* level and in spring 2016 made crosses to advance it to the 96% *vinifera* level. MAS was used to advance only genotypes with both *PdR1b* and *PdR2* for the first time on these crosses. The resistance from southeastern United States (SEUS) species is being advanced in other lines. However, the resistance in these latter lines is complex (controlled by multiple genes) and markers have not yet been developed to expedite breeding. The breeding effort with alternative resistance sources and the complexing of these resistances is being done to broaden *Xf* resistance and address *Xf*'s potential to overcome resistance.

OBJECTIVES

1. Identify unique sources of PD resistance with a focus on accessions collected from the southwestern United States and northern Mexico. Develop F1 and BC1 populations from the most promising new sources of resistance. Evaluate the inheritance of resistance and utilize populations from the most resistant sources to create mapping populations.

2. Provide support to the companion mapping/genetics program by establishing and maintaining mapping populations, and using the greenhouse screen to evaluate populations and selections for PD resistance.

3. Develop advanced lines of PD resistant winegrapes from unique resistance sources through four backcross generations to elite *V. vinifera* cultivars. Evaluate and select on fruit quality traits such as color, tannin content, flavor, and productivity. Complete wine and fruit sensory analysis of advanced selections.

4. Utilize marker-assisted selection (MAS) to stack (combine) different resistance loci from the BC4 generation with advanced selections containing *PdR1*. Screen for genotypes with combined resistances, to produce new PD resistant grapes with multiple sources of PD resistance and high-quality fruit and wine.

RESULTS AND DISCUSSION

Our PD resistance breeding activities over the last 3 calendar years are quantified and summarized in Table 1. We reached the 97% *vinifera* level in the *PdR1b* line in 2009 and finished planting out additional crosses at that level in 2011. A total of 2,911 genotypes were planted in the 2010-12 period. In 2016 we reached the 96% *vinifera* level in the *PdR1b* x *PdR2* stacked line with the planting of 126 marker tested genotypes having both *PdR1* and *PdR2*. This past spring we planted another 328 seedlings incorporating a wider range of elite *vinifera* varieties in their parentage at this same backcross level from 2017 crosses (Table 8). Our marker testing (1a) has expanded to include testing of crosses without known resistance markers for trueness-to-type and our plantings in (1b) include potential new resistance source F1, BC1and BC2 populations (Tables 2, 3 & 6). The relatively low number planted in 2016 reflects the wait for the 2014 92% *vinifera PdR1* x b42-26 intercross parents to flower for the first time in 2016. We used the interlude to complete several rounds of greenhouse testing to identify the most resistant parents for our 2016 *PdR1*x*PdR2* crosses. Fruit evaluations (1c) include new mapping crosses and stacked crosses but doesn't include spring evaluations for horticultural traits, flower sex or productivity. As we continue to advance the backcross level of various lines, especially in the absence of resistance markers for sources other than *PdR1* and *PdR2*, our greenhouse screening has steadily increased as we identify promising parents especially in lines without markers. In addition to scion genotypes, Table 1d includes rootstock breeding, mapping and germplasm testing but not any spacing or *Xf* strain trials, or the testing of biocontrol vine genotypes. As we identify particularly resistant individuals we test them multiple times (1e) to properly assess their level of resistance and insure that only the most resistant individuals are advanced. These tests are in addition to those listed in Table 1d. Five selections were sent to FPS for certification over this period shown in the Table 1f and one additional white *PdR1b* scion selection was sent in March of 2018.

Table 1. 2015-17 PD breeding activity summary.

	Calendar Year			**2015-2018**
Activity	**2015**	**2016**	**2017**	**Total**
1a. # Genotypes MAS Tested	2304	2058	2697	7059

1b. # Genotypes Planted to Field	1215	558	1279	3052
1c. # Genotypes Evaluated for Fruit	391	684	774	1849
1d. # Genotypes Tested in GH	1009	1148	935	3092
1e. # Genotypes Tested Multiple Times	124	229	517	870
1f. # Advanced Selections sent to FPS	2	3	-	5

To date over 322 wild accessions have been tested for PD resistance with the greenhouse screen, most of which were collected from the southwestern United States and Mexico. Our goal is to identify accessions with the most unique PD resistance mechanisms. To do so we evaluate the genetic diversity of these accessions and test them for genetic markers from chromosome (Ch) 14 (where *PdR1* resides) to ensure that we are choosing genetically diverse resistance sources for population development and greenhouse screening efforts. Fifteen of the most unique accessions were used to develop F1 populations with *V. vinifera* to investigate the inheritance of PD resistance in their F1 progeny and the degree to which they resist *Xf*. Most of the resistance lines we have explored from the southwestern US have PD resistance associated with Ch 14, the same region as our primary resistance line *PdR1b* (Riaz, 2016*)*. Our mapping project identified *PdR2* on Ch 8 from b42-26. *PdR2* resistance although significant, generally doesn't confer as strong a resistance as *PdR1*. Preliminary results indicate that most of the non-*PdR1* resistance sources appear to also have at least some of their resistance derived from Ch 8. Until we better understand the nature of Ch 8 PD resistance and explore additional resistance loci in these lines, it is important to continue advancing multiple sources of Ch 8 resistance. Table 2 summarizes the apparent resistance loci location, progeny tested and BC populations created from the various resistance sources. Our current focus is on exploring resistance sources without LG14 especially ANU67, b41-13 and T03-16. Crosses were made in 2018 to expand the A14 and b47-32 F1 populations to confirm our expectation that they have LG14 resistance. Although resistance in the b46-43 source is dominated by LG14, the BC1 is being explored for minor resistance genes and a BC2 has been developed should that prove necessary.

Table 2. Location of resistance loci, BC11 & BC2 plantings and progeny screening for 15 accessions evaluated for PD resistance breeding.

Resistance source	**Resistance loci**	**# F1 progeny screened**	**BC1 Progeny in Field**	**# BC1 Progeny screened**	**# BC2 Progeny in field**
A14	Inconclusive	28			
A28	LG14	42			
ANU5	LG14	60	45	3	
ANU67	Inconclusive	30	80		
ANU71	LG14	30			
b40-29	LG14	29	49	49	
b41-13	Inconclusive	301	286	42	
b43-57	LG14	51			
b46-43	LG14	60	464	454	76
b47-32	Inconclusive	89			
C23-94	LG14	44			
DVIT2236.2	LG14	30			
SAZ7	LG14	52			
SC36	LG14	35			
T03-16	non-LG14	173	76	16	

In Table 3 we show the greenhouse screen resistance distribution of the F1 progeny of our three new PD resistant sources. In contrast to our LG14 resistance sources, few genotypes are seen to manifest the highest levels of resistance. With *PdR1* lines we breed with genotypes in the 10 and occasionally 5 categories. For the b42-26 lines we have used genotypes in the 5 category as parents. Should further testing in the F1 populations fail to yield satisfactory parental material, we will approach the problem either by adding an intercross generation to regain resistance, cross to a wide range of *vinifera* parents looking for fortuitous combinations, or expand populations and look for transgressive segregants.

Table 3. Greenhouse screen results on 328 F1 genotypes from 3 new PD resistance sources. PD rating categories are based on both *Xf* titer by ELISA and degree of PD symptom expression: -1 =

Xf titer statistically higher than our U0505-01 88% *PdR1b* resistant biocontrol; 1 = R with *Xf* titer statistically the same as our U0505-01 biocontrol, 5 = Very R with *Xf* titer the same as the uninoculated Chardonnay control but having some phenotypic symptoms of PD; 10 = Immune - *Xf* titer below ELISA detection threshold and no PD symptoms.

	PD Rating category				PDR
PDR Source	**-1 = S**	**1 = R**	**5 = Very R**	**10 = Immune**	**Source Totals**
ANU67	21	9			30
b41-13	68	56	21	7	152
T03-16	58	79	9		146
Category Total	147	144	30	7	328

Early on we noticed the very limited number of highly resistant progeny in the T03-16 line. Thus, in 2016 and 2017 we made small trial populations comprised of 9 intercrosses and 3 selfs using 8 of the more resistant T03-16 F1 progeny as parents (Table 4). We have only completed greenhouse screens on 27 genotypes from 3 different crosses. Results are shown in Table 5. Admittedly the numbers tested are small but the fact that the self of 13336-018 didn't increase resistance in the progeny while the cross of 13336-046 x 13336-018 did appears promising and warrants a more complete testing of these and the rest of the cross combinations. Should further greenhouse testing validate these results and reveal other crosses that have progeny in the 5 and 10 categories, larger mapping populations can be created to identify resistance loci for future MAS.

Table 4. Small test populations of the T03-16 resistance source made by intercrosses and selfs: decimal numbers are mean parental PD R-rating, whole numbers are number of genotypes in the field for that cross combination made in 2016 and 2017. Highlight colors correspond to the same cross in Table 5.

Female x Male	**Female Ave R-rating**	**13336-018**		**13336-025**		**13336-034**		**13336-068**	
M Ave R-rating		1.0		1.0		2.3		3.0	
13302-10	3.0					2.7	30	3.0	8
13302-19	2.0					2.3	50		
13336-018	1.0	1.0	30						
13336-034	2.3					2.3	35		
13336-046	1.7	1.3	19	1.3	11	2.0	30	1.7	12
13336-068	3.0							2.3	12
13336-108	5.3					5.3	50	3.8	12

Table 5. Greenhouse PD R-rating for 27 genotypes tested from 2 intercross and one selfed populations in the T03-16 resistance background. Highlight colors correspond to the same cross in Table 4.

Female	Male	Parental mean R-rating	PD R-rating category				Count of Genotypes tested
			-1 = S	1 = R	5 = Very R	10 = Immune	
13336-046	13336-018	1.3	8	3	1	1	13
13336-046	13336-025	1.3	2	4			6
13336-018	13336-018	1.0	5	3			8
PD R-rating category total			15	10	1	1	27

In Table 6 we show the details of crosses made in 2018 to expand mapping populations of the three new SWUS resistance sources we are working with, the number of crosses made and estimated numbers of seeds produced.

Table 6. 2018 Crosses made to expand new PD mapping populations that previously had too few members to accurately determine the genetics of resistance. Estimates are in italics.

Cross PDR Source	% *vinifera*	*vinifera* Parents	# Crosses	Est. # Seeds
A14	50%	Colombard	2	*365*
ANU67	50%	F2-35	1	*80*
b47-32	50%	08326-61, F2-35	3	*465*
T03-16	50%	Palomino	1	*30*

Another area of focus and one that should produce our next PD resistant wine grape selections for release are those that stack *PdR1b* resistance from b43-17 and *PdR2* resistance from b42-26. In 2017 we planted 126 seedlings from 4 different crosses that are 96% *vinifera* and have both resistance loci. Table 7 shows the distribution of greenhouse resistance ratings for each cross. Although promising in that we see some genotypes with R-ratings above their parental means, we don't see genotypes scoring in the most resistant 10 category. However scores of 5 are adequate for release as they have ELISA titer values statistically the same as uninoculated Chardonnay. Genotypes in this category do have more phenotypic PD symptoms in our greenhouse screen than we like to see. That said, the greenhouse screen is much more severe than what the plants experience in the field and plants scoring 5 should perform well in the field.

Table 7. Greenhouse screen results from the first screening of 77 genotypes at the 96% *vinifera* level with both *PdR1b* and *PdR2*.

Female Parent	Male Parent	Parental mean R-rating	PD R-rating category				Count of Genotypes tested
			-1 = S	1 = R	5 = Very R	10 = Immune	
14309-111	Primitivo	2.2	9	12	1		22
14309-111	Cabernet Sauvignon	2.2	1	11	3		15
14388-029	Chardonnay	3.6	1	13	2		16
F2-35	14309-016	3.3	3	19	2		24
R-rating totals			14	55	7	0	77

In 2017 we expanded the diversity of elite *vinifera* parents used in the 96% *vinifera PdR1*x*PdR2* breeding line. These will give us varieties with a wide range of fruit and horticultural characteristics to present to the industry. A total of 328 MAS tested seedlings were planted from 1095 seedlings tested. This may appear low relative to previous MAS efficiencies but is the result of screening for two dominant resistance loci rather than our more typical single locus. The expected seedlings retained should be about 25%. Overall for this group we averaged approximately 30% retained with a range among the crosses from 5% to 43%. Clearly some crosses experienced significant segregation distortion, both positive and negative.

Table 8. 2017 Crosses of elite *vinifera* cultivars to three PD resistant genotypes that have both the *PdR1b* and *PdR2* loci. Progeny are 96% *vinifera*: Seeds planted, seedlings saved, MAS tested and planted to field.

Resistant Parent	***vinifera* Parent**	**Seeds planted**	**Seedlings saved**	**Seedlings MAS Tested**	**Seedlings planted**
14309-002	Alvarelhao	119	56	50	16
	Dolcetto	201	56	50	11
	Mataro	111	32	30	10
	Montepulciano	169	80	75	10
	Pinot noir FPS32	156	56	50	13
	Pinot noir FPS77	199	56	50	9
	Refosco	150	56	50	12
	Touriga Nacional	431	80	75	26
14309-111	Dolcetto	200	80	75	32
	Mataro	337	128	125	49
	Morrastel	80	56	50	13
	Refosco	223	128	120	48
14388-029	Arneis	173	56	50	9
	Morrastel	271	80	75	25
	Pedro Ximenez	316	56	50	16
	Pinot noir FPS32	75	32	25	2
	Refosco	48	24	20	1
	Sauvignon vert	296	80	75	26

A focus of our PD breeding efforts in 2018 was to stack PD resistance, either from *PdR1b* alone or in combination with b42-26 resistance, with one or more powdery mildew (PM) resistance sources in elite *vinifera* backgrounds. We have genetic markers for PM resistance derived from *V. vinifera* (*Ren1*), *V. romanetii* (*Ren4*), *V. piasezkii* (*Ren6, Ren7*), and two forms from *Muscadinia rotundifolia (Run1 and Run2.1)*. As usual we use MAS to advance only those progeny with resistance markers, the greenhouse screen to select only the most PD resistant and field and in vitro testing for PM resistance. Crosses in the 91-93% *vinifera* range were made with the goal of creating highly resistant breeding lines stacked with multiple resistances to cross one last time to a final elite *vinifera* cultivar resulting in progeny between 96-98% *vinifera*. Those in the 95-97% *vinifera* range would be candidates for release. With the exception of 9d where crosses were made directly to elite *vinifera* cultivars, the challenge of the other crosses in Table 9 are both practical, as required for rapid advance of stacking and for inheritance of typical *vinifera* characteristics, and perceptual in terms of easier market acceptance, since they, unlike those in Table 9d, don't have a most recent elite *vinifera* parent to differentiate them. These factors will require a longer period of horticultural and enological evaluation than has been our experience to date with the crosses bred for PD resistance alone where the most recent parent has always been a *vinifera* cultivar.

Table 9. Estimated number of seeds produced from PD x PM crosses made in 2018. *PdR1b* (F8909-08) is from Monterrey *V. arizonica/candicans* PD resistance b43-17; b42-26 is Baja *V. arizonica/girdiana* PD resistance source. *Ren1* and *Ren4* are PM resistance loci from *vinifera* and *V. romanetii* respectively. *Run1* and *Run2.1* are PMR loci derived from *Muscadinia rotundifolia.*

		Percent *vinifera*				
Resistances	**Recent *vinifera* parents in background**	**91%**	**93%**	**95%**	**97%**	**Total**
9a. PD - *PdR1b.* PM - *Run1*	Cabernet Sauvignon, Nero d'Avola, Zinfandel, 4 UCD *PdR1b* releases				445	445
9b. PD - *PdR1b.* PM - *Ren1 & Run2.1*	Airen, Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases			550		550
9c. PD - *PdR1b.* PM - *Ren1, Ren4 & Run1*	Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases			325		325

9d. PD - *PdR1b* with b42-26. PM - *Ren4*	Alvarelhao, Bonarda, Carmenere, Cortese, Fiano, Gouveio, Melon, Pinot blanc, Teroldego, Tinta Amarella, Tinta Cao, 3 UCD *PdR1b* releases		575	1241		1816
9e. PD - *PdR1b* with b42-26. PM - *Run1* with either *Ren1* or *Ren4*	Cabernet Sauvignon, Grenache, Touriga Nacional, Zinfandel, 1 UCD *PdR1b* release	100	295			395
9f. PD - *PdR1b* with b42-26. PM - Ren1, Ren4 & Run1	Cabernet Sauvignon, F2-35, Grenache, Zinfandel		256			256

Our rapid greenhouse screen is critical to our evaluation of PD resistance in wild accessions, new F1 and BC1 mapping populations and for selection of advanced late generation backcrosses for release. Table 10 provides a list of the PD greenhouse screens analyzed, initiated and/or completed over the reporting period.

Table 10. Greenhouse PD screens analyzed, completed and/or initiated during 2017-18. Projected in italics.

Group	Test Groups	No. of Genotypes	Inoculation Date	ELISA Sample Date	PD Resistance Source(s)
10a	2015 PD & PD-PM Crosses	155	1/5/2017	3/23/2017	*PdR1b*, b42-26
10b	b42-26 BC1 & BC2 locus refinement, 2014 Cross highly rated; b46-43, BD5-117	262	3/14/2017	6/15/2017	b42-26, b46-43, BD5-117
10c	Additional PDxPM hybrids & *V. berlandieri*	113	3/30/2017	6/29//2017	*PdR1b*, b42-26, *berlandieri*
10d	b47-32 & low severity screen retests	170	5/25/2017	8/29/2017	*PdR1b*, b42-26, b47-32
10e	14-399 b46-43 BC1 Mapping	262	8/1/2017	10/31/2017	b46-43
10f	T03-16 & b41-13 F1, *PdR1b*xb42-26 Stack, homozygous PD Stack test 1	92	8/17/2017	11/16/2017	T03-16, b41-13, *PdR1*x*PdR2*
10g	2017 Parents, *rotundifolia*, b41-13 F1s	159	10/12/2017	1/12/2018	*PdR1b*, *PdR2*, *M. rot*, b41-13
10h	17 Parents, 96% vin PD Stack, 2015-16 PDxPM crosses, T03-16 F1 Int and BC1, b41-13 BC1	256	12/19/2017	3/16/2018	*PdR1b*, *PdR2*, T03-16, b41-13
10i	2016 PD crosses, SWUS BC1s, homozygous PD Stack test 2	113	2/8/2018	5/10/2018	*PdR1b*, *PdR2*, b42-26
10j	*PdR1b*x*PdR2*^2, b41-13 F1s	171	3/22/2018	6/19/2018	*PdR1b*, *PdR2*, b46-43, b41-13
10k	*Xf* strain trial (3 strains, 7 BC genotypes, 3 time points)	7	5/24/2018	*7/19/2018, 8/2/2018, 8/23/2018*	b43-17, SEUS, *PdR1b*
10l	SWUS PD species, b41-13, 2017 parents	133	5/24/2018	*8/23/2018*	Species, b41-13, *PdR1b*

10m	Mapping Pops, 2015 PDxPM untested	115	6/23/2018	*9/22/2018*	T03-16, b41-13, *PdR1b*
10n	92 & 96% PD stack, retest of recent promising	170	*9/6/2018*	*12/6/2018*	*PdR1xPdR2*

The 10a group was our most extensive PD x PM screen up to that point where we evaluated 98 genotypes from 8 different crosses. PD resistances included *PdR1b* either alone or with b42-26 resistance and the *Ren1*, *Ren4* and *Run1* PM resistance loci. Previously, from testing of smaller PD x PM groups, we have reported some negative effect on PD resistance when PD and PM resistance loci were combined. In this trial, the percent highly resistant progeny ranged from 9% to 75%. Sample sizes were too small to make a definitive conclusion, but it appeared the selection of the PD resistant parent played a more important role to the resistance of a cross progeny than whether the cross was to a PM resistant parent. Another group tested in 10a were 50 genotypes in an alternative *PdR1b* x b42-26 line at the 93% *vinifera* level. Fifty percent were promising and one was used as a parent in 2017 crosses.

Group 10b crosses were made to refine resistance in the b42-26 line primarily associated with Ch8. We also retested eight genotypes in the b46-43 line that had anomalous greenhouse screen results relative to their Ch14 markers; these results were provided to our companion mapping/genetics program. Promising parents for breeding in novel PDR lines, including b40-14, b46-43, and ANU5, were retested, as were remnants of our BD5-117 lines (another multigenic resistance source from a Univ. Florida breeding program). One female genotype in the BD5-117 line has tested highly resistant in three screens, offering the possibility of creating outcrosses to our other lines or crossing to one of the few other BD5-117 line highly resistant genotypes. This latter strategy, however, doesn't allow us to increase the percentage of *vinifera*.

In addition to testing additional PD x PM crosses in Group 10c, we tested 20 accessions of *V. berlandieri* for the first time to evaluate PD resistance in this grape species from central Texas. High ELISA results and severe PD symptoms suggest that these aren't promising candidates for creating additional PD resistant lines. Screening in Group 10d focused on the b47-32 *V. arizonica-monticola* line to identify if resistance is unique or segregates with either Ch8 or Ch14 markers. Thirty-seven genotypes were tested, and the results were provided to our companion PD mapping project. Only one individual has the potential to create a new PD resistance line for our breeding efforts. In addition, we tested 75 genotypes in the 92% *vinifera PdR1* x *PdR2* line to confirm previous tests and identify potential parents. A third were promising, showing the benefit of stacking and careful parent selection. Four promising parents were identified from the 24 PD x PM genotypes tested.

Testing in Group 10e supports graduate student research in our companion mapping/genetics program looking for non-Ch14 PD resistance loci in b46-43, which may have additional resistance loci as we observe a range of bacterial titers in genotypes without the LG14 resistance markers. ELISA results were recently completed and analysis has begun.

In Group 10f, we tested additional F1 progeny of the new T03-16 and b41-13 PD lines to facilitate genetic mapping of their PD resistance. Results were provided to our companion mapping project. From a breeding perspective, none of the T03-16 line genotypes and only one of the 29 b41-13 line genotypes was promising for advancing breeding lines. See Table 3 for the compete overview. We also tested 33 genotypes that should complete the extensive testing of the 92% *vinifera PdR1b* x *PdR2* stack group and allow further evaluation of the resistance derived from combining Ch14 and Ch8 loci as well as minor resistance factors. In marked contrast to the two previous lines tested in this group, more than half of the genotypes from the stacking proved promising. Finally, this group included the first testing of 10 genotypes that are homozygous at both *PdR1* and *PdR2* to identify promising breeding parents which when backcrossed to elite *vinifera* would result in all progeny having both PD resistance loci. One of the ten is very promising with very low bacteria titers and no symptoms after screening. Unfortunately it didn't flower this spring so we couldn't make crosses with it.

We continue to explore PD resistance from *Muscadinia rotundifolia* with the testing of 54 genotypes in Group 10g. In the same group we tested 75 F1 genotypes to improve the map of the b41-13 resistance source, as well as a confirmatory test of the 2017 parents. ELISA showed this greenhouse screen was only moderately severe due to high temperatures in the first two weeks of the trial caused by a fan malfunction. Although little separation was

seen in the *rotundifolia*, the screen was adequately severe to separate the F1 progeny. Results were provided to our companion PD mapping project.

The first 79 genotypes from the 96% *vinifera PdR1* x *PdR2* stack line were tested in Group 10h, and represent the first multiple gene more broadly PD resistant candidates for release (Table 7). This group also included 50 PD x PM resistant genotypes from 2015 and 2016 crosses, which have *PdR1b* and various combinations of three powdery mildew resistance genes (*Ren1*, *Ren4* or *Run1*). Being tested for the first time were 28 intercross selections at the 50% *vinifera* level in the T03-16 line to check for possible complementary loci (Tables 4 & 5). Results of testing 63 BC1 selections in the b41-13 and T03-16 lines also in Group 10h are shown in Table 11. We also have another 29 BC1 genotypes crossed to a third b41-13 resistant F1 genotype and following greenhouse screening of those we'll consider further testing or whether to wait for marker results before pursuing this line any further into BC generations.

In Group 10i we retested for the second or third time promising selections that have scored well in previous greenhouse tests to confirm marker efficacy and PD resistance. We also retested the 10 double homozygeous potential breeding parents noted in Group 10f above. Since only 1 of the 10 looked promising, in Group 10j we are testing 32 selections that carry *PdR1b* and are homozygous carriers of *PdR2* to identify alternate potential parents that will, when backcrossed to elite *vinifera*, result in half the progeny having both *PdR1* and *PdR2*. In this same screen we test 32 BC1 selections in the b46-43 line looking at a different resistant parent to see if inheritance of the resistant phenotype is similar to the 14-399 line that was tested in Group 10e. To facilitate marker discovery in our companion mapping project, an additional 74 F1 genotypes in the b41-13 line are also being tested. ELISA results are in process.

Table 10k is a 3 x 3 factor matrix testing genotype, *Xf* isolate, and sample date. The genotypes being tested are our standard 7 SEUS and *PdR1b* biocontrols. The *Xf* isolates came from the SEUS cultivar Blanc du Bois, our intermediate *PdR1b* biocontrol U0505-35 and our usual culture source, Chardonnay as control. These will be sampled at 8, 10 and 13 weeks to see how *Xf* titer and phenotype scores compare across genotype, strain and sample date. The goals are twofold: to see if pathogenicity increases when the culture comes from a resistant plant and to see if our screen can be shortened to allow us to conduct more screens in a set period of time. In Group 10l we test 81 untested PD species accessions to better characterize our collection and elucidate PD resistance performance by geographical provenance and species. Twenty-six F1 genotypes in the b41-13 mapping populations are being tested for marker discovery, a retest of 5 promising PD x PM accessions from crosses made in 2015 and the second testing of 2017 PD parents. Group 10m continues testing F1 mapping populations with 50 and 27 genotypes respectively in the b41-13 and T03-16 populations. Also in testing are 11 untested genotypes from 2015 PD x PM crosses and retests on 20 genotypes identified as highly promising in recent greenhouse screens. Thirty 96% *PdR1b* x *PdR2* hermaphrodite genotypes are being tested for resistance in 10n. Should these have sufficient resistance and have adequate fruit and wine quality they would be candidates for release. An additional 55 genotypes homozygeous at either *PdR1* or *PdR2* and having the other resistance source are being tested to see if there is a pattern to high levels of resistance inheritance. Second or third screens are being conducted on 54 genotypes with PD or PD x PM to validate previous results. Confirming screens are being conducted on five 2018 genotypes used as parents that didn't already have three completed screens.

Table 11. Count of Genotype by cross type and resistance category for two BC1 genotypes in the b41-13 and T03-16 lines.

PDR type	Cross Type	Percent *vinifera*	PD Rating category				Type Totals
			-1 = S	1 = R	5 = Very R	10 = Immune	
b41-13	BC1	75%	17	22	3		42
T03-16	BC1	75%	11	10			21

CONCLUSIONS

We continue to make rapid progress breeding PD resistant winegrapes through aggressive vine training, marker-assisted selection, and our rapid greenhouse screen procedures. These practices have allowed us to produce four backcross generations with elite *V. vinifera* winegrape cultivars in 10 years. We have screened through thousands of seedlings that are 97% *V. vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to crop and others are advanced to greenhouse testing. We select for fruit and vine quality and then move the best 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 in PD hot spots around California. The best of these are being planted in vineyards at 50 to 1,000 vine trials with enough fruit for commercial scale winemaking. We have sent 20 advanced scion selections to FPS over the past five winters to begin the certification and release process. Three Pierce's disease resistant rootstocks were also sent to FPS for certification. Pierce's disease resistance from *V. shuttleworthii* and BD5-117 is also being pursued, but progress and effort is limited because their resistance is controlled by multiple genes without effective resistance markers. Other forms of *V. arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Very small-scale wines from 94% and 97% *V. vinifera PdR1b* selections have been very good, and have been received well at tastings in the campus winery, at public tastings throughout California, Texas, Georgia and Virginia.

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FUNDING AGENCY

Funding for this project was provided by the CDFA PD/GWSS Board. Additional support from the Louise Rossi Endowed Chair in Viticulture is also gratefully acknowledged.

ACKNOWLEGEMENTS

We thank Gordon Burns of ETS Labs in St. Helena, CA for continued support with grape berry chemical analysis and Ken Freeze of Brown and Miller for help arranging and coordinating the industry tastings. We also gratefully acknowledge funding from the Louise Rossi Endowed Chair in Viticulture, which helps fund our powdery mildew resistance breeding and collection trips across the southwestern US.