California Department of Food and Agriculture PD/GWSS Renewal Progress Report – July 2017

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

PROJECT TITLE: Breeding Pierce's disease resistant winegrapes.

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REPORTING PERIOD: primarily March 2017 to July 2017

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 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 19 scion and three PD resistant rootstocks have been advanced to FPS for certification. Stacking of *PdR1b* with b42-26 Pierce's disease resistance has been advanced to the 96% V. vinifera level using MAS to confirm the presence of PdR1 as well as the recently discovered (see companion report) PD resistance locus on LG8 from b42-26, PdR2. Initial selections for release will begin in 2018. Greenhouse screening is 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 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 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 eight years and from the 97% V. vinifera level for six years. They have been very good and don't have typical hybrid flaws (blue purple color and herbaceous aromas and taste) that were prevalent in red wines from the 87% V. vinifera level. b43-17 is homozygous resistant to PD. We have named it 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 *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 PdR1band 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 Chromosome 8, which we have called *PdR2*. Three years ago, in 2014, we advanced our *PdR1* x *PdR2* line to the 92% *vinifera* level and last spring made crosses to advance it to the 96% vinifera level. MAS was used to advance only genotypes with both PdRIb 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.

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 (Ch) 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 Ch 14, the same region as our primary resistance line PdR1b. From that same project we identified PdR2 on Ch 8 from b42-26. PdR2 resistance although significant, generally doesn't confer as strong a resistance as PdR1. Preliminary results indicate that most of the non-PdR1 resistance sources appear to also have at least some of their resistance derived

from Ch 8. Until we better understand the nature of Ch 8 PD resistance and explore additional resistance loci in these lines, it is important to continue advancing multiple sources of Ch 8 resistance. Now that the yearly breeding cycle is compete for the 2016 crosses, Table 1 details the crosses made to advance non-Ch 14 lines with the number of seedlings DNA tested either for trueness to type or for Ch 8 markers and the number that went to the field. Crosses in Group 1a created progeny to expand existing F1 mapping populations from the ANU67, b41-13 and T03-16 sources (all accessions from southwestern *Vitis* species). The location of PD resistance from ANU67 is not yet determined; resistance from the latter two, at least in part, appears to be associated with Ch 8. Some of the progeny from these F1 lines exhibited strong resistance, but few highly resistant progeny were detected in the T03-16 line. Crosses in Group 1b were made to examine whether complete PD resistance in this line could be recovered through a full sib crossing in the F1 generation. Two elite F1 individuals from the b41-13 line and three of the most resistant F1 genotypes in the T03-16 line were backcrossed to the indicated elite *vinifera* parents (Group 1c) to create new breeding lines at the BC1 level. Until we know more about resistance from the Ch 8 lines, will continue to advance them separately. 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.

	Cross PDR	%	vinifera Parents/	#	Act. #	# Seedlings Tested True to Type or	# to
Group	Source	Vinifera	Grandparents	Crosses	Seeds	MAS	field
	ANU67	50%	F2-35	1	1156	250	248
1a	b41-13	50%	F2-35	1	1147	250	239
	Т 03-16	50%	Palomino	1	47	35	35
1b	Т 03-16	50%	Palomino	3	160	79	60
1c	b41-13	75%	Rosa Minna, Primitivo/F2-35	2	550	307	258
	T03-16	75%	F2-35/Palomino	2	338	41	41

Table 1. 2016 Crosses made to expand new non-Ch 14 PD mapping populations and advance breeding lines to the next backcross level: *vinifera* parents, # crosses, seeds produced, # MAS tested and # planted.

Table 2 gives details of crosses made this spring to finish the expansion of our mapping populations. Table 2a crosses will complete the ANU67 and most of the T 03-16 mapping populations. The b41-13 population in Table 1a is complete. In Table 2b 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 Table 2c we broadened the elite *vinifera* parents used to advance the ANU67 and T03-16 lines and used a different promising F1 selection from the b41-13 line.

Table 2.	2017	Crosses	made	to	finish	the	expansion	of the	new	F1	PD	mapping	populations	and	advance
breeding l	ines to	the next	backer	oss	level:	vinį	fera parents	s, # cros	sses, (estir	mate	d # seeds	produced.		

	Cross				
	PDR	%			Est. #
Group	Source	Vinifera	vinifera Parents	# Crosses	Seeds
20	ANU67	50%	F2-35	1	90
2a	T 03-16	50%	Palomino	1	45
2b	T 03-16	50%	Palomino	10	285
			Montepulciano, Palomino, Pinot noir FPS77 and		
2c	ANU67	75%	Sauvignon vert	4	48
	b41-13	75%	F2-35	1	750
	T03-16	75%	F2-35, LCC	2	290

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 F1 and 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. Potential parents for 2017 crosses (Tables 5 and 6) were evaluated in Table 2a. This year was also our most extensive PD x powdery mildew (PM) screen to date and we evaluated 98 genotypes from 8 different crosses. PD resistances included PdR1b either alone or with b42-26 resistance and the Ren1, Ren4 and Run1 PM resistance loci. In previous reports we have reported some negative effect on PD resistance when PD and PM resistance loci were combined. In this trial, the percent highly resistant progeny ranged from 9% to 75%. Sample sizes were too small to make a definitive conclusion, but it appeared the selection of the PD resistant parent played a more important role to the resistance of a cross progeny than whether the cross was to a PM resistant parent. Part of Table 2a was the testing of 50 genotypes in an alternative PdR1b x b42-26 line at the 93% vinifera level. Fifty percent were promising and one was used as a parent in 2017 crosses (Table 6bam)). ELISA results are pending on the trial in Table 3b. The main effort was to refine resistance in the b42-26 line primarily around Ch 8. Similarly, we are retesting 8 genotypes in the b46-43 line that had anomalous greenhouse screen results relative to their Ch 14 markers. Promising parents for breeding in novel PdR lines including b40-14, b46-43, and ANU5 are also being retested as are untested remnants of our BD5-117 lines (another multigenic resistance source from a Florida breeding program). In addition to testing additional PD x PM crosses in Table 3c, we tested 20 accessions of V. berlandieri for the first time to evaluate PD resistance in this Texas native species. ELISA results are pending here too, but they expressed severe PD symptoms and will not be used as parents. Screening in Table 3d focuses on the b47-32 V. arizonica-monticola line to identify if resistance is unique or segregates with either Ch 8 or Ch 14 markers. Testing in Table 3e supports graduate student research in our companion mapping/genetics program looking for non-Ch 14 PD resistance loci in this predominantly Ch 14 based resistance source. In Table 3f we continue to test the F1 progeny of the new T03-16 and b41-13 lines to facilitate genetic mapping of their PD resistance. Also included are 33 genotypes that should complete the extensive testing of the 92% vinifera PdR1b x b42-26 stack group and allow further evaluation of the resistance derived from combining Ch 14 and Ch 8 loci as well as minor resistance factors.

				ELISA	
		No. of	Inoculation	Sample	PD Resistance
Group	Test Groups	Genotypes	Date	Date	Source(s)
3a	2015 PD & PDxPM Crosses	155	1/5/2017	3/23/2017	<i>PdR1b</i> , b42-26
3h	b42-26 BC1 & BC2 locus refinement, 2014 Cross highly rated: b46-43 BD5-117 lines	262	3/14/2017	6/15/2017	b42-26, b46-43,
50	Tated, 040-43, DD5-117 lines	202	5/14/2017	0/13/2017	DDJ-117
3c	Addn PDxPM HW & V. berlanderi	113	3/30/2017	6/29/2017	<i>PdR1b</i> , b42-26, berl
3d	b47-32 & low severity screen retests	170	5/25/2017	8/24/2017	<i>PdR1b</i> , b42-26, b47-32
3e	14-399 b46-43 BC1 Mapping MPP	262	8/1/2017	10/31/2017	b46-43
3f	T 03-16 & b41-13 F1, <i>PdR1b</i> xb42-26 Stack	92	8/17/2017	11/16/2017	b46-43

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

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 the University of California, Davis with industry and student tasters, and

others are at various industry gatherings with single event attendance exceeding 200 people. Overall, wines from our new PD resistant varieties have been very well received. Most recently, I presented the 5 selections that are moving through the release process at two large scale industry meetings: the ASEV-East meeting in Virginia, and an Extension class on Adaptative Wine making at UC Davis. These 5 selections are presented as an Appendix to this report.

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.

Our 2016 breeding crosses (Table 1 and 4) expanded on our 2015 efforts with increased numbers and focus on parents with superior horticultural and fruit quality traits. The numbers of relevant crosses were significantly impacted by the identification late last year of PdR2. The number of relevant crosses was reduced by 5 and the number of seeds planted fell by 1,743 saving significant time and labor. These 96% vinifera PdR1b x PdR2 progeny in Table 4a will not only be the beginning of our next round of PD resistant releases but serve as baseline populations to later quantify the value of double stacking minor resistance factors of the b42-26 resistance. The most recent elite vinifera parents include Chardonnay, Cabernet Sauvignon and Primitivo. Table 5 details the similar but much more extensive crosses made in 2017 to a more diverse group of elite *vinifera* parents. Table 4b presents intercrosses among the most resistant progeny to further evaluate compatibility and resistance in the effort to stack (combine) different resistance sources. After MAS testing for both *PdR1* and *PdR2*, the thousands of potential seedlings were reduced to only 237 plants that need to go to the field. Of these only 10 were found to be homozygeous at both PdR1 and PdR2. These plants are in greenhouse testing in group 3f above to evaluate whether they are enriched in minor b43-17 and b42-26 resistance factors. The most promising and resistant of these elite selections could be used to create populations that are 96% vinifera with all progeny inheriting both *PdR1b* and *PdR2* and all should be highly PD resistant. Table 4c-e presents the MAS testing of over 300 PD x PM cross progeny at various *vinifera* levels, the testing of which reduced the vineyard space necessary to evaluate them by 80%. Although these crosses demonstrate significant gains in fruit and horticultural quality few if any will likely have sufficient resistance and quality to be advanced for release.

Table 4. PD crosses made in 2016 with percent *vinifera*, most recent elite *vinifera* parent, number of seedlings MAS tested and planted. 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* are powdery mildew (PM) resistance loci from *V. vinifera*, *V. romanetii*, and *M. rotundifolia*, respectively.

		%	#	#	# MAS	# to
Cross PDR Type	Cross PM Type	Vinifera	Crosses	Seeds	Tested	field
4a. <i>PdR1b</i> xb42-26	None	96%	4	797	270	127
4b. <i>PdR1b</i> xb42-26	None	92%	12	3,255	985	237
4c. <i>PdR1b</i>	Ren1,Ren4,Run1	96%	1	136	25	1
4d. <i>PdR1b^2</i> xb42-26	Ren1,Ren4	94%	2	463	135	18
4e. <i>PdR1b</i> ^2xb42-26	(Ren1,Ren4)^2	90%	1	294	55	11
4g. (PdR1bxb42-26)^2	(Ren1,Ren4)^2	90%	2	595	125	31

Crosses made in 2017 in Table 5 represent 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 *vinifera* representation initiated by the seedlings planted earlier this year shown in Table 4a. The most promising selections would then be advanced to Foundation Plant Services (FPS) for certification and eventual release as the next iteration of our PD resistant winegrape breeding efforts.

Table 5. 2017 Crosses of elite *vinifera* cultivars to three resistant genotypes that have both the *PdR1b* and *PdR2* loci. Progeny will be 96% *vinifera*.

			Resistant
Desistant		Eat #	Parent Total Est
Parent	Vinifera Parent	Seeds	# Seeds
14309-002	Alvarelhao	15	
	Dolcetto	150	
	Fiano	10	
	Matero	45	
	Montepulciano	45	
	Palomino	45	
	Pedro Ximenez	45	
	Pinot noir FPS32	60	
	Pinot noir FPS77	45	
	Refosco	150	
	Sauvignon vert	150	
	Touriga nacional	150	910
14309-111	Dolcetto	150	
	Fiano	10	
	Matero	150	
	Montepulciano	10	
	Morrastel	45	
	Pinot noir FPS32	15	
	Pinot noir FPS77	10	
	Refosco	150	
	Touriga nacional	15	555
14388-029	Arneis	150	
	Montepulciano	15	
	Morrastel	150	
	Pedro Ximenez	150	
	Pinot noir FPS32	100	
	Pinot noir FPS77	115	
	Refosco	15	
	Sauvignon vert	300	995

We also completed the final BC4 generation in the PdR1c, b40-14 line (Table 6a). In Tables 6b and 6c we take two different approaches for combining PdR1b and b42-26 PD resistance. In the former, we take an approach similar to that in Table 5 but from different initial backcross generations and selections. This approach serves as insurance should we find b42-26 resistance resides significantly in genomic locations other than Ch 8. Rather than backcrossing in the PdR1b x b42-26 line as in Tables 5 and 6b, the resistance line profiled in Table 6c was backcrossed a second time to a different relatively resistant b42-26 progeny. This is with the expectation that carrying more b42-26 minor resistance factors deeper into the backcross generations may contribute a more genomically widespread PD resistance. The remaining crosses in Tables 6d-h combine PD resistance, either from PdR1b alone or in combination with b42-26 resistance with various PM resistance loci. Some of our most advanced lines in crosses represented here should be candidates for release. In Table 6d we have advanced single PdR1b PD resistance with *Ren1* and/or *Ren4* PM resistance. Crossing to these diverse elite *vinifera* should result in a wide range of possible selections. The challenges for the rest of the Table 6 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 Table 6d, 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 Table 6e and 6f integrate PM resistance from *Ren6* from *V. piasezkii* and *Run 2.1* from *Muscadina rotundifolia* into our PD resistant lines. Crosses in Table 6h are similar in result to those made last year in Table 4c, 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 5 and 6, 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 an estimated 90 seeds produced.

Table 6. 2017 Advanced PD and PD x PM resistant crosses with *vinifera* heritage, # crosses and estimated # of seeds produced. *Ren6* and *Run 2.1* PM resistance loci are rom *V. piasezkii* and *Muscadina rotundifolia* respectively. See Table 4 for details of other resistance loci.

		vinifera	%	#	Est. #
Cross PDR Type	Cross PM Type	parentgrandparents	vinifera	Crosses	Seeds
		Dolcetto, Fiano, Grenache			
		noir 224, Malvasia Bianca,			
		Montepulciano, Morrastel,			
		Pedro Ximenez and			
6a. b40-14	None	Touriga nacional	97%	9	985
		Arneis Morrastel			
6b <i>PdR1b</i> xb42-26	none	Palomino Pedro Ximenez	97%	4	90
			5170		
		Arneis, Dolcetto, Malvasia			
		Bianca, Montepulciano,			
6c. <i>PdR1b</i> xb42-26^2	none	Morrastel, Pedro Ximenez	93%	7	580
		Alvaralhao Malvasia			
		Rianca Morrastel			
6d PdR1b	Ren1 & Ren4	Sauvignon vert	98%	4	220
6e <i>PdR1b</i> xb42-26	Ren4 or Ren6	F2-35 Cab Chard Zin	90% 98%	4	490
			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		150
	<i>Ren1xRen4</i> or			_	
6f. PdR1bxPdR2	Ren1xRun2.1	Cab, Chard, Zin	93%,94%	5	107
	<i>Ren1xRen4</i> or		95%-		
6g. PdR1bxb42-26	Ren1xRun1	Cab, Chard, Zin	98%	6	700
6h. <i>PdR1b</i> xb42-26	Ren1xRen4xRun1	Cab, Chard, Zin	96%	3	495

CONCLUSIONS

We continue to make rapid progress breeding PD resistant winegrapes through aggressive vine training, markerassisted 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. Five selections are in preparation for release. They have cleared certification from Foundation Plant Services and cuttings have been sent to commercial nurseries for bulk-up, followed by commercial distribution in 203 years. 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.

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Talks at Grower Meetings (Extension/Outreach) August 2016 to July 2017

Grape breeding at UCD. Chilean table grape growers association, UCD Oct 3

Grape breeding above and below ground. Cal Poly San Luis Obispo, CA Oct 6

Grape breeding update. CDFA Industry Advisory Board, UC Davis, Nov 1.

PD resistant wines – lecture and tasting. Sacramento Private School Auction Prize, with Darrel Corti. UCD, Nov. 13

Breeding PD Resistant Winegrapes. Texas A&M, Driftwood, TX, Nov, 18

What are the next steps for the PD resistant wine grape breeding program? Vineyard Health Seminar, UCD, Nov. 29

PD Breeding program update. FPS Annual Meeting, UCD, Dec. 1, 2016

Progress in the Grape Breeding Program, Recent Advances in Viticulture and Enology, UCD, Dec. 9

Classical and molecular breeding to combat PD. CDFA PD / GWSS Board Annual Meeting, San Diego, CA, Dec. 13

The origin of grapes and grape breeding. 3rd Intl Symposium on Viticulture, Hermosillo, Mexico Jan 27 Origin of grapes and grape breeding. Daniel Roberts Grower Group Meeting, Santa Rosa, CA Jan 30

Breeding PD resistant winegrapes. Talk and tasting. DEVO Dept. Enology and Viticulture Student Organization, UC Davis, Feb. 12

Vineyard sustainability - rootstocks, irrigation, disease. Santa Cruz, Chile March 22

Breeding PD resistant winegrapes. Talk and tasting. Dept Vit & Enol. Executive Leadership Board, Santa Rosa, CA, May 5

The GRN rootstocks and breeding progress. San Pedro Winery, UC Davis, June 5

Breeding PD resistant winegrapes. Viticulture 101D.Adaptive Winemaking Extension Class, UC Davis, July 28

Presentations at Scientific Meeting

Fayyaz, L., S. Riaz and M.A. Walker. 2017. Map-based positional cloning of genes for powdery mildew resistance from the Chinese species *Vitis piasezkii*. ASEV National Meeting, Bellevue, WA, June 28

Huerta, K., S. Riaz and M.A. Walker. 2017. Evaluation of genetic diversity in wild *Vitis* material from northern and central Mexico. ASEV National Meeting, Bellevue, WA, June 28

- Uretsky and Walker. 2017. A preliminary examination of taxonomic and geographic relationships among accessions of *Vitis berlandieri* and associated taxa. ASEV National Meeting, Bellevue, WA, June 28
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Walker, MA. 2017. Vinifera hybrids and resistance to Pierce's disease. Lecture and tasting. ASEV-East Annual Meeting Charlottesville, VA, July 12

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 Foundation Plant Services and have been released to nurseries prior to patenting and sales to growers.

LAYPERSON SUMMARY

One of the most reliable and sustainable solutions to plant pathogen problems 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 elite 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 progeny 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 *Xylella 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 19 advanced selections to Foundation Plant Services (FPS) over the past five winters to begin the

certification and release process. The first 5 selections been sent to nurseries for bulk-up and then sales to growers. Three PD resistant rootstocks were also sent to FPS for certification. Other wild grape species are being studied and the resistance of some will be genetically characterized 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 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

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

ACKNOWLEGEMENTS

We thank Gordon Burns of ETS Labs in St. Helena, CA for continued support with grape berry chemical analysis and Ken Freeze of Brown and Miller for help arranging and coordinating the industry tastings.

Appendix. Details on 5 PD resistant selections that nurseries are now expanding for commercial plantings.



07355-075 – 50% Petite Sirah, 25% Cabernet Sauvignon

This red wine grape makes wines with characteristics of both Cabernet Sauvignon and Petite Sirah. It is established in large test plots along the Napa River and commercial scale wines have been made from this plot. This selection is one of the earliest to break dormancy, and it also blooms and ripens early. The berries are relatively large and the well-filled clusters are medium in size. This selection has been repeatedly tested in our greenhouse screen and is highly resistant to Pierce's disease. It is one of the few selections we will release at the 94% *V. vinifera* level, and has ranked highly at numerous tastings of both Davis and Napa grown fruit. Tasting notes include: dark-red purple color, bright red fruit, raspberry, cherry, ripe, tannic, elegant rather than dense.



09331-047 - 50% Zinfandel, 25% Petite Sirah, 12.5% Cabernet Sauvignon

This red wine grape is 97% *V. vinifera* and is highly resistant to Pierce's disease. It is established in field trials in Temecula and along the Napa River, where commercial scale wines have been made. It blooms relatively late, but ripens mid-season. The berries are medium and the clusters are well-filled and relatively large. Although this selection is spur fruitful it typically has only one cluster per shoot and is more productive with cane pruning. Wines from this selection have been ranked highly at numerous tastings of Davis and Napa grown fruit. Tasting comments include: medium dark red with purple; berry pie, cassis, black olive, herbal, dried hay, coffee, vegetal like Cabernet Sauvignon, licorice, round, moderate tannins, soft finish.



09356-235

50% Sylvaner, 12.5% Cabernet Sauvignon, Carignane, Chardonnay

This red wine grape selection is 97% *V. vinifera* and is also highly resistant to Pierce's disease in repeated greenhouse and field evaluations. It has a mid-season bloom and ripening period and has relatively large berries and loose clusters. It is highly productive. Wines have only been made from Davis fruit but they ranked very highly with tasting comments including: dark- red purple color; complex fruit with herbs and earth, plum, big wine, dense, rich middle, tannic yet balanced. This selection was also chosen by winemakers to have great blending potential with Cabernet Sauvignon. They also noted its high levels of high quality tannin.



09314-102 62.5% Cabernet Sauvignon, 12.5% Carignane, 12.5% Chardonnay

This white wine grape is 97% *V. vinifera* and highly resistant to Pierce's disease after repeated greenhouse evaluations. It has been tested in Temecula, Sonoma and along the Napa River. It has an early bloom and the fruit ripens early. It has small to medium berries and relatively large clusters, it is highly productive. The wines are reminiscent of Sauvignon blanc and tasting comments have included: light straw to clear color, citrus, lime, tropical, gooseberry golden delicious apple flavors; bright fruit, slightly bitter, textured.



09338-016 62.5% Cabernet Sauvignon, 12.5% Chardonnay and 12.5% Carignane

This white wine selection is also highly resistant to Pierce's disease after repeated greenhouse evaluations. Wines have been made from Davis fruit, but it has not been planted in other field trials yet. It has small berries and small compact clusters. It blooms relatively late and ripens mid-season. The vine has medium productivity. We have made wines from Davis-grown fruit and they have ranked well. Tasting comments include: light straw-gold color, apple-melon, lychee, floral aromas, pineapple, green apple, juicy, harmonious, well-balanced.