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Interim Progress Report – March 2019

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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 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 *PdR1b* 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 began in 2018 and our first three scion selections were sent to FPS earlier this month. 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. 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. 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 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.

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.

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 (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 six 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 through backcross generations 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 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.

Table 1 presents a summary of the greenhouse screen results for a select group of species from our recent testing of 79 wild accessions noted in (Table 3b). 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 1, 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 1. Greenhouse screen ELISA results for a subset of species from the southwestern US tested as part of Table 3b.

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 2 below provides the accession specific results for the *V. girdiana* and *V. californica* accessions summarized in Table 1. *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 2. Accession level greenhouse screen results detail for two *Vitis* species tested as part of the group in Table 3b. These data correspond to the summary of these same species in Table 1 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

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.

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 3 provides a list of the PD greenhouse screens analyzed, initiated, and/or completed over the reporting period. The trial in Table 3a 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. These were sampled 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 TD 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 TD date but interestingly 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 generally less virulent when next inoculated.

In Group 3b we tested 79 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 above. Also tested were twenty-six more F1 genotypes in the b41-13 mapping populations for marker discovery. In this moderately severe screen, although there was an order of magnitude variation in the ELISA titers, all but one were classed as resistant relative to the U0505-01 resistant biocontrol. Although this result contrasts to results reported for group 3c, our mapping uses the absolute ELISA value rather than a relative result so this shouldn't be an insurmountable problem and results were provided to our companion mapping project. Six promising PD x PM accessions from crosses made in 2015 were also tested. All were resistant by ELISA but only 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 3c continued testing of F1 mapping populations with 50 and 27 genotypes respectively in the b41-13 and T 03-16 populations. Also tested were 11 untested genotypes from 2015 PD x PM crosses and retests on 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. That said, for the b41-13 line, the R:S ratio was 3:2 and the distribution appeared bimodal. Similar outcomes were observed for the T 03-16 line with an R:S ratio approximately 3:2 and again bimodal. Results were supplied to our companion mapping program. From the 2015 PD x PM crosses, 7 were resistant, 4 susceptible; of the 7 resistant only 3 were highly so and only one had a minimal PD phenotype. It will be retested. For the retests of promising genotypes, we found 17 resistant again, 5 highly so. The 5 also carry PM resistance with the 3 at the 93% *vinifera* level candidates for future parents as fruit quality still needs improvement.

In 3d, 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. Regrettably the conditions during this trial were such that we experienced only a low severity screen which doesn't allow us to reproducibly differentiate our usual 4 categories ('immune', promising, resistant and susceptible) of PD resistance. This trial will need to be repeated. We have mentioned in previous reports the significant role temperature has on our GH screen and continue to refine the relative importance of both the absolute levels and averages of temperature and their timing on observed severity of our greenhouse screen.

In 3e, two main groups are being examined: 78 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, in the previous two generations, conferred 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 looks to be moderate to high severity. ELISA analysis is in process.

Late last month we completed the greenhouse screen for the group in 3f. 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 SWUS wild *Vitis* accessions. Based on phenotypic symptoms the screen should be of high severity. Samples are in the lab awaiting ELISA analysis.

Table 3g consists of four main groups: Similar to 3f, 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 3f. 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 T 03-16 line to support our mapping project. 3h tests 149 untested species from our collection. Similar to 3g, 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.

Table 3. Greenhouse PD screens analyzed, completed and/or initiated during the reporting period. Projected dates in italics.

3a	*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*
3b	SWUS PD species, b41-13, 2017 parents	133	5/24/2018	8/21/2018	Species, b41-13, *PdR1b*
3c	Mapping Pops, 2015 PD x PM untested	115	6/23/2018	9/25/2018	T 03-16, b41-13, *PdR1b*
3d	92 & 96% PD stack, retest of recent promising	170	8/23/2018	11/20/2018	*PdR1xPdR2*
3e	2017 PD x PM, PD Species, 2018 parents	241	10/16/2018	1/15/2019	Species, *PdR1b* x b42-26
3f	2017 PD Xs, SWUS PD species	95	11/21/2018	2/21/2019	Species, *PdR1b* x b42-26
3g	2016 & 2017 PD Crosses	171	1/10/2019	*4/11/2019*	*PdR1b* x b42-26
3h	2017 PD Xs, SWUS PD species	255	*3/28/2019*	*6/29/2019*	Species, *PdR1b* x b42-26

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 generate 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. Also in January 2018, six wines were tasted with Daniel Robert's grower group. In December we held the first tasting of our 2018 vintage wines. Results of the tasting are presented in Table 4. All of the PD whites were rated significantly higher than both Chardonnay and the traditional PD resistant control variety, Blanc du Bois. Numerically the highest scoring white wine was the 10302-178 which went to FPS in 2016. This past January it cleared FPS testing and versions exists as both classic and 2010 Protocol. With the help of Paul Skinner and Chuck Wagner at Caymus Vineyards, this past season we were able to compare wines made with Napa and Davis grown from two of our red PD varieties nearing release. All four wines were made at the UC Davis winery with the Napa wines made at the half ton scale from machine harvested fruit and the Davis versions hand harvested and fermented at the more typical 150 pound scale. For both the 07355-075 and 07331-047, the Napa versions rated higher but not significantly so being less than half a point apart. From this we are satisfied that our small scale winemaking can be taken as representative of larger ferments and that Davis grown fruit, although ranking lower than Napa serves as a good indicator of the quality potential of a new selection. Five of the 7 *PdR1* based red wines scored higher than Cabernet Sauvignon and all nine of them were ranked higher than the traditional PD resistant variety, Lenoir. This was the third and last year we will make wine from 03182-084, a 75% *vinifera* selection with resistance based on the SEUS resistance from BD5-117. It has made wine of poor quality each year and this year scored only slightly higher than the Lenoir. Another disappointment has been the low wine quality found in the 97% *vinifera* selection, 12351-03 which is our most advanced PD resistant selection deriving its resistance from *PdR1a*. Although not essential to our overall PD breeding program, having high wine quality in the *PdR1a* line would be desirable should an as yet unknown *Xf* strain susceptibility in the *PdR1b* line eventuate.

Table 4. Results of a preliminary tasting of 2018 vintage wines tasted 12/13/18 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	12/13/18 Consensus Descriptors: color; aroma; flavor-texture
09314-102	97%	W	3.7	4	3	Pale, brilliant; sweet spice, dough, slightly grassy; full, white Rhone-like.
09338-016	97%	W	3.4	4	3	Medium yellow, slight cloudy; pineapple, pear, slight spice; light body, minerality.
10302-178	97%	W	3.8	5	3	Pale, clear; candy, floral, tropical; lemon, balanced, slight astringent mid-palate
10302-238	97%	W	3.7	5	3	Light yellow, clear; very perfumy, muscat; soft, broad, attractive.
10302-293	97%	W	3.6	4	3	Very pale; lemongrass, floral; simple, candied, drying.
10302-309	97%	W	3.2	3.5	3	Pale yellow; cookie, root beer, spice; melon, apple, soft, slight bitter finish.

10317-035	97%	W	3.5	4.5	2.5	Pale yellow-green; perfume, rose, citrus; nectarine, slightly ast mid-palate, short finish.
Chardonnay	100%	W	2.3	3.5	1	Medium brown; simple, clean, apple; odd, tired, flat.
Blanc du Bois	50%	W	2.4	3	1	Pale+; floral, not muscat, estery; watermelon, slight oxidized.
03182-084	75%	R	2.5	4	1	Medium-, red, touch orange; odd, slightly sweaty, strawberry; tea, earthy, thin.
07355-075	94%	R	3.4	4	3	Dark-, purple, red; grape, blackberry; black fruit, creamy, no bitterness.
07355-075 Napa	94%	R	3.8	4	3	Dark, red-purple; more dark fruit than Davis version but similar; slightly leafy, broad.
09311-160	97%	R	2.6	3.5	1	Pale+ red; warm spice, simple, red fruit; cherry, herbal tea, light.
09330-07	97%	R	3.4	5	2	Dark, red-purple; black cherry, berry, peppery; soft entry, full middle, slightly tart cherry finish.
09331-047	97%	R	3.8	5	3	Dark-, red-purple; berry, peppery, zin-like; blackberry, medium tannins, balanced.
09331-047 Napa	97%	R	4.2	5	3	Dark, red-purple; blackberry, black pepper, celery salt; grapey, balanced full.
09331-133	97%	R	3.3	4	2	Medium+ red; ripe strawberry, cherry, simple; candied, simple tannins, fruity finish.
09333-370	97%	R	2.9	3.5	2	Dark-, red-purple; dried fruit, older dried flowers; cola, tea, limited mouthfeel.
09356-235	97%	R	3.7	5	2.5	Dark, almost black; black fruits – cherry and berry, sl. brooding; rich, quality tannins, long.
12351-03	97%	R	2.2	3	1	Pale red; vegetal, earthy, tea, tobacco, light red fruit; earthy, light, hot finish.
Cabernet Sauvignon	100%	R	3.0	4	2	Dark- red; CS veg, candied, slight sweaty; simple, stewed tomato, lacks structure.
Lenoir	50%	R	2.2	3	1	Dark, red-purple, muddy; medicinal, floral, lacks fruit; tart, no structure, limited tannins.

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.

We are part way through our yearly seed germination season. All the seeds have been planted and seedlings are emerging. 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 5d where crosses were made directly to elite *vinifera* cultivars, the challenge of the other crosses in Table 5 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 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 where the most recent parent has always been a *vinifera* cultivar.

Table 5. Number of seeds planted, saved and MAS tested 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*. Estimated quantities are in italics.

Resistances	Recent *vinifera* parents in background	% *vinifera*	# Crosses	# Seeds planted	# Seed-lings saved	# Seedlings MAS Tested
5a. PD - *PdR1b.* PM - *Run1*	Cabernet Sauvignon, Nero d'Avola, Zinfandel, 4 UCD *PdR1b* releases	97%	3	960	368	350
5b. PD - *PdR1b.* PM - *Ren1 & Run2.1*	Airen, Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases	95%	3	1219	288	300
5c. PD - *PdR1b.* PM - *Ren1, Ren4 & Run1*	Cabernet Sauvignon, Riesling, 2 UCD *PdR1b* releases	95%	3	764	*264*	*225*
5d. 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	*1338*	*1270*
5e. 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	766	*184*	*175*
5f. PD - *PdR1b* with b42-26. PM - Ren1, Ren4 & Run1	Cabernet Sauvignon, F2-35, Grenache, Zinfandel	94%	4	362	*128*	*75*

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 20 advanced *PdR1b* 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*. Our first three 96% *vinifera* scion selections went to FPS this month. 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, 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 23 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 23 advanced selections to Foundation Plant Services (FPS) over the past seven 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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