California Department of Food and Agriculture PD/GWSS
Progress Report
March 2012

Report title: Renewal Progress Report for CDFA Agreement Number 03-0282 Amend 6

Project Title: Genetic mapping of *Xylella fastidiosa* resistance gene(s) in grape germplasm from the southern United States.

.

Principal Investigator and Cooperating Staff: Andrew Walker (PI) and Summaira Riaz, Dept. of Viticulture and Enology, University of California, Davis, CA 95616; awalker@ucdavis.edu

Reporting period: October 2011 to March 2012

SUMMARY

We are genetically mapping three different genotypes that carry PD resistance genes, and then are developing markers that are tightly linked to resistance so that they can be used to expedite breeding with marker-assisted screening. Genetic mapping in two different forms of *Vitis arizonica* has identified a PD resistance region on chromosome 14, which we named *PdR1*. We have mapped two forms of *PdR1* from *V. arizonica/candicans* b43-17, identified a minor gene on chromosome 19 (*PdR2*) and have mapped a third form, *PdR1c*, originating from *V. arizonica* b40-14.

We have intensified efforts with a third resistance source – *V. arizonica/girdiana* b42-26, which is unique in that its resistance seems to be controlled by multiple genes. Mapping efforts with this resistance source have used 700 SSR markers; 125 were polymorphic and were added to the entire population of 239 seedlings. The first round of genetic mapping was done to assess the coverage across all 19 chromosomes. Markers grouped in a manner consistent with other maps, and 117 of the 125 markers mapped on 15 linkage groups. Greenhouse screening was completed for 164 seedlings, however ELISA results were highly variable, likely due to wide fluctuations in greenhouse temperatures. Preliminary QTL analysis indicated that chromosome 12 and 14 are involved in PD resistance. We are now focused on refining the genetic map to get better coverage of all chromosomes, and obtaining better greenhouse screening data for this population. The overall goal is to have markers for the b42-26 resistance source so that we can combine this source with *PdR1* to ensure broad and durable resistance to PD.

These mapping efforts are essential to physically locating and characterizing PD resistance genes. We have also expanded the search of plant material that carries resistance to PD. In the first round of testing we screened 52 accessions collected from different parts of the southern US and Mexico. Greenhouse testing identified 20 accessions that are promising enough to develop breeding populations, determine the inheritance of their resistance, create framework maps and identify resistance regions for marker development to facilitate complexing resistance from multiple sources in the PD resistance breeding program. This project provides the genetic markers critical to the successful classical breeding of PD resistant wine, table and raisin grapes. Identification of markers for *PdR1* has allowed us to reduce the seed-to-seed cycle to 2 years and attain the 97% *vinifera* PD resistant level. These markers have also led to the identification of six genetic sequences that house the PD resistance gene, which are now being tested to verify their function. These efforts will help us better understand how these genes function and could also lead to PD resistance genes from grape that would be available to genetically engineer PD resistance in *V. vinifera* cultivars

OBJECTIVES

1. Complete mapping of additional QTL for 04191 (F2-7 x F8909-17 (resistance from *V. arizonica/candicans* b43-17)) population
2. Greenhouse screen and genetically map PD resistance from other forms of *V. arizonica*: b42-26 (*V. arizonica/girdiana*) and b40-14 (*V. arizonica*).
3. Evaluate *Vitis* germplasm collected (250 accessions) from across the southwestern US to identify accessions with unique forms of PD resistance for grape breeding. Determine the inheritance of PD

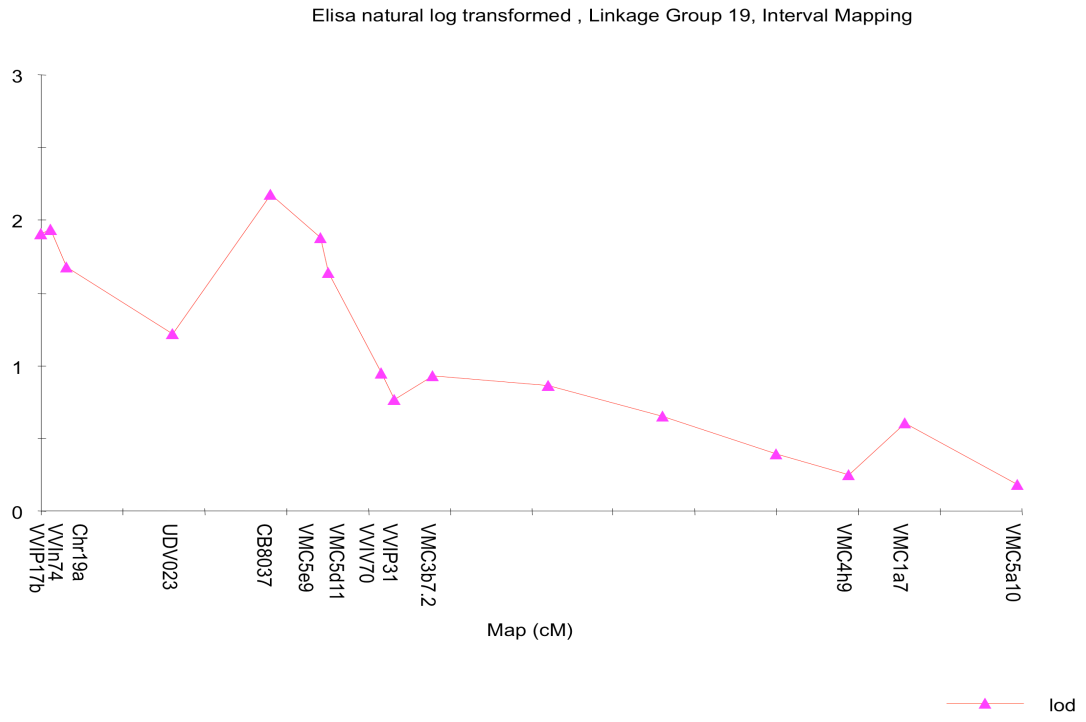
resistance from *Muscadinia rotundifolia*, develop new and exploit existing breeding populations to genetically map this resistance.

4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1a* gene candidates.

RESULTS AND DISCUSSION

Objective 1. The genetic mapping of the 04191 population with 139 SSR markers representing all 19 chromosomes confirmed a major locus *PdR1a* on chromosome 14, and identified a minor QTL (*PdR2*) on chromosome 19. The significance LOD was 2.3 for this minor QTL and it explains only 7% of the phenotypic variation that peaks at marker CB918037 (Fig 1). This QTL is within a 10 cM interval, which is likely too long for effective marker assisted screening. We have developed seven SSR markers based on the sequence of PN 40024 in this region that will be tested for polymorphism, and useful markers will be added to the entire population of 153 seedlings. These markers would allow us to reduce the gap from 10 cM so that the markers could be used in marker-assisted selection. Statistical analysis of both chromosome 14 and 19 indicated that both loci work independently of each other and do not have an additive impact. The mean ELISA values of resistant and susceptible plants with the PdR1a locus were very different, however, and the mean values of resistant plants with for *PdR2* were higher. For breeding purposes, the regions that have big impact on the phenotype are easier to manipulate via markers. Identification of minor QTLs involved in resistance is important to our understanding of possible epistatic interactions that might increase resistance levels. Further work would allow us to refine and shorten this locus, and screening of the physical sequence of PN40024 grape genome sequence will help identify the nature of genes in that region.

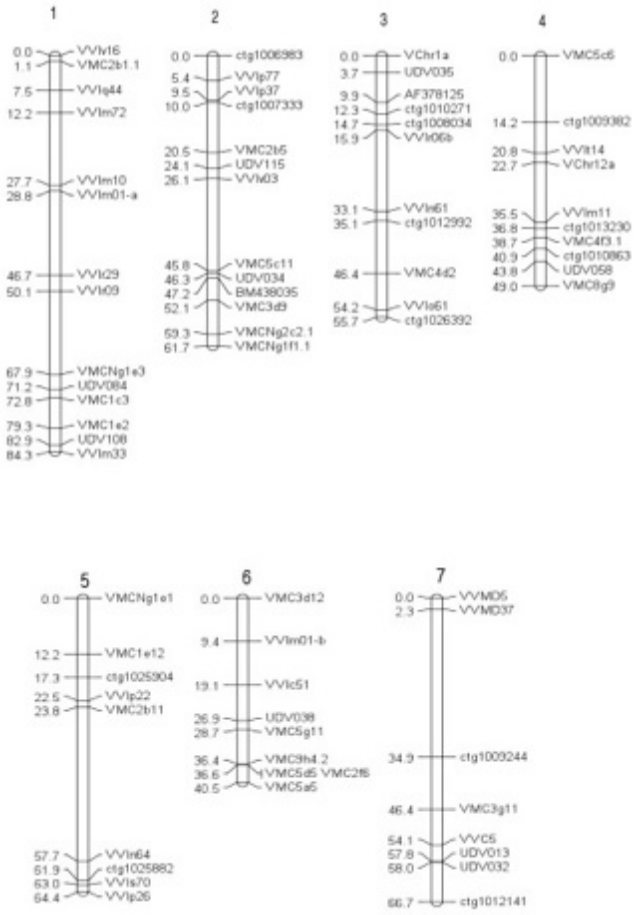
Fig. 1. Updated interval mapping analysis of the *PdR2* locus on LG 19.

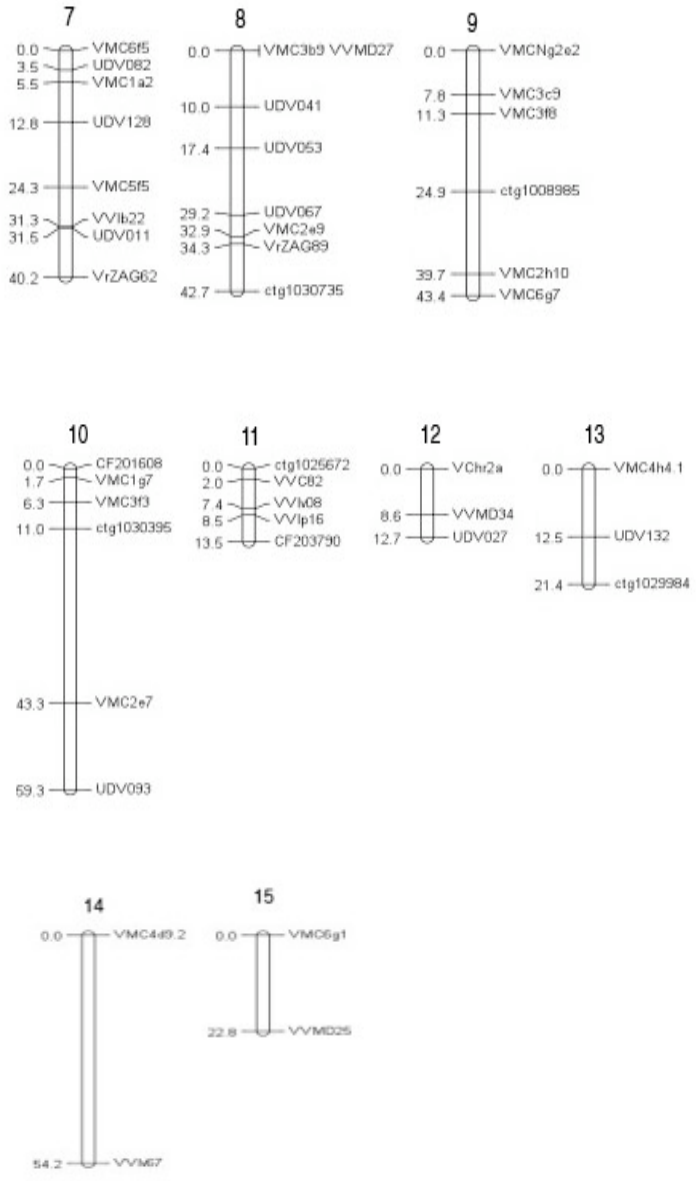


Objective 2. The genetic mapping of the F1 population 05347 (F2-35 x *V. arizonica/girdiana* b42-26) is in progress. To date we have tested 700 SSR markers that originated from a broad range of mapping efforts over the last 10 years. A total of 563 SSR primers amplified b42-26 DNA successfully, however the level of polymorphic markers is very low – only 30% of them are polymorphic. As the main focus of the work is to develop a genetic linkage map of the resistant parent, we are interested only in those

markers that could be used with b42-26. We have completed mapping 125 of the polymorphic markers on the entire population of 239 progeny. A framework map was developed in order to assess the level of coverage of all 19 chromosomes. The 125 markers grouped into 18 linkage groups; no marker was polymorphic for chromosome 6. For three chromosomes, markers were not mapped due to large distance between them. Figure 2 presents 15 linkage groups and marker order, which was comparable to that from other reference maps. Chromosomes 12 to 15 had few markers on them. Further work to refine this map is in progress and we are planning to add 30 other polymorphic markers that will cover the regions that have low marker coverage. Then more markers in regions with a significant presence of a segregating QTL will be added to allow the LOD profile to be determined more precisely in that area. Our goal is to obtain average distance between markers below 5 cM for QTL analysis.

Fig. 2. Framework genetic map of b42-26 developed with SSR markers.



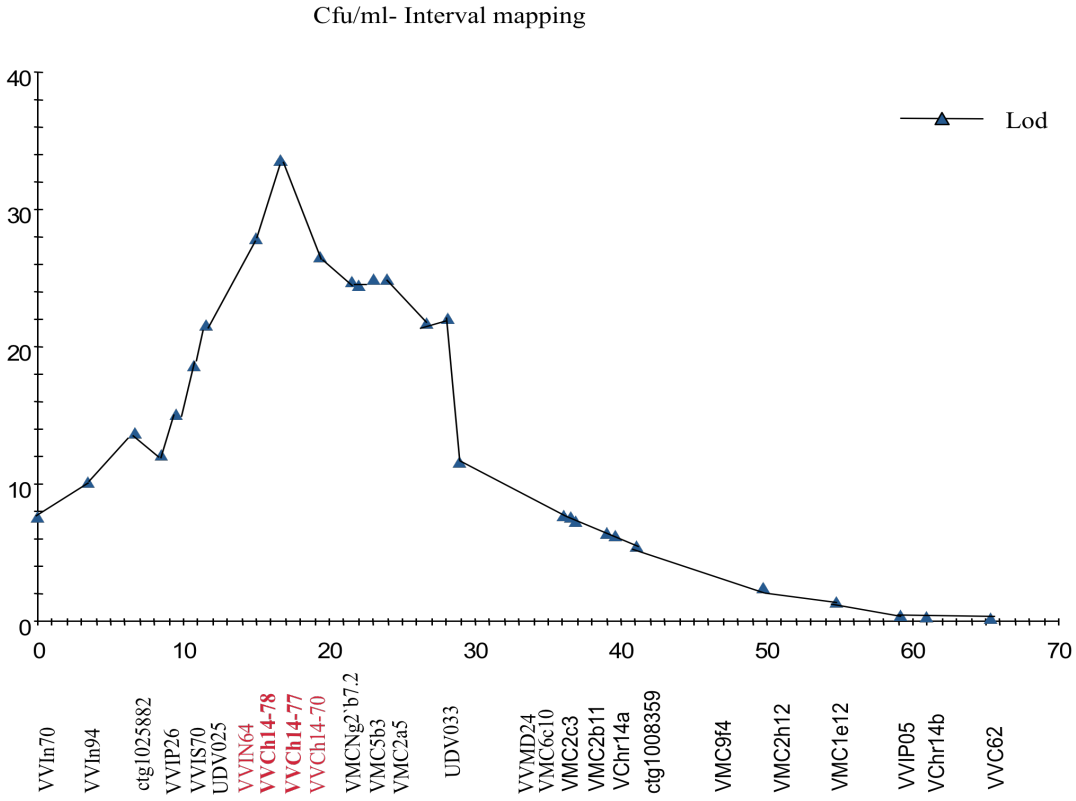


Thus far, 164 F1 progeny have been ELISA screened after greenhouse testing with 4-5 replicates of each genotype. The greenhouse screen takes 6 to 8 months depending on the cultural and environmental conditions. We have observed that the severity of this screen is much more pronounced under hotter summer conditions. We also see differences based on bench position in relation to cooling pads. The variation in test results can make the positioning of quantitative traits difficult, and it extremely important to have clean phenotypic data. The greenhouse data for the 164 progeny were compiled over two different years, and there are large standard deviations among the different reps for each genotype, as well as genotypes that were common across the two screening years. Previously, we reported preliminary QTL analysis with 64 screened genotypes using data from only 71 SSR markers. Kruskal-Wallis analysis that allows association of each marker to the phenotypic trait indicated that markers from chromosome 10, 12 and 14 are associated with PD resistance. We recently applied the same QTL analysis interval mapping approach to the updated data set with 125 markers on 164 genotypes. We did not anticipate high impact QTLs considering the quality of the phenotypic data, but we were able to identify QTLs that explained 17% and 8% phenotypic variation on chromosome 14 and 12, respectively.

We are now planning the addition of more markers on both of these chromosomes to reduce the distance between markers, and saturate other linkage groups complete the search for other QTLs. We will also have to re-screen the entire population in a hotter greenhouse to obtain more precise data.

A single dominant gene controls resistance to PD in *V. arizonica* b40-14. Two resistant siblings of this population were used to develop the 07388 (*V. vinifera* x R8918-02) and 07744 (*V. vinifera* x R8918-05) populations. Testing determined that 227 markers were polymorphic for one of the parents; 152 were analyzed on the entire set of 122 plants; a framework map of R8918-05 was produced with MAP QTL (4.0); and the Kruskal-Wallis approach was used to complete the preliminary analysis. PD resistance mapped on chromosome 14 – the same chromosome where *PdR1a* and *PdR1b* mapped. PD resistance from b40-14 (which we have named *PdR1c*) maps in the same general region as *V. arizonica/candicans* b43-17 between flanking markers VVCh14-77 and VVIN64 and within 1.5 cM. The LOD threshold for the presence of this QTL was 33 and 82% of the phenotypic variation was explained (Fig. 3). In 2009, crosses were made with F1 resistant selections from 07744 population to combine this resistance with resistance from b43-17.

Fig. 3. Interval mapping of *PdR1*from *V. arizonica* b40-14 indicating a peak at LDD 34.0 with a 95% confidence interval. The X-axis indicates the position of the markers; LOD values are plotted on the Y-axis.



Objective 3. *Vitis* species growing in the southern US have co-evolved with *X. fastidiosa* and resist this disease. To date, we have focused on accessions of *Vitis* species that Olmo collected in northern Mexico. In addition to these accessions, we maintain more than 250 accessions collected from PD hot spots in Texas, New Mexico, Arizona, Nevada and California that I have collected over the last 15 yrs. A sub set of 52 accessions from across this geographic range (including the fifteen accessions from Mexico) was evaluated for PD resistance and 22 accessions had high resistance (Table 1).

Table 1. Greenhouse screening results for PD resistance of southern US and Mexico germplasm. Accessions that are being used in our breeding program are in bold.

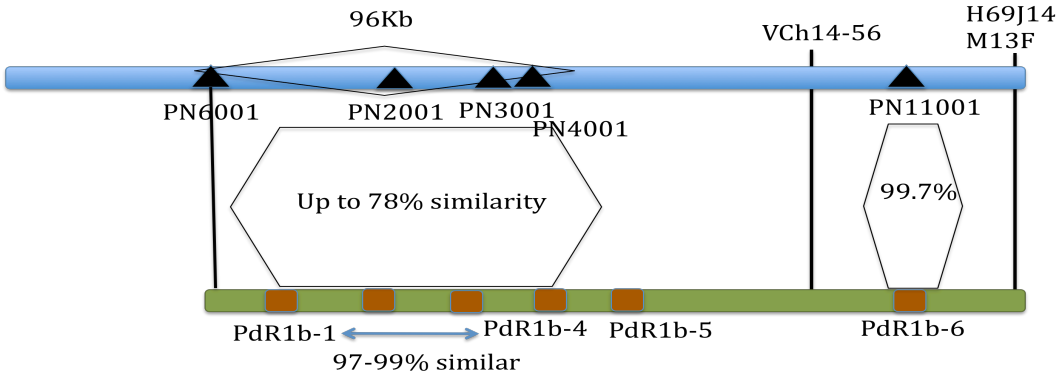
Genotype	GH Screen Result (ref b46-48)	Geometric mean (cfu/ml)	Mean (ln cfu/ml)
ANU5	R	22,190	10
ANU77	R	26,716	10.2
b40-14	**R**	**21,858**	**10**
b40-29	R	17,529	9.8
b40-61	R	25,283	10.1
b41-13	R	18,250	9.8
b41-23	R	206,282	12.2
b42-26	**R**	**27,942**	**10.2**
b42-55	R	178,136	12.1
b43-17	**R**	**135,510**	**11.8**
b43-57	R	17,967	9.8
b44-53	R	36,626	10.5
b46-43	R	13,669	9.5
b46-48	R	17,455	9.8
b47-28	R	38,227	10.6
b47-32	R	26,339	10.2
b47-5	R	35,983	10.5
SC30	R	80,170	11.3
SC36	R	27,299	10.2
SC38	R	96,327	11.5
SC39	R	139,553	11.8
T 03-06 S02	R	101,458	11.5
T 03-16 (B24-16)	R	11,544	9.4
T 03-16 (B24-26)	R	35,299	10.5
T56	R	98,726	11.5

This broader examination of PD resistance in the southern *Vitis* will expand the pool of resistance genes available for breeding, identify potentially unique sources of resistance, and identify the regions with the highest resistance so that additional accessions can be tested or collected. The inheritance of resistance and nature of resistance of the best forms of resistance will be examined in the next phase of the project.

Fig. 4. Map indicating the collection spot of the plant material recently tested for PD resistance in the greenhouse screen. All the green arrows indicate the promising resistant material.

Objective 4. We have used three categories of sequences (shotgun reads, fosmid reads and 454 sequencing) for the BAC clone H69J14 that carries PD resistant gene(s). From the assembly of this sequence, we identified 6 copies ranging from 2Kb to 3.1Kb in the resistance region. Copies 1 thru 4 are 97-99% similar and differ in size (potentially tandem repeats of one gene), they were up to 78% similar to four copies of genes on the PN40024 sequence (Fig. 4). We utilized CENSOR software to screen query sequences against a reference collection of repeats to generate a report classifying all detected repeats. All four PN40024 genes carry DNA transposons as well as LTR retrotransposon confirming the complexity of the region.

Fig. 4. Comparison of the resistance genes cloned from F8909-08 sequence to the PN40024 genomic region.



A detailed comprehensive comparison of the H69J14 clone sequence to the PN40024 sequence is not possible due to major re-arrangement of repetitive elements between the two genomes as well as due to the presence of gaps in the contigs of the H69J14 BAC clone. We are in process to using Nanopore sequence technology that produces up to a 100Kb fragment sequence with only 1% sequence error. For this purpose, we identified three overlapping BAC clones (H15B20, H69J14 and H64M16) that span approximately 450Kb of the physical sequence. Complete assembly of this region would allow comparisons to be made with the susceptible PN40024, identification of differences in the geneic and non-geneic region, and identification of the susceptible allele of the *PdR1b* gene.

We utilized different tools from www.expasy.org/tool/ to conduct pattern and profile searches of the PD resistance genes. There is very strong evidence of a LRR region in five of the candidate genes. We have cloned and verified the sequence of five candidate genes and are developing constructs for transformation experiments in tobacco and grape to determine which of these candidate genes confers resistance to PD.

PUBLICATIONS AND PRESENTATIONS

Walker M.A., Riaz S., Agüero C., Bistue C. 2011. Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*). Poster presentation Pierce's Disease Research Symposium, Dec. 13-15, Sacramento.

Presentations

UC Davis grape breeding program, Croatian Grape Growers and Wine Makers Group, UC Davis, July 13
UC Davis grape breeding program, University of Florida Horticulture Graduate Students Association visit, UC Davis, August 10
Using marker-assisted selection to optimize breeding for resistance to powdery mildew, American Vineyard Foundation Research Forum, UC Davis, February 24
Grape growing and breeding at UCD, Culinary Institute of America Foodies Tour, UC Davis, March 11
Grape research and careers, Early Academic Outreach Program, UC Davis, March 15
UCD grape breeding program, Lodi/Woodbridge Grape Growers Meeting, Lodi, CA, March 18

Sustainable winegrape growing, UC Berkeley Haas Business School Top Tech Program, Mondavi Winery, Oakville, CA, April 9

Grape breeding at UC Davis BOKU : University of Natural Resources and Life Sciences, Vienna, Austria, June 13

PD resistant winegrapes are approaching wine quality and field testing, 62nd Annual Meeting of the American Society of Enology and Viticulture, Monterey, CA, June 22

Grape breeding at UCD. Hopland Growers Meeting, Hopland, CA November 21

Grape breeding progress. Daniel Roberts Growers Meeting, Santa Rosa, CA December 5

Resistance Round Table: Breeding for resistance to grape pests and diseases. Annual Meeting of the Association of Applied IPM Ecologists, Napa, CA, December 16

Grape breeding progress update. Wilbur Ellis Viticulture Team, Santa Rosa, CA, February 9

Breeding for resistance to grape diseases. Ag Unlimited Annual Meeting, Napa, CA, March 1

STATUS OF FUNDS: These funds are scheduled to be spent by the end of the grant.

INTELLECTUAL PROPERTY: The resistance genes identified in this research will be handled by PIPRA, UC Davis.