**CDFA PD/GWSS Progress Report**
**August 2008**

**A. Project Title:** Breeding Pierce's Disease Resistant Winegrapes.

**B. CDFA Contract Number**

**C. Reporting period:** April to July 2008

**D. Principal Investigators and Cooperators**:
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**E. List of objectives and description of activities**
**Objective 1.** Breed PD resistant winegrapes through backcross techniques using high quality *V. vinifera* winegrape cultivars and Xf resistant selections and sources characterized from our previous efforts.

**Objective 2.** Continue the characterization of Xf resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations.

**F. Research accomplishments**
**Objective 1.** The breeding cycle for the development of PD resistant grapes has been reduced to 3 years (seed to seed) using marker-assisted selection (MAS) with the b43-17 resistance sources and their progeny. Our goal at this point is to introgress our PD and *PdR1* resistance sources into a large number of *V. vinifera* winegrapes backgrounds. Until we get to the backcross 4 (BC4) (96.8% *V. vinifera*), there is not much point to growing very large numbers of progeny from any given cross. With the 3-year seed-to-seed cycle we will plant BC4 progeny in 2010.

Table 1 presents the crosses made in 2007 with numbers of seedlings produced, MAS tested and planted to the field in 2008. The goals of last year's crosses were to: 1) Use the *PdR1* allele from 8909-08 to make 93.75% *vinifera* level progeny; 2) Broaden the *vinifera* winegrape lines in the 8909-08 resistance source at the 87.5% *vinifera* level; 3) Combine *PdR1* with the powdery mildew resistance gene *Run1* at the 87.5% *vinifera* level; 4) Use 8909-17 and 8909-08 based resistance with diverse *vinifera* winegrapes to produce resistant progeny at the 75% *vinifera* level; 5) Use the 8909-15 resistance source with a broad range of *vinifera* winegrapes; and 6) Produce rootstocks with *PdR1* and broad-based nematode resistance.

Table 2 presents the crosses made in Spring 2008 with the estimated numbers of seeds produced. The goals of this years crosses were: 1) Use the *PdR1* allele from the 8909-08 to broaden the *vinifera* winegrape lines at the 93.75% *vinifera* level; 2) Combine *PdR1* with the powdery mildew resistance gene *Run1* at the 90.6% *vinifera* level; 3) Combine *PdR1* with the LG13 powdery mildew resistance gene *REN1* at the 87.5% *vinifera* level; 4) Use 8909-17 based resistance with diverse *vinifera* winegrapes to produce resistant progeny at the 87.5% *vinifera* level; 5) Use the F1 progeny of the homozygous PD resistant b40-14 *V. arizonica* to produce a breeding and mapping population that is 75% *vinifera*; 6) use elite winegrapes to broaden and expand the *V. shuttleworthii* breeding lines producing progeny that are 75% and 87.5% *vinifera* and 7) Produce rootstocks with *PdR1* and broad-based nematode resistance.

To date, three groups of plants have been greenhouse screened for Xf resistance in 2008 (Table 3). Group A tests were done to verify the expression of *PdR1* from b43-17 in the 04190 (*V. vinifera* F2-7 x 8909-08) population. This group also tested advanced 87.5% *V. vinifera PdR1* carrying parents, which were used in the 2007 crosses to create 94% *V. vinifera* progeny with *PdR1*. This group also included the parents of new mapping populations: one based on single resistance from *V. arizonica* b40-14 (R89); and the other based on multigenic resistance from *V. arizonica/girdiana* b42-26 (05347). The Group B tests examined progeny of Midsouth and BD5-117 crossed to advanced *vinifera* wine types. Both of these parents continue to produce resistant progeny, but very few and in ratios that suggest a complex inheritance. The progeny of Haines City were all resistant by ELISA in the greenhouse screen, but do not contain *PdR1*. Eight promising rootstocks based on PdR1 were also tested in this group. Group C tests focused on recombinants from our 2006 breeding populations to aid fine-scale *PdR1* mapping efforts and on the F1 progeny of b40-14 crossed to *V. vinifera*.

**Objective 2.** Although resistance from other backgrounds is complex and quantitative, which results in few resistant progeny from crosses to *vinifera* cultivars, we continue to advance a number of lines. In order to better understand the limits of other PD resistance sources the following resistance sources are being studied:

***V. arizonica* b42-26** – Xf resistance in the 0023 (D8909-15 (*V. rupestris* x b42-26) x *V. vinifera* B90-116) population is strong, but is quantitatively inherited. Quantitative trait locus (QTL) analysis has identified a major QTL that accounts for about 20% of the variability (preliminary results). Previous efforts with the 0023 were focused on table grape breeding, and found that the 0023 population (F1, 1/4 b42-26) had about 30% resistant progeny. This population has a large number of weak genotypes, few females with viable seeds, and generally lacks fertility. The progeny of a cross of a resistant 0023 genotype crossed back to *vinifera* (BC1) were tested and only 7% were resistant. GH testing of 05347 (*vinifera* F2-35 x b42-26) to examine the b42-26 resistance source in a less complex background (without the confounding effect of *V. rupestris*) was completed last year. In 2007, crosses using elite *V. vinifera* wine type pollen were made to a number of females in this population and 140 genotypes were planted this Spring (Table 1e).

***V. arizonica* b40-14** – In 2006 we crossed F2-35 x b40-14, produced 1,385 seeds and established 198 seedlings for testing. We are planning on using this population, or one generated from its progeny (Table 2d) for mapping efforts to better characterize this very strong, and morphologically and genetically different source of PD resistance.

***V. shuttleworthii* Haines City** – Based on the encouraging GH screen results for this resistance source, in 2008 we made the BC1 (75% *vinifera*) and BC2 (88% *vinifera*) using a BC1 from our earlier table grape work that tested particularly well and had reasonable wine grape characteristics (Table 2e).

Given that low levels of *X. fastidiosa* exist in resistant plants it will be important to also have PD resistant rootstocks to graft with resistant scions and prevent them from dying on susceptible rootstocks. We are currently screening 8 promising progeny from crosses of 101-14 x F8909-08 (Table 3). Evaluation for grafting ability and testing against phylloxera and nematodes and finally field testing will follow.

Any new PD resistant variety should also be resistant to powdery mildew. We have been exploring powdery mildew resistance in a number of backgrounds including Olmo's VR (*vinifera* x *rotundifolia*) hybrids, which form the base of international efforts at characterizing *Run1*, the *rotundifolia*-based locus responsible for resistance to powdery mildew. 2007 season field evaluations of the 2006 crosses show the markers correlating perfectly with field resistance on leaf and cane. Berry and cluster observations will begin in Fall 2008. The goal with these individuals is to cross our advanced PD resistant selections with selections from these powdery mildew resistant progeny (Table 2b). In 2008 we also made crosses to examine powdery mildew in two other backgrounds: a source of the *REN1* locus, Karadzhandal (Table 2) and the Chinese species *V. Romanetii*. Of the later an estimated 450 seeds were produced using both 8909-08 and 8909-17. Powdery mildew resistance markers are being developed for these resistance sources by labs in Italy and the US.

**Beringer Field Testing**

Selections from the 045554 (BC2, 88% vinifera) have been made onto Dog Ridge (currently the only certified virus-free PD resistant rootstock) and were planted at Beringer on May 14, 2007. These genotypes have been marker tested and their PD resistance status confirmed by greenhouse testing. Twelve genotypes were resistant, 4 were recombinants (1 resistant in and 3 susceptible in the greenhouse test) were planted in 2007. These were needle inoculated for the first time on May 22, 2008. The A81 series (BC1, 75% *vinifera*) F8909-08 allele type was inoculated concurrently now for the second time. Both will be sampled for ELISA testing this fall.

**Wine Making**

Four of the eight advanced selections containing *PdR1* that are 87.5% *vinifera* from crosses with Syrah and Chardonnay, which we replicated for wine evaluation, have set enough fruit to again conduct small scale fermentations this Fall. All are red fruited. We plan to conduct a small-scale white wine fermentation from 87.5 % *vinifera* vines planted in 2006 and fruiting for the first time this fall. Numerous other genotypes from crosses involving elite wine cultivars have set well enough for fruit evaluation and must analysis.

**G. Publications or Reports from this Project**


Lin, H., H. Doddapanneni, Y. Takahashi and A. Walker. 2007. Comparative analysis of ESTs involved in grape responses to *Xylella fastidiosa* infection. BMC Plant Biol. 7:8, doi:10.1186/1471-2229-7-8.

Fritschi, F.B., H. Lin and M.A. Walker. 2007. *Xylella fastidiosa* population dynamics in grapevine genotypes differing in susceptibility to Pierce's disease. American Journal of Enology and Viticulture 58:326-332.

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.

Fritschi, F.B., H. Lin and M.A. Walker. 2008. Scanning electron microscopy reveals different plant-pathogen interaction pattern in four *Vitis* genotypes infected with *Xylella fastidiosa*. Plant Disease 92:276-286.

Riaz, S, A.C. Tenscher, B.P. Smith, D.A. Ng and M.A. Walker. 2008. Use of SSR markers to assess identity, pedigree, and diversity of cultivated muscadine grapes. Journal of the American Society for Horticultural Science 133(4): In Press

Doddapaneni, H., H. Lin, M.A. Walker, J. Yao and E.L. Civerolo. 2008. VitisExpDB: a database resource for grape functional genomics. BMC Plant Biology 8:23 (online http://www.biomedcentral.com/1471-2229/8/23)

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 (In Press).

Lowe, K.M., S. Riaz and M.A. Walker. 2008. Variation in recombination rates across *Vitis* species. Tree Genomics and Genetics (In Press)

Stover, E., S. Riaz and M.A. Walker. 200X. PCR screening for *Xylella fastidiosa* in grape genebank accessions collected in the south eastern United States. American Journal of Enology and Viticulture (in final revision).

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek and M.A. Walker. 200X. Using marker-assisted selection to breed Pierce's disease resistant grapes. American Journal of Enology and Viticulture (submitted)

**Presentations on PD Research**

A. Walker. Marker-assisted selection for Pierce's disease resistance. Applied Grape Genomics Meeting, UC Davis, July 16, 2007

A. Walker. Will there be GMOs in California vineyards – what are the issues and what can we expect. Lodi Woodbridge Grape Growers Meeting, Lodi, CA, July 17, 2007.

S. Riaz, A. Tenscher, and M. A. Walker. Molecular breeding: marker-assisted selection for Pierce's disease and powdery mildew resistance in grapevine. National Viticulture Research Conference, UC Davis, July 20, 2007

A. Walker. Grape breeding at UC Davis. North American Grape Breeder's Meeting, UC Davis, August 22, 2007

A. Walker. Breeding as an essential part of sustainable viticulture. Master's of Wine Course, Oakville, CA, October 22, 2007.

A. Walker. GMOs in viticulture – pollen movement and implications. Napa Farm Bureau, Napa, CA, November 19, 2007.

A. Walker. Classical and molecular breeding to combat PD. CDFA PD/GWSS Annual Meeting, San Diego, CA, December 14, 2007.

A. Walker. Progress breeding PD resistant wine, table and raisin grapes. San Joaquin Valley Consolidated Pest Control District Meeting, Delano, CA April 3, 2008.

A. Walker. Walker breeding program. American Vineyard Foundation Board Meeting, Modesto, CA, April 25, 2008.

A. Walker. Using marker-assisted selection to breed for Pierce's disease resistance in grape. American Society for Viticulture and Enology Annual Meeting, Portland, OR, June 18, 2008.

A. Walker. The hunt for resistance genes to combat Pierce's disease. National Viticultural Research Conference, Davis, CA, July 11, 2008.

J. Rubin and A. Walker. Genetic and phenotypic resistance to Pierce's disease in *Vitis arizonica/candicans* selections from Monterrey, Mexico. National Viticultural Research Conference, Davis, CA, July 11, 2008.

A. Walker. Pierce's disease: incidence, spread and control. Australian Society for Viticulture and Oenology Disease Workshop, Mildura, Australia, July 24, 2008.

## H. Research Relevance Statement

This project continues to breed PD resistant winegrapes with the primary focus on the *PdR1*resistance source so that progress can be expedited with MAS. Populations with Xf resistance from other sources are being maintained and expanded, but progress is slower with these sources. We continue to supply plant material, conduct greenhouse screens and develop new mapping populations for our companion project on fine-scale mapping of PD resistance leading to the characterization of the *PdR1* resistance. The first testing of small-scale wine from advanced selections with 87.5% *vinifera* from winegrapes was done in Fall 2007, and they scored remarkably well.

## I. Lay Summary

Progress continues on Pierce's disease (PD) resistant winegrapes and has been greatly accelerated by the incorporation of marker-assisted selection (MAS) for the Pierce's disease resistance gene, *PdR1* (see companion report). The use of MAS and our acceleration of the seed to seed breeding cycle to three years has allowed very rapid progress towards PD resistant winegrapes. Populations from the 2007 crosses were screened with MAS for both PD and powdery mildew (*Run1*) where appropriate and only those with the markers were planted in the field. The 2008 crosses were made to: *Vinifera* wine grape types with *PdR1* and 87.5% *vinifera* in were planted in a Beringer, Napa county trial. Finally, small scale wine lots were made from 4 of 8 selected 87.5% *vinifera PdR1* containing wine grape types. Fruit evaluation and must analysis were performed on numerous other promising progeny at this level.

## J. Summary and Status of Intellectual Property Produced

Thus far no selections have been released from this breeding program. When they are they will be released through UC Davis.

| Table 1. 2007 Wine & rootstock type crosses: # of seeds and seedlings produced, # marker tested and # resistant plants to field | | | | | | |
|---|---|---|---|---|---|---|
| Resistant Type | *Vinifera* Parent of Resistant Type | *Vinifera* Types used in 2007 crosses | Total Seeds | # Seedlings Produced | # Marker Tested | # Marker Tested Planted in Field (or Nursery) |
| 1a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *vinifera* parentage. | | | | | | |
| U0501 | Syrah | F2-7, F2-35 | 1055 | 222 | 94 | 20 |
| U0502 | Chardonnay | F2-7, F2-35 | 2769 | 345 | 245 | 76 |
| U0503 | Sauvignon blanc | Chardonnay, Palomino, Semillon | 126 | 92 | 55 | 15 |
| U0505 | Cabernet Sauvignon | Chardonnay, F2-7, LCC, Merlot, Palomino, Petite Syrah | 3229 | 856 | 340 | 107 |
| 1b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 87.5% *vinifera* parentage | | | | | | |
| 05310 | Alicante Bouschet | Burger, Carignane, LCC | 1666 | 110 | 25 | 0 |
| 05312 | Cabernet Franc | Zinfandel | 194 | 62 | 25 | 8 |
| 05317 | Tempranillo | Burger, LCC | 371 | 146 | 55 | 24 |
| 05319 | Zinfandel | Cabernet Franc, LCC | 144 | 93 | 45 | 9 |
| A81-17 | A38-7 | Carignane, Grenache noir, LCC | 705 | 141 | 90 | 33 |
| 1c. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Run1* powdery mildew resistance to produce progeny with 87.5% *vinifera* parentage except for the A81-17 group which is 81.5% *vinifera* (quantities in parenthesis were planted in a nursery to field screen for powdery mildew) | | | | | | |
| U0501, U0504 | Syrah | e-series, e78 and e88 allele patterns | 499 | 349 | 95 | 20 (135) |
| U0502 | Chardonnay | e-series, e78 and e88 allele patterns | 837 | 386 | 0 | (204) |
| U0505 | Cabernet Sauvignon | e-series, e78 and e88 allele patterns | 642 | 389 | 115 | 5 (118) |
| A81-17 | A38-7 | e-series, e78 allele pattern | 603 | 210 | 110 | 15 (40) |
| 1d. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 75% *vinifera* parentage. | | | | | | |
| 04373-02 12K | F2-35 (Cab x Carignane) | Alicante Bouschet, Aligote, Carignane, Chardonnay, Zinfandel | 597 | 305 | 175 | 58 |
| 1e. Other resistance sources. R89 is 50% *vinifera*, 25% resistance source. 05347 is 75% *vinifera* and 25% resistance source | | | | | | |
| R89(b40-14) | NR | Airen | 238 | 178 | NR | 163 |
| 05347 (b42-26) | F2-35 | Aligote, Chard, Grenache, Zin | 1,877 | 253 | NR | 140 |
| 1f. Rootstock crosses to combine PD and nematode resistance. | | | | | | |
| 9621-257 | 9365-85 | | 653 | 113 | 100 | 45 |
| 9365-43 | 9621-161 | | 112 | 42 | 30 | 10 |

Table 2. 2008 crosses and estimated numbers of seed produced.

| Resistant Type | Vinifera Parent of Resistant Type | Vinifera Types used in 2007 crosses | Estimated Seeds |
|---|---|---|---|
| 2a. Monterrey *V. arizonica/candicans* resistance source (F8909-08) to produce progeny with 93.75% *vinifera* parentage. | | | |
| U0501 | Syrah | Chardonnay, Colombard, Tannat, Tempranillo | 335 |
| U0502 | Chardonnay | F2-7 | 200 |
| U0505 | Cabernet Sauvignon | Tannat | 300 |
| 2b. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and *Run1* powdery mildew resistance to produce progeny with 90.6% *vinifera* parentage. | | | |
| U0502 | Chardonnay | 06353, e78 allele pattern | 60 |
| U0505 | Cabernet Sauvignon | 06717, e78 allele pattern | 70 |
| 2c. Monterrey *V. arizonica/candicans* resistance source (F8909-08) and a *vinifera* PM resistance source to produce progeny with 87.5% *vinifera* parentage. | | | |
| A81-17 | A38-7 | Karadzhandal | 350 |
| 2d. Monterrey *V. arizonica/candicans* resistance source (F8909-17 allele) to produce progeny with 87.5% *V. vinifera* parentage. | | | |
| 06324 | Chenin blanc | Airen, Cabernet Sauvignon, Chardonnay, Malaga Rosada, Tannat | 370 |
| 06372 | Malaga Rosada | Clairette blanche, Tannat | 540 |
| 06381 | F2-7 (Cab x Carignane) | Tannat | 85 |
| 2e. Other PD resistance sources: b40-14 *V. arizonica* (06339) progeny are 87.5% *vinifera.* The *V. shuttleworthii* PD resistance sources 0098-03 progeny are 87.5% *vinifera* and 04394 progeny are 75% *vinifera* | | | |
| 06339 | F2-35 (Cab x Carignane) | Tannat | 110 |
| 0098-03 | NR | Cabernet Sauvignon, Chardonnay | 175 |
| 04394 | NR | Cabernet Sauvignon, Clairette blanche, F2-35, Tannat | 1130 |
| 2f. Rootstock crosses to combine PD and nematode resistance. | | | |
| 03300-048 | 06301,Wyoming Riparia, Riparia Gloire, 44-53 mgt | | 500 |

**Table 3**. PD resistant winegrape progeny just completed or currently in greenhouse screening for PD resistance.

| Group | Genotypes | N | Inoculation Date | ELISA Date | Resistance Source(s) |
|---|---|---|---|---|---|
| A | 04190, 9621, 2007 parents | 150 | 10/18/2007 | 1/31/2008 | b43-17 (both alleles) |
| B | D89, R89, 9621, 03300/5, 03182, 03187, 04183, 04394, 2007 parents retest | 157 | 3/20/2008 | 6/26/2008 | b43-17, BD5-117, Midsouth, Haines City |
| C | 2006 recombinants, 06339 | 29 | 5/20/2008 | 9/4/2008 | b43-17, b40-14 |