California Department of Food and Agriculture PD/GWSS
Interim Progress Report – March 2018

REPORT TITLE: Interim 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 (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 Pierce's disease resistance locus, *PdR1* 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 be evaluated. 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*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and other test sites. The best of these will be advanced to field testing with commercial-scale wine production, the first of which was planted in Napa in June 2013. To date 19 scion and 3 PD resistant rootstocks have been advanced to Foundation Plant Services for certification. Stacking of *PdR1b* with b42-26 PD 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*. Greenhouse screening is still used to select for advancement only genotypes with higher than usual 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 long-lived 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 in Texas, Virginia and Georgia.

The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xylella fastidiosa* 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 *Vitis rupestris x V. arizonica* selections, as well as an extensive collection of southwestern grape species, which allows the introduction of extremely high levels of *X. fastidiosa* resistance into commercial grapes. We genetically mapped and identified what seems to be a single dominant gene for *X. fastidiosa* 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 seven 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. There are two forms of *PdR1* that descend from sibling progeny of b43-17 and they have different alleles of *PdR1*, designated *PdR1a* and *PdR1b*. Screening results reported previously showed no significant difference in resistance levels in genotypes with either one or

both alleles. We have narrowed our focus to *PdR1b*, 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 *PdR1c* 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 *PdR1c* 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 last year our companion project identified the location of a significant PD resistance locus from b42-26 on LG8, which we have called *PdR2*. Three years ago, 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 *X. fastidiosa* resistance and address *X. fastidiosa'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.

DESCRIPTION OF ACTIVITIES

Objective 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.

See Objective 2.

Objective 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.

To date over 293 wild accessions have been tested for PD resistance with the greenhouse screen, most of which were collected from the southwestern United States and Mexico (SWUS). 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 14 (where *PdR1* resides) to ensure that we are choosing genetically diverse resistance sources for population development and greenhouse screening efforts. Over the last five years, 15 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 *X. fastidiosa*. We have

reported previously the surprising result from our companion PD mapping project that most of the resistance lines we have explored from the southwestern US have PD resistance associated with chromosome 14, the same region as our primary resistance line *PdR1b*. From that same project we identified *PdR2* on chromosome 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 the resistance locus on chromosome (Ch) 8. Until we better understand the nature of Ch8 PD resistance and explore additional resistance loci in these lines, it is important to continue advancing multiple sources of Ch8 resistance.

We are part way through our yearly seed germination season. About half the seeds have been planted and seedlings are emerging. Table 1 gives details of crosses made in 2017 to finish or expand our mapping populations. Group 1a crosses expand the ANU67 and most of the T03-16 mapping populations. The b41-13 population was completed with crosses made in 2016. In Group 1b we expand the number of T03-16 progeny used in full sib F1 intercrosses in an attempt to recover the strong resistance of the parent. In Group 1c we broadened the elite *vinifera* parents used to advance the ANU67 and T03-16 lines to the BC1 level and used a different promising F1 selection from the b41-13 line to complement the BC1 cross made in that line last year. Ultimately they could be combined either individually or in combination with the b42-26 *PdR2* line to enhance and broaden PD resistance in our main *PdR1b* resistance crosses.

Table 1. 2017 Crosses made to expand the new F1 Pierce's disease mapping populations and advance breeding lines to the next backcross level: *vinifera* parents, # crosses, actual # seeds produced, estimated seedlings produced and MAS tested (estimates in italics).

Group	Cross PDR Source	% *vinifera*	*vinifera* Parents	# Crosses	Act. # Seeds	Est # Seedlings Produced	Est # Seedlings Tested True to Type or MAS
1a	ANU67	50%	F2-35	1	68	*24*	*24*
	T03-16	50%	Palomino	1	73	*55*	*55*
1b	T03-16	50%	Palomino	10	717	*250*	*185*
1c	ANU67	75%	Montepulciano, Palomino, and Sauvignon vert	3	123	*43*	*30*
	b41-13	75%	F2-35	1	1061	*69*	*50*
	T03-16	75%	F2-35, LCC	2	184	*65*	*60*

Our rapid greenhouse screen is critical to our evaluation of PD resistance in wild accessions, new F1and BC1 mapping populations, and for selection of advanced late generation backcrosses for release. Table 2 provides a list of the PD greenhouse screens analyzed, initiated, and/or completed over the reporting period. The main focus in Group 2A was to refine resistance in the b42-26 line primarily associated with Ch8. Similarly, in this same group, we retested eight genotypes in the b46-43 line that had anomalous greenhouse screen results relative to their Ch14 markers, and provided the results to our companion PD mapping project. 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 Florida breeding program). One female genotype in the BD5-117 line has tested highly resistant in all 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 powdery mildew crosses in Group 2B, we tested 20 accessions of *V. berlandieri* for the first time to evaluate PD resistance in this Texas grape species. High enzyme-linked immunosorbent assay (ELISA) results and severe PD symptoms suggest that these aren't promising candidates for creating additional PD resistant lines. Screening in Group 2C 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 best were provided to our companion PD mapping project. Only one individual has the potential to create a new PD resistance line. 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 powdery mildew genotypes tested.

Testing in Group 2D 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 are pending.

In Group 2E, 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. 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. If it flowers this spring we will make crosses with it.

We continue to explore PD resistance from *Muscadinia rotundifolia* with the testing of 54 genotypes in Group 2F. 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. Phenotypic scores were taken and ELISA results are pending.

The first 79 genotypes from the 96% *vinifera PdR1* x *PdR2* stack line were tested in Group 2G, and represent the first multiple gene more broadly PD resistant candidates for release. Twenty-two genotypes have Primitivo as their most recent *vinifera* parent, 17 have Cabernet Sauvignon, 16 have Chardonnay and 24 have F2-35 (Cabernet Sauvignon x Carignan). As of this writing at 8 weeks post inoculation, PD symptoms are already showing in control plants and some of the new selections with stacked resistance are completely symptomless. Sampling for ELISA is scheduled for late March 2018 and evaluation of symptoms (phenotyping) a week earlier. This group also includes 50 PD x powdery mildew 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 are 28 intercross selections at the 50% *vinifera* level in the T03-16 line to check for possible complementary loci and 20 and 44 BC1 selections in T03-16 and the b41-13 lines to advance them for possible future stacking. In Group 2H we retest for the second or third time promising selections that have scored well in previous greenhouse tests to confirm marker efficacy and PD resistance. We are also retesting the 10 double homozygeous potential breeding parents noted in Group 2E above. Since only 1 of the 10 looked promising, in Group 2I we test 32 selections that carry *PdR1b* and are homozygous carriers of *PdR2* to identify alternate potential parents that will, when backcrossed to elite *vinifera*, results 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 2D. To facilitate marker discovery in our companion project, an additional 74 F1 genotypes in the b41-13 line are also being tested.

Table 2. Greenhouse PD screens analyzed, completed and/or initiated during the reporting period. Projected dates in italics.

Group	Test Groups	No. of Genotypes	Inoculation Date	ELISA Sample Date	PD Resistance Source(s)
2A	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
2B	Addn PDxPM hybrids & *V. berlandieri*	113	3/30/2017	6/29//2017	*PdR1b*, b42-26, *berlandieri*
2C	b47-32 & low severity screen retests	170	5/25/2017	8/29/2017	*PdR1b*, b42-26, b47-32
2D	14-399 b46-43 BC1 Mapping	262	8/1/2017	10/31/2017	b46-43
2E	T03-16 & b41-13 F1,	92	8/17/2017	11/16/2017	T03-16, b41-13,

	*PdR1b*xb42-26 Stack, homozygous PD Stack test 1				*PdR1*x*PdR2*
2F	2017 Parents, *rotundifolia*, b41-13 F1s	159	10/12/2017	1/12/2018	*PdR1b*, *PdR2*, *M. rot*, b41-13
2G	17 Parents, 96% vin PD Stack, 2015-16 PDxPM crosses, T03-16 F1 Int and BC1, b41-13 BC1	256	12/19/2017	*3/20/2018*	*PdR1b*, *PdR2*, T 03-16, b41-13
2H	2016 PD crosses SWUS BC1s, homozygous PD Stack test 2	113	2/8/2018	*5/10/2018*	*PdR1b*, *PdR2*, b42-26
2I	*PdR1b*x*PdR2*^2, b41-13 F1s	171	*3/17/2018*	*6/16/2018*	*PdR1b*, *PdR2*, b46-43, b41-13

Objective 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.

We continue to present our PD resistant wines at the 94% and 97% *V. vinifera* levels to grower and vintner groups. Some of these tastings are at UC Davis with industry and student tasters, and others are at various industry gatherings including the American Society of Enology and Viticulture East Section meetings in Charlottesville, Virginia, and at the January 2018 meeting of the Georgia Wine Producers in Braselton, Georgia. The wines were very well received and generated a lot of discussion and excitement. We have three trials with 88% and 94% *vinifera* selections in Texas (in cooperation with Jim Kamas of Texas A&M) and they presented small-scale wines from their trials. The three 88% *vinifera* selections planted in Alabama have been expanded to 1,000 vines each. This plot is in cooperation with Randall Wilson of White Oak Cellars. The vines are thriving and commercial scale wines are being made. This January, six wines were tasted with the Daniel Robert's grower group. In November we held the first tasting of our 2017 vintage wines. Results of the tasting are presented in Table 3. One of the PD whites was rated equivalent to Chardonnay and another nearly so. That wine, the Emerging White Blend, is comprised predominantly of 10302-293 (see Figure 1) and 10302-309. Both are full sibs that share Riesling as their most recent *vinifera* parent. The former went to FPS in 2016 and the latter is being sent this spring. Six of the 7 *PdR1* based red wines scored higher than Cabernet Sauvignon, the red *vinifera* control, and all were rated higher than the traditional PD resistant variety, Lenoir. This was the second year we made wine from 03182-084, a 75% *vinifera* selection with resistance based on the SEUS resistance from BD5-117. It performed poorly last year scoring at the bottom of the reds in the preliminary tasting this year. The selection will be abandoned if its 2017 vintage wine doesn't come around before bottling in a few weeks.

Table 3. Results of a preliminary tasting of 2017 vintage wines tasted 11/03/17 at UC Davis by 5 tasters comprised of the staff winemakers and the authors. Wines were produced from grapes grown in Davis. Wines were rated on a hedonic quality scale from 1 = poor to 5 = v. good. Selections in yellow are nearing release.

Wine Name	% *vinifera*	Color	Average Score	Max Score	Min Score	10/31/17 Consensus Descriptors: color; aroma; flavor-texture
09314-102	97%	W	3.4	4	3	pale, sl cloudy, citrus, floral; melon, fruity, balanced, sl thin.
09338-016	97%	W	3.9	5	3	pale yellow, clear; tropical, rich, sl green pepper; light, creamy, astringent finish.
10302-178	97%	W	3.1	4	1	yellow, sl brown?, clear; pear, melon, volatile, vanilla; floral, rich, sl phenolic.
Blanc du Bois	66%	W	1.7	3	1	gold-brown, cloudy; perfume, rubber; oxidized, medicinal.
Chardonnay	100%	W	4.0	5	3	pale, sl cloudy; apple, citrus, lemon; balanced, short.
Emerging White Blend	97%	W	4.0	5	3	pale, brilliant; intense rose floral more than muscat; tropical, balanced, crisp, sl coating.
03182-084	75%	R	2.0	4	1	med red, sl cloudy; weedy, fruity, dough, tea warm; flat, sl mousy and chemical.
07355-075	94%	R	3.5	4	3	med red, sl cloudy; fruity, jam, candied; licorice, balanced, sl cloying.

09311-160	97%	R	3.2	4	2	med- red; cherry, strawberry, candied; light, sweet mid-palate, sl astringent.
09330-07	97%	R	3.8	5	3	dark-, red-purple; clean, grapey, black fruit; sweet (but not), rich and balanced.
09331-047	97%	R	3.2	5	2	dark red-purple; dark fruit, licorice; black fruit, astringent, hot.
09331-133	97%	R	3.9	4.5	3	dark-, red sl purple, clear; cherry, jam; sl cough syrup, cloying.
09333-370	97%	R	2.9	4	2	dark- red purple, cloudy; grapey, chocolate, rich; fruity, cloying, tch mousy.
09356-235	97%	R	3.2	5	2	inky, red, sl muddy; dark fruit, blackberry, jammy; earthy, tannic, warm.
Cabernet Sauvignon	100%	R	3.0	4	2	light+, cloudy; strong veg, candied; cloying, mousy.
Lenoir	50%	R	2.5	4	1	inky, red-brown, muddy; chemical, medicinal; tea, tobacco, oxidized.

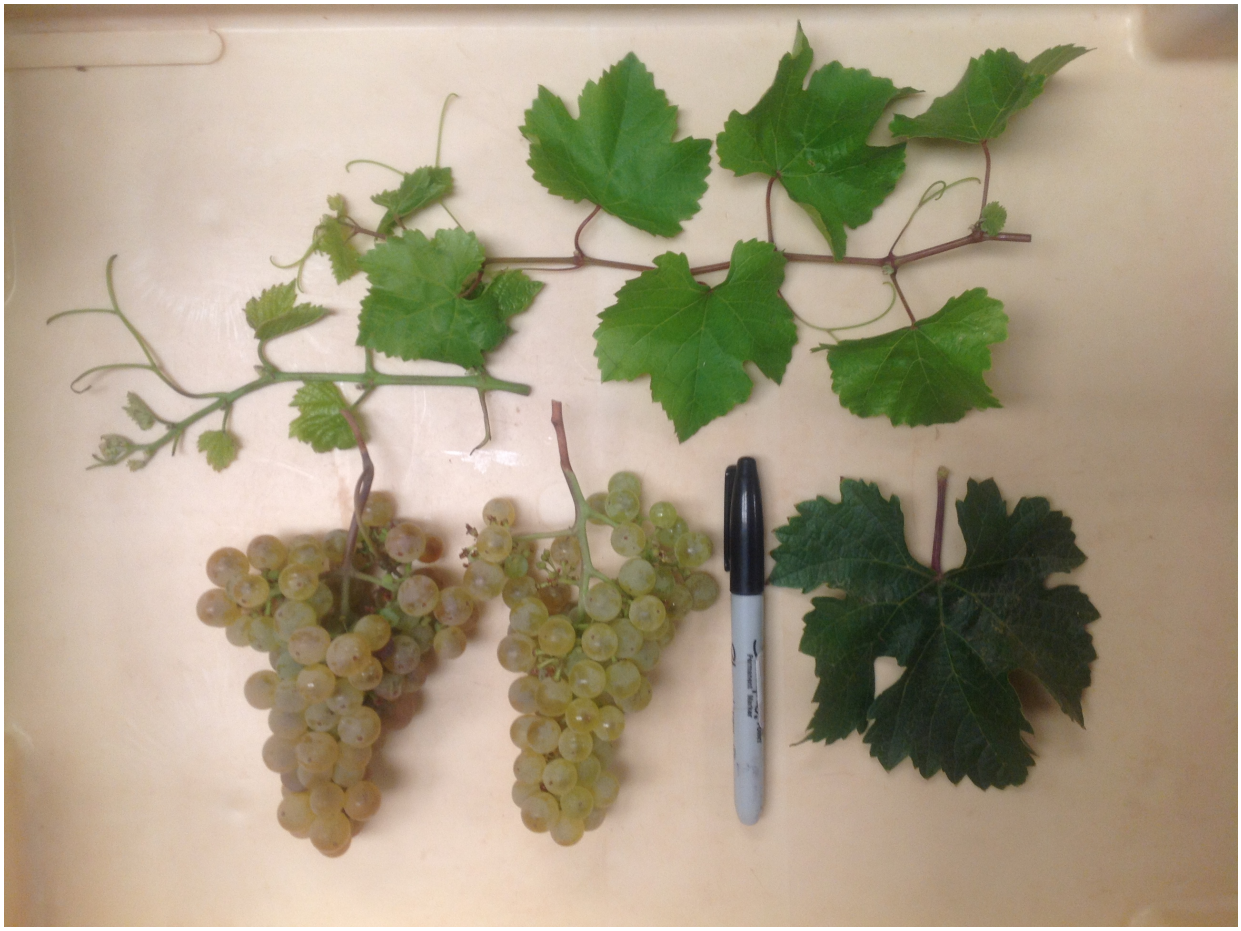

Figure 1. 97% *vinifera* PD resistant selection 10302-293 (07370-028 x Riesling) that was sent to FPS in 2016.

Objective 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.

Crosses made in 2017 (Table 4) reflect our primary focus of 96% *vinifera* backcrosses to a diverse selection of elite *vinifera* wine varieties to three of our most resistant parents carrying both *PdR1b* and *PdR2*. This will expand and broaden the range of vinifera wine styles we breeding for. The most promising selections will be advanced to Foundation Plant Services (FPS) for certification and eventual release as the next iteration of our PD resistant winegrape breeding efforts.

Table 4. 2017 Crosses of elite *vinifera* cultivars to three PD resistant genotypes that have both the *PdR1b* and *PdR2* loci. Progeny are 96% *vinifera*. Estimates in italics.

Resistant Parent	*vinifera* Parent	# Seeds planted	# Seedlings saved	# Seedlings MAS Tested
14309-002	Alvarelhao	119	56	50
	Dolcetto	201	56	50
	Mataro	111	32	30
	Montepulciano	169	80	75
	Pinot noir FPS32	156	56	50
	Pinot noir FPS77	199	56	50
	Refosco	150	56	50
	Touriga Nacional	431	80	75
14309-111	Dolcetto	200	80	*75*
	Fiano	75	40	*35*
	Mataro	337	128	*125*
	Montepulciano	11	0	0
	Morrastel	80	56	50
	Pinot noir FPS32	34	0	0
	Refosco	223	128	120
14388-029	Arneis	173	56	*50*
	Morrastel	271	80	*75*
	Pedro Ximenez	316	*60*	*50*
	Pinot noir FPS32	75	32	*25*
	Refosco	48	24	*20*
	Sauvignon vert	296	*90*	*75*

We also completed the final BC4 generation in the *PdR1c*, b40-14 line (Table 5, Cross 5a). The Cross 5b progeny were discarded because the parent did not contain *PdR2* and was not adequately PD resistant in subsequent greenhouse screens. The resistance from Cross 5c involves two full sib *PdR1b* x b42-26 resistant parents that had been backcrossed a second time to a different relatively resistant b42-26 progeny. This was done with the expectation that carrying more b42-26 minor resistance loci through subsequent backcross generations may contribute a genetically wider base of PD resistance. After the discovery of *PdR2* and subsequent marker testing, one resistant parent carries *PdR2* and the other, enriched in other resistance factors doesn't. Since both are highly resistant in our GH screen, we are advancing both lines with the expectation that eventually another PD resistant locus in the b42-26 line will be identified. The remaining crosses in Table 5 (Crosses 5d-5h) combine PD resistance, either from *PdR1b* alone or in combination with b42-26 resistance with various sources of powdery mildew resistance. We have genetic markers for powdery mildew resistance derived from *V. vinifera* (*Ren1*), *V. romanetii* (*Ren4*), *V. piasezkii* (*Ren6, Ren7*), and two forms from *Muscadinia rotundifolia* (*Run1* and *Run2.1*). Some of our most advanced lines in crosses represented here should be candidates for release. In Cross 5d we have advanced single *PdR1b* PD resistance with *Ren1* and/or *Ren4* powdery mildew resistance. Crossing to these diverse elite *vinifera* should result in a wide range of possible selections. The challenges for the rest of the Table 5 PD x powdery mildew (PD x PM) crosses 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, and they, unlike those in Cross 5d, 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. For the first time, some of the crosses in 5e and 5f integrate powdery mildew resistance from *Run 2.1* (*Muscadina rotundifolia*) into our PD resistant lines. Crosses in 5h are similar in result to those made last year, however, we have selected for parents with better germination and anticipate a higher percentage of progeny with desirable MAS results. In addition to the 2017 crosses presented in Tables 4 and 5, we also made crosses in the b46-43 line to advance to the BC2 level using Alvarelhao and Muscat Blanc as elite *vinifera* parents with 222 seeds produced and 65 resistant seedlings expected.

Table 5. PD crosses made in 2017 with percent *vinifera*, most recent elite *vinifera* parent and number of seeds produced, planted, saved and MAS tested. The PD resistance in *PdR1b* originated b43-17 a Monterrey, Mexico *V. arizonica/candicans;* b42-26 (*V. arizonica/girdiana)* has a multigenic form of PD resistance from Loreto, Baja California. *Ren1*, *Ren4* and *Run1* and *Run2.1* are powdery mildew (PM) resistance loci from *V. vinifera*, *V. romanetii*, and *M. rotundifolia*, respectively. Estimated numbers are in italics.

Cross PDR Type	Cross PM Type	% *vinifera*	*vinifera* parent...grandparents	# Crosses
5a. b40-14	None	97%	Dolcetto, Fiano, Grenache noir 224, Malvasia Bianca, Montepulciano, Morrastel, Pedro Ximenez and Touriga Nacional	7
5b. *PdR1b*xb42-26	None	97%	Arneis, Morrastel, Palomino, Pedro Ximenez	4
5c. *PdR1b*xb42-26^2	None	93%	Arneis, Dolcetto, Malvasia Bianca, Montepulciano, Morrastel, Pedro Ximenez	7
5d. *PdR1b*	*Ren1* & *Ren4*	98%	Alvarelhao, Malvasia Bianca, Morrastel, Sauvignon vert	4
5e. *PdR1b*xb42-26	*Ren4*	98%	F2-35,…Cab, Chard, Zin	5
5f. *PdR1b*x*PdR2*	*Ren1xRen4* or *Ren1xRun2.1*	93%,94%	...Cab, Chard, Zin	4
5g. *PdR1b*xb42-26	*Ren1xRen4* or *Ren1xRun1*	95%-98%	...Cab, Chard, Zin	6
5h. *PdR1b*xb42-26	*Ren1xRen4xRun1*	96%	...Cab, Chard, Zin	3

Table 5. Continued.

Cross PDR Type	Act. # Seeds	# Seeds planted	# Seedlings saved	# Seedlings MAS Tested
5a. b40-14	1,004	829	368	300
5b. *PdR1b*xb42-26	235	235	0	0
5c. *PdR1b*xb42-26^2	1,556	1106	352	327
5d. *PdR1b*	505	*507*	*176*	*160*
5e. *PdR1b*xb42-26	1,404	197	*8*	*0*
5f. *PdR1b*x*PdR2*	300	*253*	*88*	*70*
5g. *PdR1b*xb42-26	1,251	1082	*318*	*275*
5h. *PdR1b*xb42-26	446	345	*119*	*105*

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. We select for fruit and vine quality and then move the best 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 in Pierce's disease 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 19 advanced winegrape selections to FPS over the past four winters to begin the certification and release process. Three PD resistant rootstocks were also sent to FPS for certification. The first selections have cleared certification from FPS and we are currently working through the UC patent and release process. We have also identified PD resistance on chromosome (Ch) 8 from *V. arizonica-girdiana* accession b42-26 and designated it *PdR2*. Numerous selections with *PdR1b* and *PdR2* combined together at the 92% *vinifera* level have been greenhouse screened and used in crosses to increase the percentage of *vinifera*. 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 and at public tastings throughout California, and Texas, Virginia and Georgia.

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RESEARCH RELEVANCE

The goal of this research is two-fold: to produce PD resistant winegrapes 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 have 19 winegrape selections at FPS and five are in a pre-release status at grape nurseries to prepare for their commercial distribution in 2020.

LAYPERSON SUMMARY

One of the most reliable and sustainable solutions to controlling plant disease is to create resistant plants. We use a traditional plant breeding technique called backcrossing to bring PD resistance from wild grape species into a diverse selection of classic and high quality winegrape backgrounds. We identified an area on a chromosome that carries a very strong source of PD resistance from a grape species native to Mexico and the southwestern United States (*Vitis arizonica*). Because we were able to locate this resistance gene/region, which we named *PdR1*, we have been able to use marker-assisted selection (MAS) to screen for DNA regions associated with *PdR1* allowing us to select resistant seedlings shortly after seeds germinate. MAS and aggressive growing 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 PD bacteria, 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 19 advanced selections to Foundation Plant Services (FPS) over the past four winters to begin the certification and release process. Five of these now certified selections were pre-released to grape nurseries in 2017 so that they can be multiplied and prepared for commercial release. Three PD resistant rootstocks were also sent to FPS for certification. New sources of PD resistance have been identified and they are being added to *PdR1* resistance so that a broader range of resistance genes is available to control PD. The small-scale wines made from our advanced *PdR1* selections have been very good, and have been received well at professional tastings throughout California.

STATUS OF FUNDS: These funds are schedule 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.

FUNDING AGENCY
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