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

**REPORT TITLE:** Interim Progress Report for CDFA Project 14-0137-SA

**PROJECT TITLE:** Molecular breeding support for the development of Pierce's disease resistant winegrapes.

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## **INTRODUCTION**

This project provides molecular support to the PD resistance grape breeding project – "Breeding Pierce's disease resistant winegrapes" by acquiring and testing a wide range of resistant germplasm, tagging resistance regions with markers generated from genetic mapping and functionally characterizing the resistance genes from different backgrounds. To meet key objectives of the program, we have surveyed over 250 accessions of Vitis species growing in the southern US and Mexico in an effort to identify new PD resistant accessions. Analysis using population genetics tools allowed us to better understand gene flow among resistant species and their taxonomic and evolutionary relationships. Twenty resistant accessions were identified from a screening of the above 250 accessions. Markers were used to determine their genetic diversity and relationship to each other. Small breeding populations were developed and more than 700 seedlings were marker tested to ensure correct parentage and identity. We used a limited mapping strategy with markers from chromosome (Ch) 14 in conjunction with greenhouse screen data of small breeding populations to determine if resistance to PD is different from the previously identified locus PdR1 (Riaz et al. 2018 "Genetic Characterization of Vitis Germplasm Collected from the Southwestern US and Mexico to Expedite Pierce's Disease Resistance Breeding" recently accepted for publication in Theoretical and Applied Genetics). Three new unique resistance sources (T03-16, ANU67 and b41-13) were identified as having a resistance region on a chromosome other than Ch14. More crosses were made in Spring 2016 and 2017 to expand these breeding populations for map-based identification of genomic regions that contribute to resistance.

The identification and characterization of resistance genes and their regulatory sequences will help determine the basis of resistance/susceptibility in grape germplasm, and help predict their longevity as resistance genes. In addition, these genes and their promoters could be employed in production of 'cisgenic' plants. Cisgenesis is the transformation of a host plant with its own genes and promoters (Holmes et al. 2013). We have completed the physical map of PdR1a and PdR1b locus for b43-17 to clone and characterize resistance genes (see earlier reports). The physical map of the PdR1c locus (from b40-14) is also complete. Development of V. vinifera plants transformed with our PD resistance genes will allow us to better understand the function of PdR1 and might allow a crop improvement strategy that would be less disruptive of the winegrape it was inserted into (although it may not provide the same levels of resistance). Cisgenic strategies might also be less objectionable in terms of a transformation procedure.

Upstream and downstream sequences as well as gene sequences of two candidate genes, ORF14 and ORF18, from PdR1b were verified and constructs were developed. Transformation experiments with the PdR1 resistance gene with a native grape promoter were completed with ORF18 and transgenic lines are being developed and

maintained for later resistance verification. A large scale multiple time point gene expression project was completed in the greenhouse and RNA extractions were completed for over 400 samples. We used qPCR to test the expression of candidate genes. Embryogenic callus cultures of *V. vinifera* cvs. Chardonnay and Thompson Seedless and *V. rupestris* St. George are being maintained. These efforts will help us to identify candidate resistance genes by complementation (transforming them into susceptible grape varieties) and better understand how they function.

# **OBJECTIVES**

- 1. Provide genetic marker testing for mapping and breeding populations produced and maintained by the PD resistance-breeding program, including characterization of novel forms of resistance.
- 2. Complete a physical map of the *PdR1c* region from the b40-14 background and carry out comparative sequence analysis with b43-17 (*PdR1a* and *b*).
- 3. Employ whole genome (WGS) sequencing (50X) of recently identified PD resistant accessions and a susceptible reference accession; use bioinformatics tools to identify resistance genes, perform comparative sequence analysis and develop SNP markers to be used for mapping.
- 4. Clone *PdR1* genes with native promoters.
- 5. Compare the PD resistance of plants transformed with native vs. heterologous promoters.

## **RESULTS AND DISCUSSION**

**Objective 1.** Provide genetic marker testing for mapping and breeding populations produced and maintained by the PD resistance breeding program, including characterization of novel forms of resistance.

Greenhouse testing was completed for over 250 southwestern and northern Mexico Vitis, which included accessions, collected from multiple collection trips from States bordering Mexico or previously collected from Mexico by Olmo. Both SSR (simple sequence repeat) and chloroplast markers were used to establish relationships with known sources of resistance currently being used in the breeding program (Riaz and Walker 2013). Small breeding populations were developed with the 14 most promising resistant accessions by crossing to highly susceptible V. vinifera. In Spring 2016, we extracted DNA from the 704 individuals obtained from these breeding populations that been greenhouse screened. We carried out a limited mapping strategy by utilizing markers from Ch14 that are linked to the PdR1 locus (See previous reports for details of the PdR1 locus). This strategy allowed us to identify resistance sources whose resistance is similar to PdRI and sources that are different among the newly identified accessions. Twelve SSR markers that cover a 3.5 Mb (megabase) region including the PdRI locus and genotypic data with 22 markers from 19 chromosomes were used to analyze how genetically distinct the resistant accessions were from each other. Based on the polymorphic markers for each breeding population, a genetic map was created to determine the relative marker order and then QTL analysis for each population was carried out. The results from this study identified nine accessions with a major resistance locus within the genetic window that the PdR1 locus from accession b43-17 maps to (Table 1). Results were not conclusive for two accessions A14 and b47-32 due to small population size an/or lack of polymorphic markers. The phenotypic data of three accessions, ANU67, b41-13, and T03-16 did not correlate with the resistance markers from Ch14. These three accessions were identified as candidates for further work to develop framework maps with larger populations to detect new unique loci for PD resistance breeding. The small breeding populations used in this study effectively identified the presence or absence of a major resistance locus. This approach is being used to enhance the PD resistant winegrape breeding program by rapidly identifying new resistance loci and broadening the genetic base of resistance. The major findings of this work were recently accepted for publication in Theoretical and Applied Genetics. We completed propagation of 4-5 replicates for the subset of crosses mentioned in Table 1. These plants will be inoculated with X. fastidiosa in September and the results of the assay will be available in Winter 2018.

Table 1. Resistant accessions used for the 23 breeding populations. Accessions marked as Ch14 possess the PdR1 locus. Those noted as Inconclusive could not be determined because of small populations, limited marker data or possession of a unique resistance source.

Resistance	Species description	Populations tested	Number of	<b>Results of</b>
source			Screened	Limited

			Genotypes	mapping
ANU5	V. girdiana	12-314	60	CH14
b40-29	<i>V. arizonica</i> , brushy	12-340, 12-341, 14- 367, 14-368	29	CH14
b46-43	<i>V. arizonica</i> , glabrous with <i>V. monticola</i>	12-305, 14-308, 14- 321, 14-322, 14-324, 14-336	159	CH14
b41-13	<i>V. arizonica-mustangensis</i> and <i>champinii</i> hybrid, red stem with hairy leaves	13-355	47	Inconclusive
b47-32	<i>V. arizonica</i> glabrous with <i>monticola</i> , small clusters, red stem	13-344	13	Inconclusive
SC36	V. girdiana	13-348	35	CH14
T03-16	<i>V. arizonica</i> glabrous	13-336	62	Inconclusive
A14	V. arizonica	14-313	25	Inconclusive
A28	V. arizonica	14-347, 14-364	42	CH14
ANU67	V. arizonica glabrous	14-362	28	Inconclusive
ANU71	<i>V. arizonica-riparia</i> hybrid	14-340	30	CH14
C23-94	<i>V. arizonica</i> glabrous and brushy	14-303	44	CH14
DVIT 2236.2	<i>V. cinerea</i> like, long cordate leaves, short wide teeth, small flower cluster	14-360	30	CH14
SAZ 7	V. arizonica	14-363	52	CH14

We have also identified a new locus *PdR2* in the *V. arizonica/girdiana* b42-26 background. To create a genetic map of the F1 population 05347 (F2-35 x b42-26), we expanded the population to 352 seedling plants and tested more than 1,000 markers. The level of polymorphism in b42-26 is very low likely because of its geographic isolation and resulting inbred genetic nature (this accession was collected in an isolated area of Baja California). The genetic map was developed with 163 markers from 17 chromosomes. Chromosome 10 and 19 were not represented. We carried out analysis with this map and identified resistance on Ch8, which was also verified on the basis of linked alleles in the pBC1 and pBC2 populations. We named the resistance locus *PdR2* and it resides between markers FAM82 and VMC 7h2.

In Spring 2017, we began using closely linked markers to assist the breeding program with marker-assisted selection (MAS) to stack the *PdR1*b and *PdR2* loci together. Additional markers from Ch10 and 19 were also tested to get complete representation of the genome for the final genetic map and QTL analysis. A manuscript detailing genetic mapping in b42-26 and b40-14 is approaching publication.

This project also provides molecular support to the companion PD breeding project by marker testing seedling plants. In Spring 2017, we marker tested 1,895 seedling plants, from 23 different crosses, for the PdR1 and PdR2 loci. A total of 1,380 seedlings were tested for both loci and 515 seedlings were tested for the PdR1 locus alone. A total of 902 seedling plants from 14 different crosses were tested for trueness to type. In total, we extracted DNA from 2,797 seedling plants for different PD resistance breeding projects.

**Objective 2.** Complete a physical map of the PdRlc region from the b40-14 background and carry out comparative sequence analysis with b43-17 (source of PdRla and PdRlb).

QTL analysis with the SSR-based genetic map of *V. arizonica* b40-14 identified a major PD resistance locus, PdR1c, on Ch14 (see previous reports for details). The genomic location of the PdR1c locus is similar to the PdR1a and PdR1b loci. An additional 305 seedlings were marker tested to identify unique recombinants using

new SSR markers developed from the b43-17 sequence to narrow the genetic mapping distance. Four recombinants were identified between Ch14-81 and VVIn64, and one recombinant between the Ch14-77 and Ch14-27 markers. The new markers positioned the *PdR1c* locus in a 325 Kb (kilobase) region based on the sequence of b43-17.

A BAC (bacterial artificial chromosome) library from b40-14 genomic DNA (see details in previous reports) was screened and 30 BAC clones were identified with two probes, Ch14-56 and Ch14-58. BAC clones that represent PdR1c were separated from the other haplotype and four overlapping BAC clones VA29E9, VA57F4, VA30F14, and VA16J22 were selected for sequencing. Probes in common with the PdR1c and PdR1b regions were used to align the sequences. The assembly of four BAC clones is presented in Figure 1 and Figure 2 represents the sequence analysis of PdR1b and the reference grape genome PN40024 region. A manuscript entitled "The physical map of the PD resistance locus, PdR1c" is in preparation.



Fig. 2. The sequences of four BAC clones were assembled and full-length open reading frames were identified. Sequences were compared with the reference genome and checked for synteny in that region. Analysis is currently being carried out with the Cabernet Sauvignon genome sequence.



The assembly of H43-I23 from the b43-17 BAC library, which represents the PdR1a haplotype (F8909-17) was also completed. The length of the assembled sequence was 206Kb. The open reading frames (ORF) of the PdR1b region and the BAC clone H69J14 were used to make comparisons. There was complete homology between the over-lapping BAC clone sequences that reflect the two different haplotypes. The BAC clone H43I23 has ORF16 to ORF20 and all five ORFs have identical sequences to the PdR1b haplotype. Based on these results, we concluded that there is complete sequence homology between haplotype a, and b of the PdR1 locus; therefore cloning and functional characterization of genes from any one haplotype will be sufficient for future work. Complete sequence homology also suggests that the parents of b43-17 must be closely related and may have a

first-degree relationship and acquired resistance from shared parents. This also explains why we observed complete homozygosity of SSR markers for the *PdR1* locus in the resistant accession b43-17.

**Objective 3.** Employ whole genome sequencing (WGS) (50X) of recently identified PD resistant accessions and a susceptible reference accession; use bioinformatics tools to identify resistance genes, perform comparative sequence analysis and develop SNP markers to be used for mapping.

In this proposal and as detailed in previous reports, we proposed to use WGS to genetically map two new resistant accessions, b46-43 and T03-16, both of which have very strong *X. fastidiosa* resistance in repeated greenhouse screens. Next generation sequencing using IIlumina HiSeq and MiSeq platforms to carry out SNP discovery and identification of SNP markers linked to resistance would only be used with those resistant lines for which we have strong greenhouse screen information, information on the heritability of their PD resistance, and the potential to screen the population using a limited mapping strategy.

The *V. arizonica* accession b46-43 is homozygous resistant to PD. Multiple crosses to *V. vinifera* were made to develop BC1 populations in 2014 and 2015. Breeding populations were tested with markers to verify the integrity of the crosses. Greenhouse screening of the BC1 populations with b46-43 and other resistance sources was completed (see companion project report) and results were used in conjunction with markers from Ch14 to evaluate the correlations between markers and resistance. Preliminary results indicate that there is a major PD resistance locus on Ch14. However, our breeding program has already identified two other accessions that have a major PD resistance locus on this chromosome. In order to optimize the development of broadly resistant PD winegrapes, we need to use PD resistance sources that map to different regions so that we have the greatest chance of stacking resistance genes from multiple and diverse sources. Test results suggest that b46-43 is not a unique source of PD resistance since it maps to the same location as *PdR1*, although it does have very strong resistance to *X. fastidiosa*. In the light of these results, we will not pursue WGS to map in the b46-43 background.

We completed a map of Ch14 for the BC1 mapping population, and completed greenhouse screening of the 121 seedling plants in this population. QTL analysis results indicated that the locus explains only ~42% phenotypic variation indicating that there might be another resistance locus on a different chromosome (Fig. 3). Figure 4. presents the correlation of different phenotypic parameters we have used in the pBC1 population. Currently we are repeating the greenhouse screen and expanding the mapping effort to develop a framework map of all chromosomes to identify any other genomic region that contributes to the resistance.

Fig. 3. QTL analysis results of interval mapping in the pBC1 14399 population for Ch14. The arrow represents the maximum LOD for marker ch14-78 and the percentexplained variation for PD resistance. The red dotted line is the LOD threshold for a significant QTL call. Mapped markers are on the x-axis



Fig. 4. Comparison between resistant (R) and susceptible (S) genotypes in each measured phenotypic parameter. Significant differences with Tukey's test are indicated with letters a and b. The letter 'n' denotes the number of genotypes screened.



**Objective 4.** Cloning of *PdR1* genes with native promoters.

We employed PAC BIO RSII sequencing approach to sequence H69J14 and three other overlapping BAC clones containing both markers flanking the *PdR1b* resistance locus. The assembled sequence data generated a 604 Kb long fragment without any gaps. Multiple ORFs (open reading frames) of the Leucine-Rich Repeat Receptor Kinase gene family were identified. These genes regulate a wide range of functions in plants including defense and wounding responses for both host and non-host specific defense. With the help of molecular markers, we limited the genetic region to 82 Kb – with 5 ORFs associated with disease resistance and other plant functions described above. ORF sequences found outside the 82 Kb window are also highly similar. Two ORFs V.ari-RGA14 and V.ari-RGA18, within the resistance region boundaries, are the most likely candidates for *PdR1b*. The other 3 sequences, V ari-RGA15, 16 and 17, are shorter and contain a large number of transposable elements (TE).

Both RGA14 and 18 (resistance gene analogs) have a very similar sequence profile except that RGA-18 is 2.946bp in size and lacks the first 252 bp of sequence that is part of RGA14. Functional analysis of both RGAs revealed that RGA-14 lacks a signal peptide in the amino terminal of the protein. This result was verified using 3'RACE (rapid amplification of cDNA ends) to specifically amplify RNA from grapevines transformed with V.ari-RGA14 under the 35S promoter. The results found that mature mRNA does not contain an N-terminal signal peptide necessary for proper membrane localization, thus leaving RGA-18 as the strongest candidate. Sequence verification for RGA14 and RGA18 and flanking sequences was completed and fragments that contain the entire coding region plus  $\sim$ 3 Kb upstream and  $\sim$ 1 Kb downstream sequences were synthesized and cloned into pCLB2301NK at Genewiz. Inc. pCLB2301NK is an optimized vector (Feechan et al. 2013), capable of carrying large DNA sequences, thus allowing us to insert the candidate genes plus surrounding sequences.

New plasmids, called pCLB2301NK-14 and pCLB2301NK-18, were verified by restriction analysis in our lab (Figure 5). Besides the corresponding 7 Kb fragment, containing RGA14 or RGA18, these plasmids contain a 35S:mGFP5-ER reporter cassette and a kanamycin-selectable marker gene with the NOS promoter.



Figure 5. (a) Restriction analysis of plasmids pCLB2301NK-14 (lanes 2,3, 4) and pCLB2301NK-18 (lanes 5, 6, 7) after digestion with Nhe1 (lanes 2, 5), Sac1 (lanes 3, 6) and Sal1 (lanes 4, 7). Gel image includes a 1kb ladder (lane 1) with the 3 kb fragment having increased intensity to serve as a reference band. The results on the gel match the predicted sizes inferred from the plasmid information; (b) pCLB2301NK-14 restriction map; (c) pCLB2301NK-18 restriction map.

We sequenced genotype U0505-22, which is used as a biocontrol in our greenhouse screenings. This genotype was originally selected for the presence of *PdR1b* markers in our breeding program. However. U0505-22 is susceptible to PD despite being positive for the markers, which then offers the opportunity to explore the changes that could explain this behavior at the DNA level. Primers were designed to produce 3 Kb fragments that include sequences upstream and downstream of RGA-14 or RGA-18 in order to increase the specificity of the amplification and facilitate cloning. Results obtained with U0505-22 showed the amplification of fragments of the predicted size, but with sequences that differ from RGA14 and RGA18 in several bases. On the other hand, sequencing of cDNA from b43-17, the original source of resistance, 16 days after inoculation, resulted in the amplification of fragments that comprise sequences identical to RGA-14 but different from RGA-18.

A large experiment with resistant and susceptible plants using multiple replicates, and time points for control (uninoculated) and inoculated plants (see details in previous report) was completed. To date, we have completed RNA extractions from 450 samples in the above-mentioned experiment. We have also designed primers and determined primer efficiency for gene expression studies with both RGA14 and RGA18. Two different primer pairs with efficiency of greater than 90% were selected to carry out preliminary analysis with un-inoculated and inoculated samples of Chardonnay and F8909-17 (source of PdR1). Preliminary results with samples from six time points indicates that the expression level of both RGA14 and RGA18 in F8909-17 increases after day 8 in comparison to un-inoculated, which peaks at day 23 and then decreases. Un-inoculated and inoculated susceptible Chardonnay did not show any change in expression. Gene expression and cDNA sequence analysis is underway.

**Objective 5.** Comparing the PD resistance of plants transformed with native vs. heterologous promoters.

We have established an *Agrobacterium* mediated transformation system followed by regeneration of plants from embryogenic callus. We have streamlined the protocol and have established cultures of pre-embryogenic callus derived from anthers of *V. vinifera* Thompson Seedless (TS), Cabernet Sauvignon, Chardonnay (CH) and the rootstock *V. rupestris* St. George (SG) (Agüero et al. 2006). In an earlier phase of this project, we transformed these varieties with five candidate genes containing the 35S cauliflower mosaic virus promoter, the nopaline synthase terminator and an *hptII*-selectable marker gene (see previous reports for details). We completed testing and found that the transgenic plants did not confer PD resistance or tolerance. These results are in accordance with the latest assembly obtained using PAC BIO SRII system. They show that only one of the sequences tested, V.ari-RGA-14, lays within the more refined resistance region of 82 Kb. The 3'RACE technique was used to amplify RNA from V.ari-RGA14 transformed grapevines and results showed that mature mRNA does not contain the N-terminal signal peptide needed for proper membrane localization. However, this could result from a lack of effect of 35S on splicing. In addition to the embryogenic calli of TS, CH, CS and SG we have available for transformation, we developed meristematic bulks (MB) of these genotypes plus 101-14 Mgt for transformation via organogenesis (Figure 5). Slices of MB can regenerate transformed shoots in a much shorter period of time than somatic embryos. We have tested different media and selective agents and established protocols for the initiation, maintenance and genetic transformation of MB from these 5 genotypes (Xie et al. 2016). Meristematic bulk induction in non-*vinifera* genotypes is less efficient, but still high, with about 80% of the explants producing MB after 3 subcultures in medium containing increasing concentrations of cytokinins.



Figure 6. Embryogenic cultures (top) and meristematic bulks (bottom) of CH, TS, CS, SG, 101-14 and accession 29-42 from the 04-191 population.

In order to include native promoters and terminators in constructs for future genetic transformations, we verified sequences upstream and downstream of V.ari-RGA14 and 18, the two most likely *PdR1b* candidates. Sequence verification was completed up to 4-6 Kb in the upstream region and 1 Kb in the downstream region. In silico analysis of the upstream regions with PlantCare, a database of plant cis-acting regulatory elements, showed that upstream sequences contain several motifs related to drought and defense responses.

Transformations with *Agrobacterium tumefaciens* carrying binary plasmids that contain hygromicin (pCLB1301NH) or kanamycin (pCLB2301NK) selectable marker genes showed that both antibiotics are effective selection agents for embryogenic calli. However. MB regeneration has mainly occurred in selection with kanamycin, confirming our previous observation that MB are highly sensitive to hygromicin. Thus, pCLB2301NK was chosen to carry RGA14 and RGA18 expanded sequences and named pCLB2301NK-14 and pCLB2301NK-18 thereafter.

*Agrobacterium tumefaciens* strain EHA 105 pC32 was chemically transformed with pCLB2301NK-14 or pCLB2301NK-18 and subsequently used to transform embryogenic calli of Chardonnay, Thompson Seedless and St. George. Transformation experiments with pCLB2301NK-18 and pCLB2301NK-14 were initiated in March and July 2016 respectively, after synthesis and cloning was completed. In addition, *Agrobacterium* was used to transform meristematic bulks (MB) of PD susceptible\_genotypes selected from the 04-191 population, which are 50% *vinifera*, 25% b43-17 and 25% *V. rupestris* A. de Serres (as in the original population used for *PdR1b* mapping). These genotypes can provide an additional genetic background for analysis of expression of *PdR1* candidate genes. Two of these genotypes, designated 29-42 and 47-50 exhibited great potential for the development of MB (Figure 6) and transformation experiments with *Agrobacterium* have been initiated. Table 2 shows the number of independent lines regenerated up to date. Transformation was checked through PCR in plants transferred to the greenhouse. Primers that bind the promoter and coding regions of RGA14 or RGA18 were used for amplification. DNA fragments amplified successfully in all the lines tested (Figure 7). Transformation was also verified by fluorescence microscopy to visualize GFP, since pCLB2301NK-18 and pCLB2301NK-14 also contain a 35S:GFP5-ER cassette.

Genotype	No. lines <i>in</i> <i>vitro</i>	No lines in greenhouse
pCLB2301NK-18		
Chardonnay	13	11
T. Seedless	30	11
St. George	4	4
29-42	1	-
pCLB2301NK-14		
Chardonnay	20	11
T. Seedless	18	10
St. George	4	4

Table 2. Number of independent lines regenerated after transformation with Agrobacterium carrying pCLB2301NK-18 or pCLB2301NK-14.



Figure 7. Transgene detection of RGA18 through PCR (left) and GFP fluorescence (right), UN: untransformed Chardonnay (CH) and Thompson Seedless (TS), 1-10: transgenic lines.

V.ari-RGA18 lines in the greenhouse were multiplied from green cuttings and were inoculated with the Beringer strain of *X. fastidiosa* in August 2017. Symptoms of PD were evaluated using a 0-5 score for leaf scorch-leaf loss (LS-LL) and a 0-6 score for cane maturation index (CMI). For ELISA, plants were sampled 12 weeks post-inoculation by taking 0.5 g sections of stem tissue from 30 cm above the point of inoculation (Krivanek and Walker 2005, Krivanek et al. 2005). Table 3 shows that all RGA18 transgenic lines tested displayed disease symptoms with different degrees of intensity. Lines CH-18-2 and CH-18-7 showed the lowest cane maturation index and leaf scorching (Figure 8). Bacteria concentration in these lines was lower than in the untransformed control but not as low as the resistant biocontrols (Table 3). Thompson Seedless was considerably more susceptible than Chardonnay.

Table 3. Greenhouse screen results for Chardonnay and Thompson Seedless transformed with V.ari-RGA18. The first six genotypes correspond to the negative control and resistant biocontrols. CH-0 and TS-0 are untransformed Chardonnay and Thompson Seedless, respectively.

			Std				
		ln	Error	CMI	CMI	LS-LL	LS-LL
Gentoype	cfu/ml	cfu/ml	(cfu/ml)	Mean	Std Err	Mean	Std Err
CH uninoc	10.034	9,2	0,00	0,0	0,00	0,0	0,00
b43-17	23.416	10,1	0,32	2,1	0,48	2,9	0,48
U0505-01	37.499	10,5	0,62	0,3	0,15	1,8	0,41
U0505-35	100.798	11,5	0,83	0,3	0,33	1,5	0,43

Blanc du Bois	194.385	12,2	0,46	2,1	0,49	1,9	0,49
Roucaneuf	245.426	12,4	0,78	0,3	0,33	0,8	0,40
U0505-22	760.190	13,5	0,27	2,3	0,71	2,6	0,43
CH-0	6.119.118	15,6	0,42	3,6	0,60	1,6	0,53
CH-18-1	4.636.369	15,3	0,18	1,0	0,63	1,8	0,20
CH-18-2	2.078.921	14,5	0,42	0,8	0,58	2,4	0,51
CH-18-3	6.152.629	15,6	0,04	2,6	0,81	2,4	0,40
CH-18-4	4.686.410	15,4	0,06	3,4	0,82	0,9	0,11
CH-18-5	5.562.260	15,5	0,10	1,6	0,24	2,8	0,58
CH-18-6	4.888.719	15,4	0,18	1,0	0,55	3,0	0,32
CH-18-7	1.786.455	14,4	0,60	0,0	0,00	1,8	0,58
CH-18-8	6.500.000	15,7	0,00	2,2	0,58	3,2	0,37
TS-0	6.500.000	15,7	0,00	4,2	0,20	5,0	0,00
TS-18-1	6.500.000	15,7	0,00	4,6	0,40	4,6	0,40
TS-18-2	6.500.000	15,7	0,00	4,0	0,32	5,0	0,00
TS-18-3	6.500.000	15,7	0,00	4,0	0,32	4,4	0,24
TS-18-4	6.500.000	15,7	0,00	5,0	0,45	4,8	0,20
TS-18-5	6.500.000	15,7	0,00	4,0	0,00	4,6	0,24
TS-18-6	6.500.000	15,7	0,00	4,2	0,66	4,2	0,20
TS-18-7	6.500.000	15,7	0,00	3,6	0,75	4,6	0,24
TS-18-8	6.500.000	15,7	0,00	5,0	0,45	5,0	0,00
TS-18-9	6.500.000	15,7	0,00	4,2	0,37	4,8	0,20
TS-18-10	6.500.000	15,7	0,00	4,6	0,40	5,0	0,00
TS-18-11	6.500.000	15,7	0,00	4,2	0,73	4,8	0,20
СНО		CI	4 18 2		СН 18-7		



Figure 8. Lignification observed in nodes collected 40 cm above POI, 3 months after inoculations. CH-0 is untransformed Chardonnay.

qPCR analysis to determine the correlation between the level of transgene expression and symptom/bacteria concentrations is underway.

Testing of V.ari-RGA14 lines began this winter; 11 Chardonnay and 10 Thomson Seedless V.ari-RGA14 lines, plus 3 additional lines of Chardonnay RGA18, were inoculated with the Beringer strain of *X. fastidiosa* in March 2018. Testing of transformed St George will start in July, 2018.

## CONCLUSIONS

We completed greenhouse screening, marker testing and QTL analysis of breeding populations from 15 new resistance sources including b46-43 and T03-16. We identified two accessions, T03-16 and b41-13, with resistance on a different region than chromosome (Ch) 14, on which our PdR1 resistance gene resides. Crosses were made to expand these breeding populations for framework map development in order to identify other genomic regions of resistance. Our primary goal was to identify new sources of resistance that do not reside on

Ch14 so that we can facilitate stacking of these resistance sources with PdR1 from b43-17, since the incorporation of multiple resistances should make resistance more durable. We have also identified a new resistance locus PdR2 from the b42-26 background and closely linked markers are being used in marker-assisted selection (MAS) to stack resistance loci from these different backgrounds. We have completed the genetic and physical mapping of PD resistance from b40-14. This resistance source maps within the PdR1b locus, but it may be an alternative gene within this complex replicated locus. Finally, we verified the sequence of two candidate genes from the PdR1b locus, completed transformations with ORF18 and ORF14 and obtained transgenic lines for complementation tests in the greenhouse, some of which were completed last Fall and others will be completed later this Spring. This effort is also identifying the promoters of these genes so that we can avoid the use of constitutive non-grape promoters like CaMV 35S.

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#### Presentations that included PD breeding and genetics of resistance

UCD grape breeding program update. FPS Annual Meeting, Dec 1, 2016

Progress in the grape breeding program. Vine Health Seminar, UCD ARC, Dec 9, 2016

Breeding grapes to adapt to climate change. 3<sup>rd</sup> International Symposium on Grapes, Hermosillo, Sonora, Mexico, Jan 27, 2017

- The origin of winegrapes. Daniel Roberts Client Group Seminar, Martinelli Winery, Santa Rosa, CA, Jan 30, 2017
- Vineyard challenges, Wine Executive Program, UCD Business School, Mar 28, 2017
- Breeding PD resistant winegrapes/**Tasting**, Executive Leadership Board, Lyn-Mar Winery, Sebastopol, May 5
- Grape breeding update, CDFA IAB meeting, June 2, 2017
- Grape breeding in CA, Vina San Pedro growers, UCD, June 5, 2017
- Grape breeding in CA. Provedo Nursery Spain, UCD, July 17, 2017
- Grape breeding and UCD tour, Lake County Growers tour (Paul Zellman), UCD, July 19, 2017
- Breeding Pierce's-disease-resistant winegrapes, Wine 101: Getting the Most Out of Your Site, UCD Conference Center, UCD, July 28, 2017
- What are the next steps for the PD resistant wine grape program? Current Issues in Vineyard Health, UCD Conference Center, Dec. 5, 2017
- PD resistant winegrapes/Tasting, Roberts Viticultural Group, Martinelli Winery, Santa Rosa, Jan. 12, 2018.
- PD resistant winegrapes: development and tasting/**Tasting**, Georgia Winegrape Association, Chateau Elan, Braselton, GA, Jan. 24, 2018.
- Understanding Plant Material Selection for Vineyard Redevelopment: including rootstock and plant material selection and soil pest and virus considerations. Gallo Growers, Fresno, CA Feb. 15, 2018
- Understanding Plant Material Selection for Vineyard Redevelopment: including rootstock and plant material selection and soil pest and virus considerations. Gallo Growers, Lodi, CA, Feb. 16, 2018
- UCD Grape Breeding Program Update. Current Grape and Wine Research. UC Davis, Feb 21

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- Fayyaz, L., S. Riaz, R. Hu, M.A. Walker. 2017. Map-based positional cloning of genes for powdery mildew resistance from the Chinese species *Vitis piasezkii*. 68<sup>th</sup> ASEV National Meeting, Bellevue, WA, June 29
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- Walker, M.A. 2017. *Vinifera* hybrids and resistance to Pierce's disease/**Tasting**. ASEV Eastern Section Meeting, Charlottesville, VA, July 12
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#### **RESEARCH RELEVANCE**

The goal of this research is to understand the genetics of PD resistance and provide genetic support to our PD resistance breeding of wine grapes. We successfully mapped the resistance genes from a form of *V. arizonica* and used the linked markers to greatly expedite our breeding program. We are now searching for additional forms of PD resistance in other species from a variety of geographic locations across the southern US and Mexico, with the goal of combining different resistance from several species to ensure durable resistance

#### LAYPERSON SUMMARY

Our main focus is to identify and genetically characterize unique PD resistance sources from our southwestern US and Mexican *Vitis* species collections. In order to carry out the task, we create genetic maps that associate regions of chromosomes with PD resistance. These regions (markers) are used to expedite screening for resistance since they can be used to test seedlings for resistance as soon as they sprout. Markers developed from different sources of resistance allow us to combine multiple resistance forms and therefore produce offspring that will have broader

and more durable resistance against an evolving bacterial pathogen. These markers also allow us to identify resistance genes and engineer them into susceptible grapes, which we are doing to better understand the genetics and mechanisms of resistance.

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.

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