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PROJECT TITLE: Breeding Pierce's disease resistant winegrapes.

Principal Investigator:
Andrew Walker
Department of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Cooperating Staff:
Alan Tenscher
Department of Viticulture and Enology
University of California
Davis, CA 95616
actenscher@ucdavis.edu

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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 use 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 select for fruit and vine quality and then move the best 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 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*. In the current grant period, the first three scion selections that employ both *PdR1* and *PdR2* resistance were delivered to FPS. 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 public tastings, most recently at the 2019 Unified Symposium.

LAYPERSON SUMMARY
We use a classical plant breeding to combine PD resistance from wild grape species with high quality winegrapes. To date we have identified two different chromosomal 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 *PdR1*-based resistant rootstocks were also sent to FPS for certification. In the current grant period the first three scion selections that employ both *PdR1* and *PdR2* resistance were delivered to FPS. 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 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. 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.* In the current grant period the first 3 scion selections that employ both *PdR1* and *PdR2* resistance were delivered to FPS. 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 continue to be made.

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 ten years and from the 97% *V. vinifera* level for seven years. They have been very good and do not 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 a 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.

Objective of Proposed Research: The overall objective of this proposal is to develop lines representing different Pierce's disease (PD) resistance sources with diverse and elite *vinifera* backgrounds, stack different lines for durable

field resistance, and continue to evaluate new resistant germplasm to optimize the breeding of PD resistant winegrapes.

1. Identify PD resistant germplasm for breeding and provide support to the companion mapping/genetics project.
   a. Test new accessions primarily collected from the southwestern United States and northern Mexico.
   b. Establish and maintain mapping populations, and evaluate them for PD resistance to support our companion genetics project.
   c. Develop, maintain and evaluate breeding populations from the most promising new sources of resistance.

2. Develop and select advanced lines of PD resistant winegrapes.
   a. Establish resistance lines with different resistance sources through four backcross generations to elite *V. vinifera* cultivars.
   b. Evaluate and select on fruit quality traits.
   c. Complete wine and fruit sensory analysis of advanced selections.

3. Stack (combine) different resistance loci.
   a. Combine multiple resistances by making crosses of BC4 generation with advanced selections containing *PdR1* and validate with marker testing.
   b. Test for PD resistance and high quality fruit and wine.
   c. Field trials, wine tastings and outreach efforts

RESULTS AND DISCUSSION

Our PD resistance breeding activities over the grant period are quantified and summarized in Table 1. Percentage of MAS tested going to field (1b.) is only about 27% because these were also screened in the GH for resistance to powdery mildew. Fruit evaluations (1c) include new PD crosses and PD x PM stacked crosses but does not 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. Three selections were sent to FPS for certification over this period as shown in Table 1f.

Table 1. July 2018 thru June 2019 PD breeding activity summary.

Activity	2018-19
1a. Genotypes MAS Tested	2,400
1b. Genotypes Planted to Field	722
1c. Genotypes Evaluated for Fruit	192
1d. Genotypes Tested in GH	1,343
1e. Genotypes Tested Multiple Times	52
1f. Advanced Selections sent to FPS	3

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 trial in Table 2a was a 3 x 3-factor matrix testing genotype, *Xf* isolate, and sample date. The genotypes tested were 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. Samples were taken at 8, 9 and 13 weeks to see how *Xf* titer and phenotype scores compare across genotype, strain and sample date. The goals were 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. Results indicated that genotype was by far the most important effect ($p<0.0001$) followed a distant second by sample date

(p=0.0346). We plan to keep the latter as is, at 13 weeks, since we observed a slight but steady increase in *Xf* titer with time and we want to be able to compare and validate genotype results across the extensive data set from trials conducted to date. *Xf* strain (p=0.0418) was not far behind sampling date but interestingly the CH strain had the highest mean for the U0505 alone group. This may indicate that rather than making *Xf* more pathogenic, strains derived from a plant with a PD resistant background may actually be somehow weakened and be less virulent when next inoculated.

In Group 2b, we tested 78 untested PD species accessions to better characterize our collection and elucidate PD resistance performance by geographical provenance and species. A summary of the majority of species tested and details of two are presented in the two Tables 3 and 4 below. Also tested were twenty-six more F1 genotypes in the b41-13 mapping population and results helped identify resistance on LG14. Six promising PD x powdery mildew (PM) accessions from crosses made in 2015 were also tested. All were resistant by ELISA and four also had low phenotype scores. They will be tested again to confirm their high levels of resistance. In the second testing of 2017 PD parents, all were highly resistant.

Group 2c continued testing of F1 mapping populations with 50 and 27 genotypes respectively in the b41-13 and T03-16 populations. We also evaluated 11 untested genotypes from 2015 PD x PM crosses and retested 18 genotypes identified as highly promising in recent greenhouse screens. This screen was of very high severity with clear separation of our U0505 *PdR1b* biocontrols. This is an ideal severity for selecting highly PD resistant advanced candidates for possible release but perhaps a little high in severity for mapping. Still these results helped locate PD resistance in these two lines on LG14. For the retests of promising genotypes, we found 17 resistant, five highly so. The five also carry PM resistance with the three at the 93% *vinifera* level candidates for future parents as fruit quality still needs improvement.

In 2d, thirty 96% *PdR1b* x *PdR2* hermaphrodite genotypes were tested for resistance with the intent, that should they have sufficient resistance and have adequate fruit and wine quality, they would be candidates for release. An additional 55 genotypes homozygous at either *PdR1* or *PdR2* and having the other resistance source were tested to see if there is any pattern to high levels of resistance inheritance. Second or third screens were conducted on 54 genotypes with PD or PD x PM to validate previous results and confirming screens were run on five genotypes used as parents that didn't already have three completed screens. Unfortunately, this was a low severity screen and did not differentiate our usual 4 categories (‘immune’, promising, resistant and susceptible) of PD resistance; it will be repeated.

In 2e, two main groups were examined: 77 untested species to better characterize our collection and further elucidate PD resistance performance by geographical provenance and species; and 148 PD x PM crosses from 2017. The latter is of interest as the lines involved have conferred, in the previous two generations, an exceptionally high level of resistance on an exceptionally large percentage of their progeny. Resistance comes from *PdR1b* and b42-26 but with genotypes not having *PdR2*. Based on phenotypic symptoms, the screen is moderate to high severity. ELISA analysis is in process.

In February of this year, we completed the greenhouse screen for the group in 2f. Fifty genotypes in this trial tested two 93% *vinifera* crosses from highly resistant *PdR1b* x b42-26 line parents (13329-09 and 13329-20) crossed back to elite *vinifera* to see if this high level of resistance carries forward another backcross generation. Eighteen *PdR1b* x *PdR2* genotypes at the 94% *vinifera* level that also carry PM resistance were tested for the first time as well as 15 southwestern US wild *Vitis* accessions. Based on phenotypic symptoms the screen is high severity. Samples are in the lab awaiting ELISA analysis.

Table 2g consists of four main groups: Similar to 2f, we are testing twenty 93% *vinifera* genotypes from the highly resistant *PdR1b* x b42-26 line parent (13329-20) crossed to Dolcetto and Pedro Ximenez to further validate results in 2f. Fifty-four genotypes from the 96% *PdR1b x PdR2* 2017 crosses are also included. *Vinifera* parents include Arneis, Montepulciano, Morrastel, Pedro Ximenez, Pinot noir and Sauvignon vert. Fifty-five PD x PM genotypes are also being tested. Filling out this group are 22 untested F1 genotypes in the T03-16 line to support our mapping project. 2h tests 149 untested species from our collection. Similar to 2g, an additional eighty 96% *vinifera PdR1b* x *PdR2* genotypes from 2017 crosses are tested. Elite *vinifera* parents, in addition to those mentioned above, include Alvarelhao, Mataro, and Refosco. The balance consists of biocontrols and the parents of the 2018 crosses. Samples are in the lab awaiting ELISA analysis. In 2i we are testing 42 untested wild *Vitis*

accessions from our collection and, as in 2e testing or retesting 61 genotypes from the 2017 PD x PM crosses from 2017 that have, in the previous two generations, conferred an exceptionally high level of resistance on an exceptionally large percentage of their progeny.

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	*Xf* strain trial (3 strains, 7 BC genotypes, 3 time points)	7	5/24/2018	7/19/2018, 8/2/2018, 8/21/2018	b43-17, SEUS, *PdR1b*
2b	SWUS PD species, b41-13, 2017 parents	133	5/24/2018	8/21/2018	Species, b41-13, *PdR1b*
2c	Mapping Pops, 2015 PD x PM untested	115	6/23/2018	9/25/2018	T 03-16, b41-13, *PdR1b*
2d	92 & 96% PD stack, retest of recent promising	170	8/23/2018	11/20/2018	*PdR1xPdR2*
2e	2017 PD x PM, PD Species, 2018 parents	241	10/16/2018	1/15/2019	Species, *PdR1b* x b42-26
2f	2017 PD Xs, SWUS PD species	95	11/21/2018	2/21/2019	Species, *PdR1b* x b42-26
2g	2016 & 2017 PD Crosses	171	1/10/2019	4/11/2019	*PdR1b* x b42-26
2h	2017 PD Xs, SWUS PD species	255	3/28/2019	6/27/2019	Species, *PdR1b* x b42-26
2i	Species, 2017 *PdR1b* x b42-26 promising or untested	112	5/7/2019	*8/6/2019*	Species, *PdR1b* x b42-26

To date over 370 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 unique PD resistance mechanisms. We first 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 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*. Fourteen of these lines have now been confirmed to have PD resistance associated with Ch 14, the same region as our primary resistance line *PdR1b* (Riaz, 2016*)*. Of the fifteen lines, only the ANU67 line remains a possibility for non-LG14 resistance. Cuttings have been made and testing is underway. Although resistance in the b46-43 source is dominated by LG14, we continue to explore the BC1 for minor resistance genes and a BC2 has been developed should that prove necessary. Our mapping project identified *PdR2* on Ch 8 from b42-26. *PdR2* resistance although significant, generally does not confer as strong a resistance as *PdR1*.

We continue to explore additional wild accessions for novel sources of PD resistance to incorporate into our breeding program. Table 3 presents a summary of the greenhouse screen results for a select group of species from our recent testing of 78 wild accessions noted in (Table 2b). For comparison, the reference genotypes (biocontrols) in this trial had the following mean cfu/ml values: b43-17 18,616; U0505-01, our 88% *PdR1b* resistant standard that served as Dunnett's reference to determine R & S, 78,503; and Chardonnay 4,114,426. Although the *treleasei* had the lowest average *Xf* titer of all the species shown in Table 3, all the accessions were collected from one relatively small geographic area near Ruidoso Downs, NM. Judging the general species wide PD resistance of *treleasei* as a species from these results should be with that caveat. Across these and the other species tested in this group, generally the further north a genotype was collected, the higher its *Xf* titer regardless of species. For breeding lines from wild species, we select genotypes with lowest ELISA values and least PD phenotypic symptoms.

Table 3. Greenhouse screen ELISA results for a subset of species from the southwestern US tested as part of the greenhouse screen conducted in Table 2e.

Vitis species	R	S	Average Geometric mean (cfu/ml)	Maximum Geometric mean (cfu/ml)	Minimum Geometric mean (cfu/ml)	of Genotypes
acerifolia	1	11	5,004,481	6,500,000	134,797	12
arizonica	16	4	266,155	1,932,322	10,335	20
californica	3	4	2,299,805	6,500,000	113,160	7
girdiana	7	5	2,490,613	6,500,000	11,368	12
riparia	3	5	3,241,542	6,500,000	78,724	8
treleasei	5		69,646	265,915	10,413	5
Sum or value	35	29	2,150,678	6,500,000	10,335	64

Table 4 below provides the accession specific results for the *V. girdiana* and *V. californica* accessions summarized in Table 3. *Vitis girdiana* is a species endemic to the southern parts of California, Nevada and Utah where PD has historically been more common. *Vitis californica* has a broad range from central to northern California and into southern Oregon. Except for the North Coast of California, PD is typically less common in its range. In both species we see ELISA titers for accessions spanning nearly the range. However, we see three accessions of *girdiana* with titers below the level of our U0505-01 resistant biocontrol and one (NV11-119) numerically lower that b43-17, the source of *PdR1*. Were it not for the high phenotype scores of this accession, it could be a promising source for a new breeding line. It is also interesting to note that two of the three *girdiana* accessions collected from Death Valley are highly resistant while the third is highly susceptible, perhaps indicating some introgression of *vinifera* in its lineage.

Table 4. Accession level greenhouse screen results detail for two *Vitis* species tested as part of the group in Table 2b. These data correspond to the summary of these same species in Table 3 above. Cfu/ml are from ELISA; CMI is the cane maturity index from 0 (no PD symptoms) to 6 (high level of PD symptoms); and LS-LL is a 0-5 scale reflecting the extent of leaf scorch and leaf loss.

Genotype	Species	Location name	GH Screen Result	Geometric mean (cfu/ml)	Reps	CMI Mean	LS-LL Mean
C70-01	*californica*	Lake County, CA	R	113,160	5	3.0	0.8
C118-95	*californica*	Mix Canyon, CA	R	233,388	5	1.6	2.2
NC34	*californica*	LaMoine, CA	R	255,160	5	1.8	1.8
CC11	*californica*	Three Rivers, CA	S	1,015,137	4	3.8	3.3
NC44	*californica*	Dunsmuir, CA	S	1,481,784	5	4.2	2.8
C19-95	*californica*	Corning, CA	S	6,500,002	5	5.2	5.0
CC4	*californica*	Three Rivers, CA	S	6,500,002	4	3.8	1.5
NV11-119	*girdiana*	NV	R	11,368	4	3.3	1.5
girdiana -22	*girdiana*	Death Valley, CA	R	27,083	3	0.7	1.3
girdiana -1	*girdiana*	Death Valley, CA	R	42,441	5	0.4	0.2
UT12-084	*girdiana*	St. George, UT	R	286,204	5	2.8	2.4
NV12-057	*girdiana*	Kershaw-Ryan SP, NV	R	587,351	5	3.6	3.0
SC40	*girdiana*	Casino, CA	R	627,733	5	3.0	1.2
SC27	*girdiana*	Grapevine, CA	R	666,320	5	3.6	2.2
UT12-094	*girdiana*	St. George, UT	S	2,214,761	5	4.2	3.4
girdiana -8	*girdiana*	Death Valley, CA	S	5,924,086	5	4.0	3.6
SC21	*girdiana*	Kern Co., CA	S	6,500,002	4	4.0	4.0
SC1	*girdiana*	Nye, NV	S	6,500,002	3	4.0	5.0
UT12-075	*girdiana*	St. George, UT	S	6,500,002	3	4.3	4.0

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 four different crosses that are 96% *vinifera* and have both resistance loci. Although promising in that we saw some genotypes with R-ratings above their parental means, we did not see genotypes scoring in the most resistant 10 category. However, scores of five 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. That said, the greenhouse screen is much more severe than what the plants experience in the field and plants scoring five should perform well in the field. Three of the most promising selections (Figure 1) were sent to FPS in March and multi-vine trials for small-scale winemaking were established in Davis this spring.

Figure 1. Three promising 96% *vinifera PdR1b* x *PdR2* PD resistant selections sent to FPS in March (l – r): 16353-072, 16329-015, and 16333-022.

Fruit evaluations of the three selections conducted last fall demonstrated promising fruit and horticultural characteristics. Results of these are shown in Tables 5a-c. These and other selections are currently being retested (Table 2) in the greenhouse to verify the high level of PD resistance.

Table 5a. Three promising 96% *vinifera PdR1b* x *PdR2* PD resistant selections: background and fruit characteristics.

Genotype	Parentage	2018 Bloom Date	2018 Harvest Date	Berry Color	Berry Size (g)	Ave Cluster Wt. (g)	Prod 1=v low, 9=v high
16353-072	14388-029 x Chardonnay	5/25/2018	8/30/2018	W	1.0	160	6
16329-015	14309-111 x Primitivo	5/29/2018	8/30/2018	B	1.3	199	8
16333-022	14309-111 x Cab Sauvignon	5/22/2018	8/30/2018	B	1.3	286	4

Table 5b. Juice analysis of three promising 96% *vinifera PdR1b* x *PdR2* PD resistant selections.

Genotype	°Brix	TA (g/L)	pH	L-malic acid (g/L)	potassium (mg/L)	YAN (mg/L, as N)	catechin (mg/L)	tannin (mg/L)	Total antho-cyanins (mg/L)
16353-072	25.4	8.2	3.28	2.4	1780	167			
16329-015	25.6	6.4	3.64	3.3	2060	260	97	649	2344
16333-022	23.4	6.6	3.53	3.5	2020	223	146	589	1618

Table 5c. Three promising 96% *vinifera PdR1b* x *PdR2* resistant selections: berry sensory analysis.

Genotype	Juice Hue	Juice Intensity	Juice Flavor	Skin Flavor	Skin Tannin Intensity (1=low, 4= high)	Seed Color (1=gr, 4= br)	Seed Flavor	Seed Tannin Intensity (1=high, 4= low)
16353-072	Green touch yellow	Med-	Green apple, pear, slight spice	spicy, slight green hay	2	4	Spicy, woody, warm	3
16329-015	Red	Dark-	Strawberry jam, sweet spices	Berry, fruity	1	3	Spicy, hot	2
16333-022	Red-orange	Light+	Fruity, like PN	spicy, slight grass	2	2	Woody, spicy	3

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 the 1095 seedlings tested. Table 6 shows preliminary greenhouse screen results based solely on phenotypic symptoms expression of PD for a subset of these 2017 crosses. Interestingly we see some crosses with level 10 resistance, something we have not yet observed in the 2016 crosses. Once ELISA results are complete, analysis of both resistant and *vinifera* parents will be conducted to identify any patterns of inheritance for this highest level of resistance.

Table 6. Preliminary GH screen results based on phenotypic PD symptoms expression of a subset of the 2017 crosses of elite *vinifera* cultivars to three PD resistant genotypes that have both the *PdR1b* and *PdR2* loci. Progeny are 96% *vinifera*. ELISA results are pending.

Resistant Parent	*vinifera* Parent	Seedlings planted	PD R-rating Category				Count of Genotypes tested
			-1 = S	1 = R	5 = Very R	10 = Immune	
14309-002	Alvarelhao	16	1	3	3	2	9
	Mataro	10	3	4	3		10
	Montepulciano	10	1	1	4	1	7
14309-111	Mataro	49	7	19	10	1	37
14388-029	Arneis	9	1	1	1		3
	Morrastel	25	1	5	3	6	15
	Pedro Ximenez	16		3	3	4	10
	Pinot noir FPS32	2	1				1
	Sauvignon vert	26	7	7	5	1	20

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, greenhouse 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 7d where crosses were made directly to elite *vinifera* cultivars, the challenge of the other crosses in Table 7 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 7d, 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.

Table 7. Number of seeds planted, MAS tested and number making it into the field from our PD x PM crosses made in 2018. *PdR1b* (F8909-08) is from Monterrey *V. arizonica/candicans* PD resistance b43-17; b42-26 is the Baja California *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*.

Resistances	Recent *vinifera* parents in background	% *vinifera*	Crosses	Seeds planted	Seedlings MAS Tested	Seedlings Planted in field
7a. PD - *PdR1b.* PM - *Run1*	Cabernet Sauvignon, Nero d'Avola, Zinfandel, 4 UCD *PdR1b* releases	97%	3	960	350	98
7b. PD - *PdR1b.* PM - *Ren1 & Run2.1*	Airen, Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases	95%	3	1219	275	60
7c. PD - *PdR1b.* PM - *Ren1, Ren4 & Run1*	Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases	95%	3	756	240	54
7d. 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	93%, 95%	15	3730	1270	359
7e. PD - *PdR1b* with b42-26. PM - *Run1* with either *Ren1* or *Ren4*	Cabernet Sauvignon, Grenache, Touriga Nacional, Zinfandel, 1 UCD *PdR1b* release	91%, 93%	4	765	175	78
7f. PD - *PdR1b* with b42-26. PM - *Ren1*, *Ren4* & *Run1*	Cabernet Sauvignon, F2-35, Grenache, Zinfandel	94%	4	357	90	73

No crosses were made in the spring of 2019.

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 PD resistant rootstocks were also sent to FPS for certification. In 2019, the first three scion selections that employ both *PdR1* and *PdR2* resistance were delivered to FPS. PD 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 well received at tastings throughout California, Texas, Georgia and Virginia.

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