CDFA PD/GWSS Progress Report
August 2009

I. Project Title: Map-based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce's disease resistance in grapes.

Reporting period: March 2009 to August 2009

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III. List of objectives and description of activities
Objective 1. Completely characterize and refine the *X. fastidiosa* resistance locus on chromosome 14 by genetic mapping in four populations 04190 (*V. vinifera* F2-7 x F8909-08), 9621 (D8909-15 x F8909-17), 04191 (F2-7 x F8909-17), and 04373 (*V. vinifera* F2-35 x *V. arizonica* b43-17).
Objective 2. Genetic mapping of PD resistance from resistant accessions b42-26 and b40-14.
Objective 3. Develop a BAC library for the homozygous resistant genotype b43-17 (parent of F8909-08, and F8909-17) and screen the library with closely linked markers.
Objective 4. Complete the physical mapping of *PdR1a* and *PdR1b* and initiate the sequencing of BAC clones that carry *PdR1a* gene candidates.

IV. Summary of Research Accomplishments
Objective 1. Previously we reported the genetic position of the *PdR1a* resistance locus between marker VVCh14-56 and VVCh14-70. In the course of past three months, we have developed three additional SSR markers derived from Pinot noir genome sequence that allowed us to narrow down the physical distance from 300Kb to 200 Kb. These new markers are named VVCh14-77, VVCh14-78 and VVCh14-81. We tested these markers on the composite set of recombinants from the 9621 population. Table 1 shows the key recombinants from this population.

The new markers VVCh14-77 and VVCH14-78 were also tested on the resistant and susceptible parents used for crosses in 2008 to determine if the resistance allele is unique and not present in susceptible selections. The resistance allele was unique in size and made these markers very valuable and robust for Marker-assisted screening. We also marker screened a subset of 49 recombinant plants that were selected based on two flanking markers (VMC2a5 and VVIP26). Tightly linked markers (VVCh14-56 and VVCh14-70) were added to the set of recombinant plants. For sixteen of the plants, the recombination event happened between VVIP26 and VVCh14-56, five plants had recombination between markers VVCh14-70 and VVCh14-29; twenty-three plants had recombination between marker VMCNg2b7.2 and VMC2a5. There were four potential key recombinants where the recombination event happened between *PdR1a* and the flanking markers (VVCh14-70 and VVCh14-56). Greenhouse screening of all 49 plants was completed and results were compared with marker data. Both marker data and

greenhouse data was in complete agreement. Twenty-four additional recombinant plants were also selected from five different crosses with the F8909-17 background (180 plants); greenhouse screening is underway.

We previously reported that *PdR1b* mapped between VvCh14-02 and VVCh14-70 in the 04190 population (Fig. 1). Additional markers VvCh14-28/VVCh14-29/VVCh14-30 were added to the entire set of 397 plants from the 04190 population. The greenhouse screen was repeated for key recombinants, which helped refine the data. In addition, marker analysis identified 14 recombinants from 15 different crosses (1,000 plants) that carry F8909-08 background. We also completed greenhouse screening 35 recombinants (including seedlings from *PdR1b* background crosses). The screen indicated four key recombinants: in two plants a recombination event happened between *PdR1b* and VVCh14-02, another plant had a recombination event between the resistance locus and VVCh14-70. Table 1 presents the updated data after the addition of three new markers on the composite recombinant set. Greenhouse screening data was not conclusive for four plants (marked as "?" for PdR1b data), two of these plants are key recombinants and their greenhouse screening is being repeated. In the most updated map, we have located the *PdR1* locus between markers VVCh14-81 and VVCh14-78. Both of these markers are less than 200Kb apart from each other based on the Pinot noir genome sequence.

In addition to these mapping populations, we also retained recombinants from both *PdR1a* and *PdR1b* backgrounds from our other project 'Breeding of PD resistant winegrapes". This new batch of plants will be tested again with flanking markers and will be useful to test any new markers that we develop from the sequencing of b43-17 BAC clones.

A *V. vinifera* F2-35 x F8909-17 cross, generated a third mapping population, 04191, with 153 progeny. This population provides genotypes with a 50% *vinifera* background for breeding and more recombinant plants for genetic mapping. It also provides a population where resistance from F8909-17 can be examined without possible confounding effects from D8909-15 (since D8909-15 has a multigenic resistance from b42-26). This population will also be critical for the identification of any minor genes that might contribute to resistance. Therefore, we are expanding the framework genetic mapping to all 19 chromosomes. All 153 plants were greenhouse screened and ELISA results will be available in November 2009. The framework map of all 19 chromosomes is also in process. The completed results of this project will be presented in our next report.

Objective 2. The preliminary data from the b42-26 resistance source indicate that resistance is quantitative in this form of *V. arizonica*. We initiated genetic mapping in the F1 population from the b42-26 background (population 05347 –Table 2). Greenhouse screening of a subset found 48 genotypes were resistant and 13 were susceptible. A total of 337 markers were tested on a small parental data set. Results found a high level of homozygosity for b42-26 (only 113 markers were polymorphic); 184 markers were homozygous for the male parent b42-26, 40 markers did not amplify. We completed 70 markers on a set of 64 genotypes, however 64 genotypes is not an adequate population size to map a quantitative trait. In Spring 2009, 100 seedlings were

selected and transferred to the field. Currently the core size of the 05347 population is 165 genotypes. DNA extractions were completed on the additional plants to initiate marker analysis. Additional seedling plants are too small to greenhouse screen this year, but are scheduled for testing in Summer 2010. Meanwhile the framework map will be developed on a core set of 165 seedlings.

V. arizonica b40-14 is third promising homozygous resistant genotype. We previously reported that all F1 progeny from a cross of *V. rupestris* x b40-14 were resistant except three genotypes with intermediate results. Two resistant F1 plants were used to develop the 07744 and 07386 populations (Table 2). We completed DNA extractions from 122 seedlings from the 07744 and 105 seedlings for the 07386. Marker screening was completed on small subset of 8 genotypes including parents and progeny (VMC series, VVI, VMCNg, VVMD, and VVS markers). A total of 277 markers were polymorphic for one or the other parent in preliminary screen. One hundred fifty two polymorphic markers were completed on entire set of 122 plants of the 07744 population. Mapping analysis was carried out on each parent separately. The framework map of R8918-05 (the resistant parent) was carried out with 152 markers on 121 genotypes with JOINMAP (3.0). Only three markers were unlinked and the remaining 149 markers were placed on 19 chromosomes as per the international reference maps. Quantitative trait loci analysis was performed with program MAP QTL (4.0) and the Kruskal-Wallis approach was used to conduct preliminary analysis. No association of PD resistance was found on any other chromosome except 14 – the same chromosome that contains *PdR1a* and *PdR1b* from the b43-17 background (Fig. 2). The resistance to PD in b40-14 background also maps in the same region (flanking markers VVCh14-78 and VVIN64 and within 1.5 cM), thus we named this locus *PdR1c* to distinguish it from other backgrounds. The LOD threshold for the presence of QTL was very high. Next, interval and MQM analysis will be carried out after selection of markers as cofactors and determinations of the level of variance contributed by this region.

Objective 3 and 4. Two BAC libraries (each generated with a different restriction enzymes) from the homozygous resistant b43-17 were developed. These BAC libraries were screened twice with two markers (VVCh14-10 and VVCh14-56), both tightly linked to *PdR1*. This identified 24 positive clones– four clones were positive with both markers: H23-P13, H34-B5 and H64-M16 and H45-J22. The inclusion of new marker and greenhouse screen information moved the *PdR1* locus between markers VVCh14-56/VVCh14-02 and VVCh14-70 (Fig.1), which required additional BAC library screening to find the clones in the genomic region at the end of VVCh14-70 marker. The 14 positive BAC clones that were selected with flanking marker were amplified with marker VVCh14-56, which is polymorphic (with two alleles) for b43-17 and can be used to distinguish and group clones. In an attempt to develop more markers, we utilized the 695Kb region from the Pinot noir genome sequence that covers the marker VVCh14-56 and VVCh14-27/VMCNg2b7.2 (Fig 1). It is important to note that this region is from two different scaffolds (groupings of sequences) of Pinot noir genome sequence (9 and 21). In a previous report we described the development of markers for library screening. We also developed SSR markers from this region that were placed in between these flanking markers (Fig. 2). Currently the resistance locus resides between Ch14-81 and

Ch14-78; a physical distance of ~200Kb. Based on the genetic map from the 9621 population, the physical and genetic distance correlates as 1cM is equal to about 216Kb. The second round of library screening was carried out with the Ch14-58 marker. A total of 17 clones were positive, five of them were also positive with the VVCh14-56 marker (Fig. 3). We selected the H69J14 clone for sequencing (454) and results are expected in about a month. The additional 12 clones that were positive with Ch14-58 were isolated and high quality DNA extractions were carried out for BAC end sequencing. Clean BAC end sequences would allow us to align and position the clones in comparison to the Pinot noir genome sequence.

V. Publications or Reports from this Project

Baumgartel, J.E. 2009. Optimizing screening technology for breeding Pierce's disease resistant *Vitis*. M.S. Thesis. University of California, Davis.

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek, D.W. Ramming and M.A. Walker. 2009. Using marker-assisted selection to breed Pierce's disease resistant grapes. Amer J Enol Viticult 60:199-207,

Vezzulli, S., D. Micheletti, S. Riaz, M. Pindo, R. Viola, P. This, M.A. Walker, M. Troggio and R. Velasco. 2009. A SNP transferability survey within the genus *Vitis*. BMC Plant Biol 8:128

Cheng, D.W., H. Lin, M.A. Walker, E.L. Civerolo and D. Stenger. 2009. Transcriptional regulation of the grape cytochrome P450 monooxygenase gene, CYP736B expression in response to *Xylella fastidiosa* infection. DOI 10.1007/s10658-009-9473-8

VI. Presentations on PD Research

"Creating *Xylella* resistant grapevines by conventional breeding", CDFA Pierce's Disease Symposium, San Diego, CA, December 17

"Grape breeding with an emphasis on flavor" Recent Advances in Viticulture and Enology, UC Davis, March 19

"Lessons learned about plant materials & PD resistant winegrapes coming soon", Temecula Grape Day, Temecula, CA, April 2

"Grape breeding at UC Davis" American Vineyard Foundation Board Meeting, Modesto, CA, April 25.

"20 years of grape breeding at UC Davis" Honorary Research at ASEV meeting, Napa, June 24

"Breeding winegrapes with resistance to Pierce's disease" ASEV Annual Meeting, Napa, June 25

"Walker grape breeding program" North American Grape Breeder's meeting, FAMU, Tallahassee, FL, August 6

VII. Research Relevance Statement

This research is studying the genetic basis of resistance to *X. fastidiosa* from several sources. It is developing fine-scale genetic maps of PD resistance loci, and physically locating these loci on chromosomes so that the genes that control resistance can be studied and utilized. This research project also provides the molecular genetic support and marker-assisted selection for breeding efforts to produce PD resistant wine grapes and collaborative efforts to produce PD resistant table and raisin grapes.

VIII. Lay Summary

This report presents updated results on the refined mapping of the Pierce's disease resistance locus, *PdR1*, in the 04190 (397 plants) and 9621 populations (433 plants). We have now mapped resistance to within 1cM (a single mapping unit) in both populations. The flanking markers VVCh14-78 and VVCh14-81 were added to key recombinants from both populations and greenhouse screening was repeated to avoid any error. Genetic mapping was initiated in three more populations (07744, 04191 and 05347) to better understand the genetics of resistance. A total of 152 markers were completed for the 07744 population to develop the framework map and greenhouse screening was conducted for both the 07744 and 04191 populations. Preliminary analysis of the 07744 population indicated that PD resistance from *V. arizonica* b40-14 resides on chromosome 14 in the same general area as *PdR1a* and *PdR1b* but different enough to distinguish it as *PdR1c*. Two BAC libraries (collections of thousands of segments of DNA from b43-17) were screened to identify regions of the chromosomes that contain *PdR1*. In the first phase of library screening, 24 positive BAC clones were identified (segments of DNA that should contain some part of *PdR1*. The Pinot noir genome sequence was used to develop markers that were used for the second round of BAC library screening, and other SSR markers were developed to reduce the physical distances between markers and *PdR1*. These new SSR markers are also very useful for marker-assisted selection. In the second round of screening, marker Ch14-58 was used and we isolated 17 more clones, five of these clones were also positive with Ch14-56, which was used for screening in the first round. One relatively large clone 'H69J14' (> 200Kb) was selected and is currently being sequenced at Amplicon Express.

IX. Status of Funds

These funds will be spent over the course of the project.

X. Summary and Status of Intellectual Property Produced

This project may identify resistance genes that will be patentable under UC regulations. The DNA markers are also valuable breeding tools, but are closely associated with specific breeding sources.

Table 1. Key recombinants from the 9621 (*PdR1a*) and 04190 (*PdR1b*) populations. The genotypes in bold red font are key recombinants with a recombination event between the marker and the resistance locus. "0" indicates a susceptible allele and "1" indicates a resistant allele.

Genotypes with *PdR1a* background	A010	VVCh 14-56	VVCh 14-81	*PdR1 a*	VVCh 14-78	VVCh 14-77	VVCh 14-70	VVCh 14-29	VMC Ng2b7 .2
-416	0	0	0	0	0	0	0	1	1
-426	0	0	0	0	0	0	0	1	1
-470	0	0	0	0	0	0	0	1	1
-554	0	0	0	0	0	**0**	**1**	1	1
-1064	1	1	1	1	1	**1**	**0**	0	0
-8	0	0	0	**0**	**1**	1	1	1	1
-194	0	0	0	**0**	**1**	1	1	1	1
-38	0	0	**0**	**1**	1	1	1	1	1
-15	1	1	1	1	1	1	1	0	0
-23	1	1	1	1	1	1	1	0	0
-901	1	1	1	1	1	1	1	0	0
-915	1	1	1	1	1	1	1	0	0
-919	0	0	0	0	0	0	0	1	1
Genotypes with *PdR1b* background	VVCh 14-10	VVCh 14-02	VVCh 14-81	PdR1 b	VVCh 14-78	VVCh 14-77	VVCh 14-70	VVCh 14-30	VVCh 14-27
06314-24	0	0	0	0	0	0	0	1	1
06328-05	0	0	0	0	0	0	0	1	1
04190-026	0	0	0	0	0	0	0	1	1
06317-50	1	1	1	1	1	1	1	0	0
04190-383	1	1	1	1	1	1	1	0	0
06317-50	1	1	1	1	1	1	1	0	0
04190-320	1	1	1	?	1	1	1	0	0
04190-065	1	1	1	?	1	1	1	0	0
04190-109	1	1	1	1	1	**1**	**0**	0	0
04190-381	1	1	1	**1**	**0**	0	0	0	0
06711A-60	0	0	0	?	1	1	1	1	1
04190-236	1	1	1	?	0	0	0	0	0
06315-49	1	0	0	0	0	0	0	0	0
06326-23	1	0	0	0	0	0	0	-	-

Table 2. Parentage and species information for populations and genotypes being used to map PD resistance.

Population / Genotype	Species / Parentage
b42-26	*V. arizonica/girdiana*
b43-17	*V. arizonica/candicans*
b40-14	*V. arizonica*
D8909-15	*V. rupestris* A. de Serres x b42-26
F8909-08 and F8909-17	*V. rupestris* A. de Serres x b43-17
F2-7 and F2-35 (females)	*V. vinifera* (Carignane x Cabernet Sauvignon)
9621	D8909-15 x F8909-17
0023	F8909-15 x *V. vinifera* B90-116
03300/5	101-14Mgt (*V. riparia* x *V. rupestris*) x F8909-08
04190	F2-7 x F8909-08
04191	F2-7 x F8909-17
04373	F2-35 x b43-17
05347	F2-35 x b42-26
07744	R8918-05 x *V. vinifera* Airen
07386	R8917-02 x *V. vinifera* Airen

Table 3. *PdR1c* regions, as determined by Kruskal-Wallis analysis, on chromosome 14 where potential genes for PD resistance are present. K is the LOD score for the presence of a QTL and '*' indicates the level of significance. Yellow area indicates the region that have LOD value greater than 20. The first peak is the same region where *PdR1a* and *PdR1b* from b43-17 background mapped.

Map position	**Locus name**	**K* (df)**
0.0	VVIN70	5.392 (1) **
3.5	VVIn94	9.323 (1) ****
9.5	ctg1025882	16.293 (1) *******
10.4	VVIP26	12.764 (1) ******
10.7	VVIS70	17.315 (1) *******
11.6	UDV025	16.160 (1) *******
15.0	VVIN64	21.081 (1) *******
16.7	VVCh14-78	22.692 (1) *******
16.7	VVCh14-77	22.946 (1) *******
17.7	VVCh14-70	19.350 (1) *******
20.3	VMCNg2b7.2	17.282 (1) *******
21.5	VVMD24	20.496 (1) *******
22.0	VMC5b3	20.631 (1) *******
22.5	VMC2a5	22.915 (1) *******
22.5	VVIV69	21.978 (1) *******
23.2	UDV033	22.857 (1) *******
28.9	VMC6c10	15.577 (1) *******
36.2	VMC2c3	8.872 (1) ****
36.5	VMC2b11	8.057 (1) ****
36.9	VChr14a	7.229 (1) ***
39.0	ctg1008359	8.772 (1) ****
39.8	VMC9f4	9.360 (1) ****
41.1	VMC2h12	8.967 (1) ****
49.8	VMC1e12	3.507 (1) *
59.2	VVIP05	1.714 (1) -
61.1	VChr14b	0.398 (1) -
65.4	VVC62	0.386 (1) -

Fig. 1. SSR-based genetic map of chromosome 14 from the 9621 (left) and 04190 (right) populations.