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

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

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

Principal Investigator and Cooperating Staff: M. Andrew Walker and Summaira Riaz, Dept. of Viticulture & Enology, University of California, One Shields Ave., Davis, CA 95616-8749, awalker@ucdavis.edu, 530-752-0902

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: The results reported here are from work conducted from March 2012 – July 2012

LAYPERSON SUMMARY
Three different genetic resources are being exploited to identify resistance for Pierce's disease. We made the most progress with b43-17 and b40-14 that carry major as well as minor locus for disease resistance. Genetic mapping from these two different forms of *V. arizonica* identified a PD resistance region on chromosome 14, which we termed *PdR1*. We have mapped two forms of *PdR1* from *Vitis arizonica/candicans* b43-17, identified a minor gene on chromosome 19 (PdR2) and have mapped a third form, *PdR1c*, that originated from *V. arizonica* b40-14. For the third resistance source, *V. arizonica/girdiana* b42-26, a total of 916 markers were tested and 170 polymorphic markers were added to the whole population of 239 seedlings – 45 more markers were added since the previous report. An initial genetic map was developed to *assess the level of coverage* on all 19 chromosomes. All markers were grouped and 15 linkage groups were established. The entire mapping population will be screened for PD resistance a second time because of the variation in the previous results. Currently, four to five reps of 86 plants have been inoculated and results are expected in Nov 2012. Preliminary QTL analysis with previous screen results determined that chromosome 12 and 14 are involved in PD resistance. Our focus is to refine the genetic map to get better coverage of all chromosomes, and obtain consistent greenhouse screen data in the b42-26 background. We plan to combine these multiple resistance sources in our breeding program to ensure broad and durable resistance to PD. We also expanded the search for PD resistant *Vitis* species by examining 52 accessions collected from the southern US and Mexico. Greenhouse screen results identified 20 other accessions that are promising. Next, breeding populations will be developed to determine the inheritance of resistance, and framework mapping will be used to identify resistance regions for marker development that will allow stacking new PD resistance genes with *PdR1*. Crosses were made with 5 of the most resistant accessions in Spring 2012. We have also genotyped set of 96 accessions from the southern US and Mexico to determine the genetic diversity of PD resistance genes and the extent to which gene flow is occurring. Identification of markers for *PdR1* has allowed us to reduce the seed-to-seed cycle to 2 years and produce PD resistant hybrids with 97% *vinifera,* demonstrating the value of integrating classical and molecular breeding while producing PD resistant wine, table and raisin grapes. These markers have also led to the identification of 6 genetic sequences that house the PD resistance gene, which we will soon be testing to verify their function. These efforts will help us better understand how these genes contribute to PD resistance, and could lead to PD resistance genes from grape that would be available to genetically engineer PD resistance into *V. vinifera* cultivars

INTRODUCTION

Identification, understanding and manipulation of novel sources of resistance are the basis for a successful breeding program. We are well-equipped to examine multiple genetic backgrounds for Pierce's disease resistance grape, develop and test breeding populations via a well-tuned greenhouse screen, carry out genetic mapping of segregating populations to identify genomic regions that carry disease resistance genes, and develop physical sequence maps of resistance regions leading to the identification and characterization of grape resistance genes. This project greatly facilitates our companion project of breeding program. We have initiated and completed mapping of a major PD resistance locus from *Vitis arizonica/candicans* b43-17, which serves as the backbone for our PD resistant grape-breeding program. b43-17 is highly resistant with very low bacterial titers in the stem and no disease symptoms. The breeding part of the program produces and greenhouse screens the seedling populations. While the tightly linked genetic markers generated in these mapping efforts are used to optimize and greatly accelerate the PD breeding program. These markers are essential to the successful introgression of resistance from multiple sources, and therefore the production of durably resistant grapevines. In response to recommendations from the CDFA-PD Review Board to broaden resistance, we are characterizing resistance from two additional sources and making good progress. We are pursuing two other resistant forms of *V. arizonica*: b42-26 *V. arizonica/girdiana* from Loreto and b40-14 *V. arizonica* from Chihuahua, Mexico. Although they are morphologically different than b43-17, both possess strong resistance to PD and greatly suppress *X. fastidiosa* levels after inoculation. We have also expanded the search for additional resistance sources by screening germplasm collected from the southern US and Mexico. Initial greenhouse screen results indicate that we have **ten** other accessions that possess very strong PD resistance.

OBJECTIVES

1. Fine scale mapping of additional QTL for PD resistance PD resistance in the 04191 ((F2-7 x F8909-17) 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 (250 accessions) collected 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.

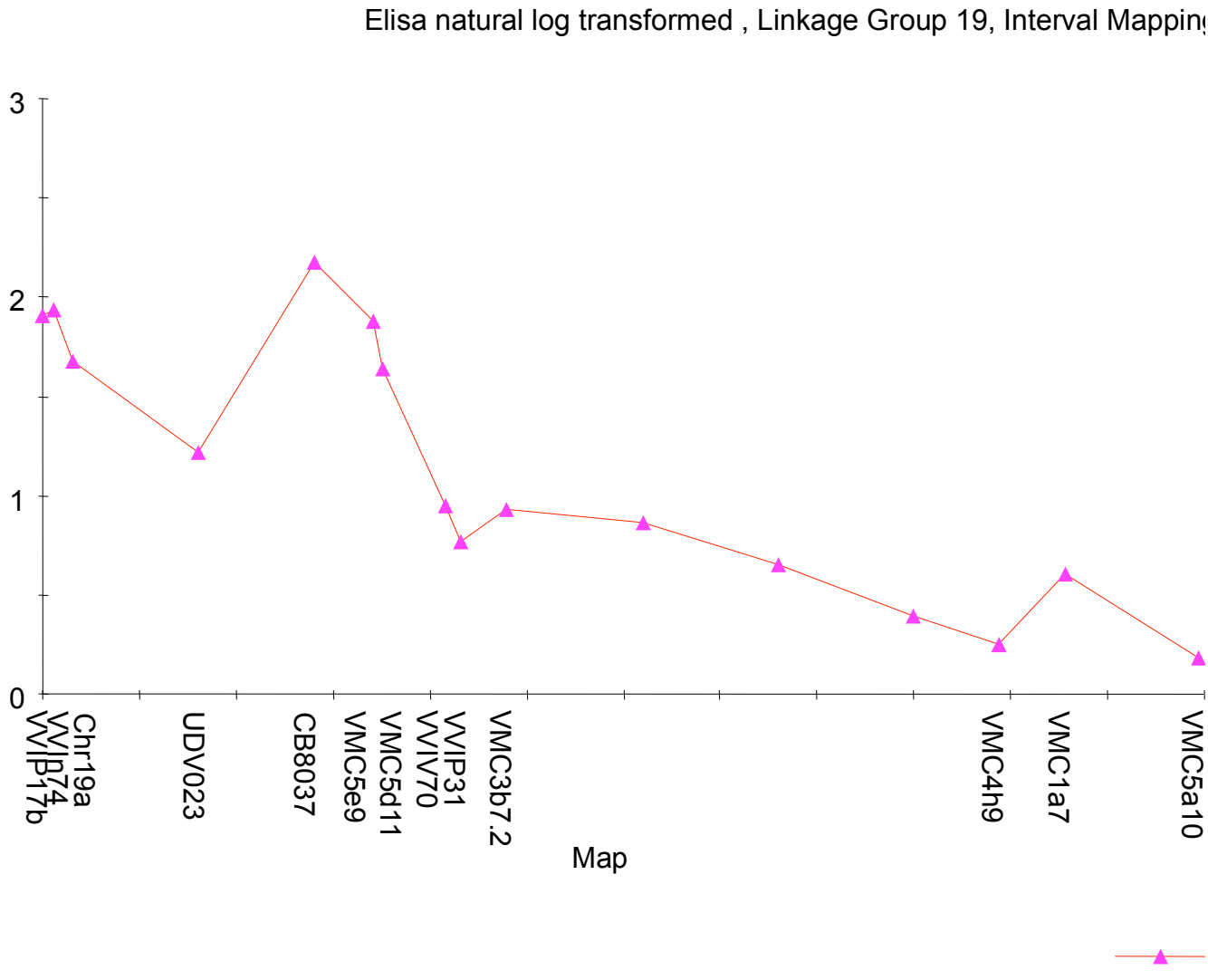
RESULTS AND DISCUSSION

Objective 1. We developed a framework genetic map of the 04191 (F2-7 x F8909-17) that carries the major locus *PdR1a*. The main objective of this map was to identify minor QTL contributing to this form of PD resistance. A total of 139 SSR markers representing all 19 chromosomes were added to set of 150 genotypes. QTL analysis confirmed a major locus *PdR1a* on chromosome 14, and identified a minor QTL (*PdR2*) on chromosome 19. The significance LOD for the minor QTL was 2.3 and it explains 7% of the phenotypic variation that peaks at marker CB918037 (Fig 1). This QTL is within 10 cM interval too wide an area for effective marker assisted screening. To shorten the genetic distance between the markers, we utilized the PN40024 Pinot sequence for that region to develop more markers. The positions of marker UDV023, CB918037, and VMC5e9 on the genome sequence are at 2,346,119, 2,974,668 and 4,182,806 bp, respectively. This provided us with a 1.783 Mbp distance to develop 7 SSR primers. These primers are in the process of testing for polymorphism. Useful markers will be added to the entire population of 150 seedlings. These markers would allow us to reduce the gap from 10 cM.

Analysis with the SAS Jump program for both LG 14 and LG 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, the mean values of resistant and susceptible for the *PdR2* locus were higher for the resistant plants. For breeding purposes, regions with the largest genotypic impact on the phenotype are easier to manipulate via markers. The identification of this minor QTL is important for better understanding of genetic and epistatic interactions. Further work will allow us to narrow the genetic distance and facilitate comparisons of this region with that of the PN40024 genome sequence to help characterize the nature of genes in that region. In order to study the impact of the minor QTL, we made two crosses with 04373-02 and 04373-22 lines with Pinot blanc. The goal is to discard all those plants that carry the *PdR1a* locus, greenhouse screen all other plants to test their level of PD resistance and use these populations to study and verify the *PdR2* region without any interaction from the *PdR1* locus. A total of 100 plants were screened with SSR markers and 43 plants were planted in the field in Spring 2012. These plants will be greenhouse assayed in 2013.

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



Objective 2. We have 918 SSR markers in our database for mapping the F1 population 05347 (F2-35 x b42-26). A total of 916 were tested, 763 SSR primers amplified b42-26 DNA successfully, and 180 markers were polymorphic; a relatively low level of 23% polymorphic markers. We have not observed this low a level of polymorphism while mapping in any other background. As the main focus of the work

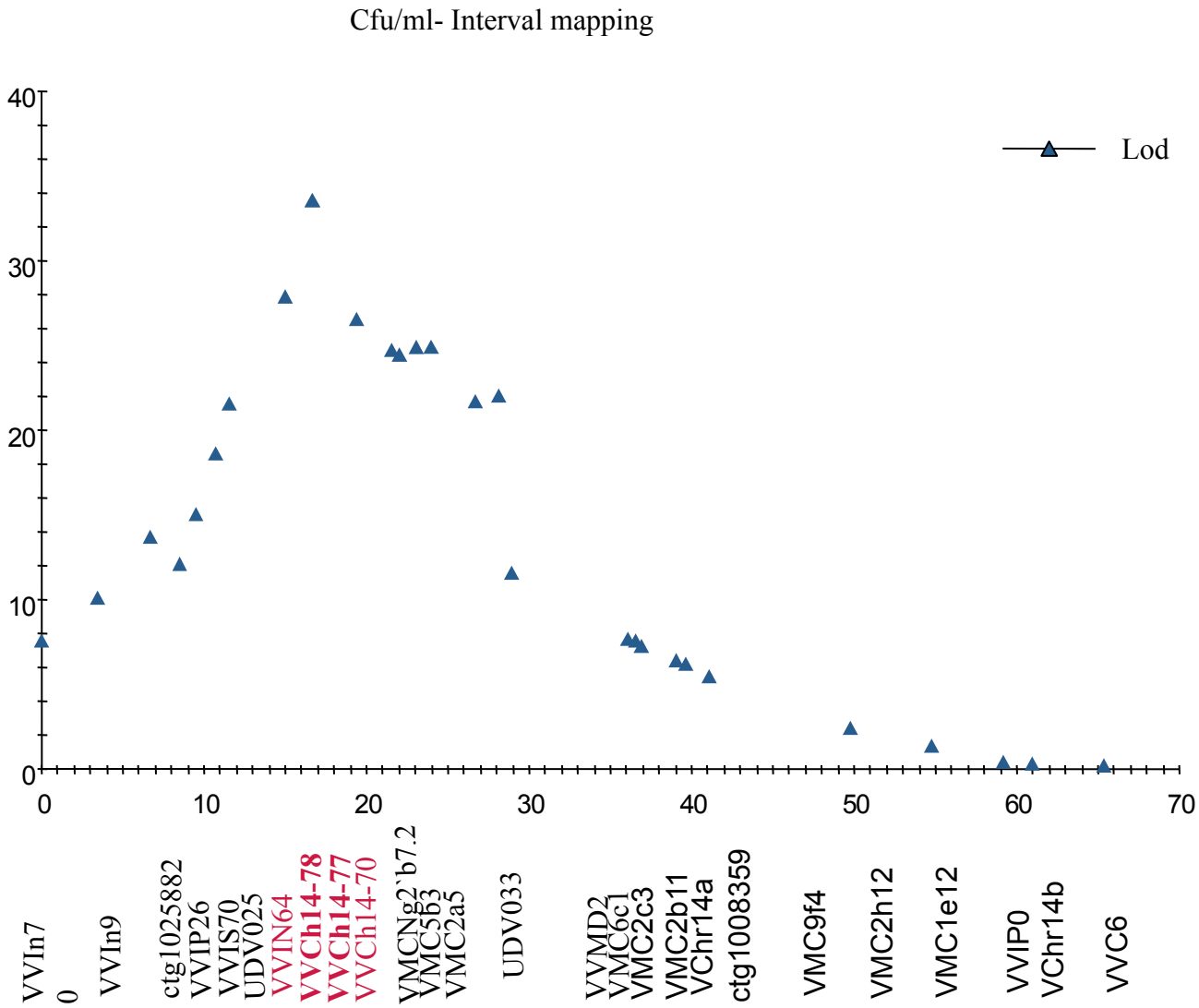
is to develop genetic linkage map of the resistant parent, we are interested only in those markers that could be used for b42-26. We have completed 173 of the polymorphic markers on entire population of 239 progeny; 48 additional markers from the previous report. We are currently analyzing and verifying the scores of newly added markers. We have also developed a framework map in this population with 125 markers (see Figure 2). All 125 markers were grouped into 18 linkage groups; no marker was polymorphic for chromosome 6. For three of the linkage groups, markers were not mapped due to the large distance between them. A genetic map with 180 markers will be completed by the next report period. Our goal is to obtain average distance between markers below 5 cM to enable QTL analysis.

In the previous report, we carried out the QTL analysis with ELISA results on 164 F1 progeny. The greenhouse screen takes 6-8 months depending on the cultural and environmental conditions. We have observed that the greenhouse screen is much more severe in hot summer months. Any plant material that exhibits low bacterial counts in a severe screen is considered highly resistant. Apart from the temperature influence, the greenhouse condition and temperature variation within different benches as well as sections of greenhouse also impart significant differences in the ELISA values. When quantitative traits are examined, it is extremely important to phenotypic data with low variability. The greenhouse data on the 164 05347 progeny was produced over two different years and the data has relatively high standard deviations and biocontrol genotypes also had variable responses. Using this data we were not able to identify a major effect QTL for PD resistance. We carried out QTL analysis utilizing Kruskal-Wallis as well as the interval mapping approach to identify genomic regions on the updated data set with 125 markers on 164 genotypes. We were able to identify QTLs that explains 17% and 8% phenotypic variation on chromosome 14 and 12 respectively.

After these results, we are decided to repeat the greenhouse screen on the entire population. At this point four to five replicates of 82 of the progeny have been propagated and inoculated with ELISA testing scheduled for October 2012. As more greenhouse space will become available, we will screen the rest of the population so results could be used for the QTL analysis.

A single dominant gene controls PD resistance in *V. arizonica* b40-14. Two resistant siblings of this population were used to develop the 07388 (R8918-02 x *V. vinifera*) and 07744 (R8918-05 x *V. vinifera*) populations. Two hundred and twenty-seven 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 only 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 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 to develop a pseudo-BC1 in this line to vinifera advancing it to the 75% vinifera level. In 2012 we made additional crosses to vinifera to advance this line to the 88% vinifera level (see details in the PD breeding report).

Fig. 2. Interval mapping of *PdR1c* indicating a peak at LDD 34.0 with the 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. Previously, we have focused on accessions of *Vitis* species Olmo collected in northern Mexico in 1960. I have collected more than 250 accessions collected from PD hot spots in Texas, New Mexico, Arizona, Nevada and California, which we are now beginning to evaluate. Fifty-two accessions from across this geographic range (including the fifteen accessions from Mexico) were evaluated in the greenhouse screen, and 22 had strong resistance. These new resistance sources will expand the germplasm pool for PD breeding and will hopefully include unique sources of resistance for study and eventual pyramiding with *PdR1*. We will also go back to the US regions with the most resistant accessions to collect more and examine the extent of this resistance. Table 1 presents crosses made in 2012 to determine the inheritance of resistance from 5 of the most resistant accessions. The best of these will be advanced into new breeding lines.

Table 1. Crosses made in 2012 to develop genetic maps of PD resistance from in new accessions from southern US and Mexico germplasm. Crosses 08-319 and 08-326 are selfs of Zinfandel and Cabernet Franc respectively and 100% *vinifera*.

Resistant Parent	Geographic Origin of Resistance	Pure Vinifera Types used in 2012 Crosses	Estimated # of Seed
ANU5	Littlefield, AZ	Alicante Bouschet	140

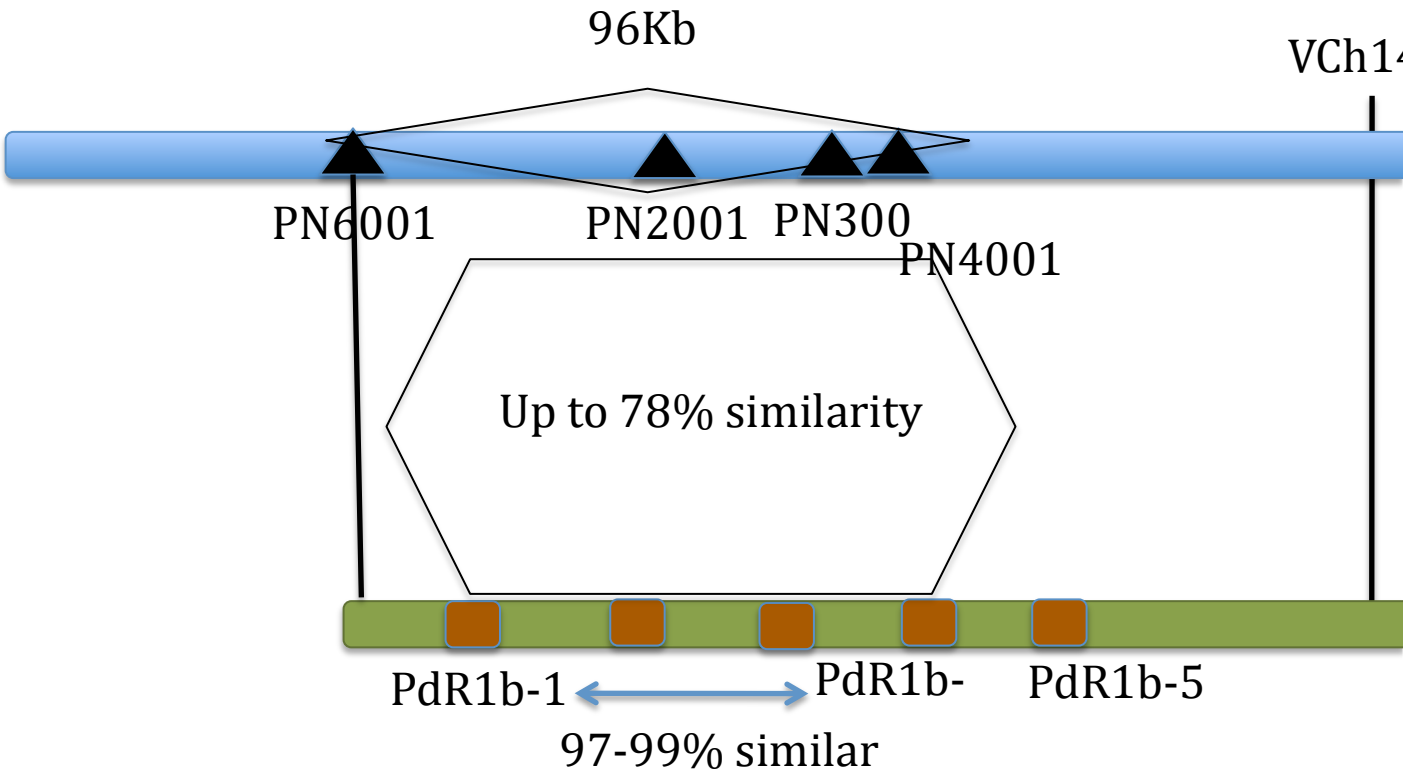
		Grenache	100
b40-29	Chihuahua, MX	08319-07	280
		08319-29	45
b31-13	Ciudad Mante, MX	08319-07	250
		08319-29	95
b46-43	Big Bend, TX	08319-07	320
		08319-12	150
		08326-61	200
b47-32	Big Bend, TX	08319-07	190
		08319-12	90
		08326-61	140

Fig 3. Map indicating the collection location of the plant material recently tested for PD resistance in the greenhouse screen. Green dots were accessions with strong resistance.

We are also examining the genetic diversity and gene flow in these southern US and Mexican accessions. DNA was collected from all genotypes that were screened with ELISA. DNA was extracted from 96 genotypes and they were tested with 22 SSR markers. These markers cover all 19 chromosomes, and include the 8 international reference markers that are used for germplasm maintenance in collections worldwide. The results of this work will be available later this year.

Objective 4. We have used three categories of sequence data (shotgun reads, fosmid reads and 454) to assemble the BAC clone H69J14 that carries *PdR1*. From the assembly of this sequence, we have 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 the four copies of genes on the Pinot noir (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. The comparison of the resistance genes cloned from the F8909-08 sequence to the PN40024 genomic region.



A complete 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 sequence gaps in the contigs of H69J14 BAC clone. We are now using Nanopore sequence technology to examine this region. It can obtain up to 100Kb fragment sequence with only 1% sequence error. For this purpose, we identified three overlapping BACx (H15B20, H69J14 and H64M16) that span approximately 450Kb physical sequence. Complete assembly of this region would allow us to make comparisons with the susceptible PN40024 sequence, identify difference in the geneic and non-geneic regions, and identify the susceptible allele of the *PdR1b* gene. These sequencing results are expected this Fall.

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 (leucine rich repeat region, common resistance responses) 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 (see Companion Project).

PUBLICATIONS AND PRESENTATIONS

Riaz, S., Hu, R. and Walker, M.A. 2012. A framework genetic map of *Muscadinia rotundifolia*. Theoretical and Applied Genetics (on line) DOI 10.1007/s00122-012-1906-7

Agüero C.B., Riaz S., Hwang C-F, He R., Hu R., Bistue C., Walker M.A. Map-based cloning of Pierce's disease and X*iphinema index* resistance genes from *Vitis arizonica*. 63rd Annual Meeting of the American Society of Viticulture and Enology, Portland, OR, June 21, 2012.

Roig, D., Nakao, Y., Boulton, R. and Walker, M.A. 2012. Anthocyanin composition of Pierce's disease resistant winegrapes. 63rd Annual Meeting of the American Society of Viticulture and Enology, Portland, OR, June 21, 2012.

Walker, M.A. 2012. Breeding programs from around the world: what is their likely contribution. 63rd

Annual Meeting of the American Society of Viticulture and Enology, Portland, OR, June 19, 2012

Presentations by Walker
Breeding disease resistant winegrapes. Ag Unlimited Grower's Meeting. Napa, CA March 1, 2012.
Breeding resistant grapevines. Recent Advances in Viticulture and Enology, UC Davis, March 15, 2012.
Grape breeding at UC Davis. University of Chihuahua, Mexico, March 30, 2012.
PD resistant winegrapes are approaching wine quality and field testing. Temecula Grape Day, Temecula, CA, April 19, 2012.
Advances in scion and rootstock breeding. Sonoma Vit Tech Group, Santa Rosa, CA, May 16, 2012.
Progress breeding rootstocks and fruiting varieties. Board of Visitors and Fellows, UC Davis, May 18, 2012.

RESEARCH RELEVANCE
This research project provides the molecular breeding tools used in our PD resistance breeding program and companion project entitled "Breeding Pierce's disease resistant winegrapes". It has created genetic maps for two sources of PD resistance and the tightly linked markers are in use selecting for resistance. These genetic maps lay the foundation for physical maps that allow the resistance genes to be sequenced. We have identified 6 candidate PD resistance genes, all of which appear to be responsible in the detection of and response to pathogens. The function of these candidate genes is being studied in our other companion project "Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*)". This study may lead to the identification of PD resistance genes that can be used to genetically engineer PD resistance into *V. vinifera* cultivars.

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.

LITERATURE CITED

Buzkan, N. L. Kocsis and M.A. Walker. 2005. Detection of *Xylella fastidiosa* from resistant and susceptible grapevine by tissue sectioning and membrane entrapment immunofluorescence. Microbiological Research 160:225-231.

Doucleff, M, Y. Jin, F. Gao, S. Riaz, A.F. Krivanek and M.A. Walker. 2004. A genetic linkage map of grape utilizing *Vitis rupestris* and *Vitis arizonica*. Theoretical and Applied Genetics 109:1178-1187.

Hwang, C-F., K. Xu, R. Hu, R. Zhou, S. Riaz and M.A. Walker. 2010. Cloning and characterization of *XiR1*, a locus responsible for dagger nematode resistance in grape. Theoretical and Applied Genetics 121:780-799

Krivanek, A.F. and M.A. Walker. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44-52.

Krivanek, A.F., J.F. Stevenson and M.A. Walker. 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology 95:36-43.

Krivanek, A.F., S. Riaz and M.A. Walker. 2006. The identification of *PdR1*, a primary resistance gene to Pierce's disease in *Vitis*. Theoretical and Applied Genetics 112:1125-1131.

Krivanek, A.F., T.R. Famula, A. Tenscher and M.A. Walker. 2005b. Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* x *Vitis arizonica* hybrid population. Theoretical and Applied Genetics 111:110-119.

Riaz, S., Hu, R. and Walker, M.A. 2012. A framework genetic map of *Muscadinia rotundifolia*. Theoretical and Applied Genetics 123: (on line) DOI 10.1007/s00122-012-1906-7

Riaz, S., A.C. Tenscher, D.W. Ramming and M.A. Walker. 2011. Using a limited mapping strategy to identify major QTLs for resistance to grapevine powdery mildew (*Erysiphe necator*) and their use

in marker-assisted breeding. Theoretical and Applied Genetics (In press).
Riaz, S., A.C. Tenscher, J. Rubin, R. Graziani, S.S. Pao and M.A, Walker. 2008. Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theoretical and Applied Genetics 117:671-681.
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. American Journal of Enology and Viticulture 60:199-207
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. American Journal of Enology and Viticulture 60:199-207
Riaz, S., A.F. Krivanek, K. Xu and M.A. Walker. 2006. Refined mapping of the Pierce's disease resistance locus, *PdR1*, and *Sex* on an extended genetic linkage map of *Vitis rupestris* x *V. arizonica*. Theoretical and Applied Genetics 113:1317-1329.
Riaz, S., S. Vezzulli, E.S. Harbertson, and M.A. Walker. 2007. Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's disease. American Journal of Enology and Viticulture 58:494-498.
Riaz, S., S. Vezzulli, E.S. Harbertson, and M.A. Walker. 2007. Use of molecular markers to correct grape breeding errors and determine the identity of novel sources of resistance to *Xiphinema index* and Pierce's disease. American Journal of Enology and Viticulture 58:494-498.
Ruel, J.J. and M.A. Walker. 2006. Resistance to Pierce's Disease in *Muscadinia rotundifolia* and other native grape species. American Journal of Enology and Viticulture 57:158-165.
Xu, K., S. Riaz, N.C. Roncoroni, Y. Jin, R. Hu, R. Zhou and M.A. Walker. 2008. Genetic and QTL analysis of resistance to *Xiphinema index* in a grapevine cross. Theoretical and Applied Genetics 116:305-311.