THE GENETICS OF AND BREEDING FOR PIERCE'S DISEASE RESISTANT GRAPES

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ABSTRACT
We continue to make many crosses, produce thousands of seeds and embryos, and about four thousand plants in the field each year. We have been increasing the number of seedlings and high fruit quality selections we test under our greenhouse screen. This screening is very severe, but material that passes the screen is reliably resistant and dramatically restricts *Xylella fastidiosa* movement. We are also co-screening for powdery mildew resistance. The heritability of *Xf* resistance from a range of resistant Southeast US (SEUS) cultivar and species parents is not consistent – some parents produce few resistant offspring, while others produce a large percentage – making careful parental screening very important. We have been able to expand our *Xf* screening this year and are currently testing 178 potential parents (selected backcross progeny, new SEUS parents, and Olmo VR hybrids) and will have resistance results before the 2004 pollination season so that crosses can be optimized. The USDA embryo rescue process has produced a large number of progeny from crosses of resistant males to seedless females including 265 plants from second generation backcrosses. Culture efforts this year (2,702 ovules) produced 484 embryos that are now germinating. Crosses continue to be made with SEUS resistant wine grape selections to *vinifera* wine grapes including Merlot, Syrah, and Olmo selections. Rootstock crosses are also being made and seedlings evaluated and will be used in joint efforts to examine possible inducible tolerance to PD via rootstocks.

INTRODUCTION
This project is a collaborative effort between UC Davis and the USDA/ARS –Parlier, and is focused on breeding new PD resistant cultivars of table, raisin and wine grapes. The project integrates with efforts to develop genetic maps for resistance to *Xylella fastidiosa* (*Xf*) in segregating populations containing resistance from *Vitis arizonica* and a number of southeastern US (SEUS) *Vitis* species and cultivars. The goal of these mapping efforts is the development of strongly linked DNA markers to expedite breeding with the long term goal of characterizing and localizing *Xf* resistance genes leading to genetic transformation efforts.

A noteworthy discovery this year involved our *Vitis rupestris* x *Muscadinia rotundifolia* selections. These selections were produced from crosses made by Dr. H.P. Olmo and the seedlings were raised and evaluated in the Walker lab. The Walker lab has been evaluating these selections for *Xf* and nematode resistance and using progeny from them in mapping efforts for over 13 years. The mapping efforts have focused on a cross of two siblings from Olmo's *V. rupestris* 'A. de Serres' x *M. rotundifolia* 'Cowart' – D8909-15 x F8909-17 which generated the 9621 mapping population. Mapping efforts with this population are the basis of the Walker/Riaz proposal "Map-based Identification and Positional Cloning of *X. fastidiosa* Resistance Genes from Known Sources of Pierce's Disease Resistance in Grape". This year simple sequence repeat (SSR) DNA markers were placed on the map to allow comparisons with other maps being created within the lab and internationally. SSR markers are also ideal for parentage analysis, and once they were applied it became clear that D8909-15 and F8909-17 were not full siblings and that the male parents were incorrect. It is now clear that this cross was contaminated by wind-blown pollen from five male vines in addition to the applied *M. rotundifolia* 'Cowart' pollen. D8909-15 is a cross of *V. rupestris* 'A. de Serres' x *V. arizonica* and F8909-17 is a cross of the same female by what appears to be a hybrid of *V. arizonica* x *V. champinii*. F8909-08 is a true sibling of F8909-17 and the resistance source for a number of crosses in the breeding program.

This discovery has many positive implications for PD breeding. The *Xf* resistance of these *V. arizonica* hybrids is remarkably strong (as good as selections of *M. rotundifolia*) and has been integrated into a large number of *V. vinifera* grapes and is now in the third backcross (BC) generation. The Walker lab has reported on the resistance of these selections to both *Xf* and *Xiphinema index* (Walker and Jin 1998 Acta Hort 473:113-120., Krivanek and Walker 2003 Acta Hort 603:429-432). The horticultural characteristics of *V. arizonica* are far preferable to *M. rotundifolia* and include relatively neutral flavors, normal clusters with fruit that does not abscise when ripe and reasonable rooting ability, although its berry size is small. Most importantly there are no genetic barriers to breeding because *V. arizonica*'s chromosome number is the same as *V. vinifera*, as opposed to *M. rotundifolia*.

OBJECTIVES
1. Develop PD resistant table and raisin grapes by crossing a variety of *Xf* resistance sources with large berried and seedless *V. vinifera* table and raisin grapes.
2. Evaluate existing PD resistant wine-type cultivars for suitability in PD hotspots in California.
3. Breed PD resistant wine-type cultivars by crossing a selected number of *Xf* resistance sources with traditional wine varieties.

4. Investigate the potential of inducing *Xf* resistance by using various *Xf* resistant selections as rootstocks.

RESULTS AND CONCLUSIONS

Resistant Table/Raisin Varieties

We completed the screening of 126 additional genotypes in the last year: 102 were recent crosses of SEUS types backcrossed to advanced *vinifera* table and raisin types; 16 were from the USDA disease resistant table grape program (focused on powdery mildew); and 8 were new SEUS resistant sources added to our collections. In the first group, several resistant genotypes were identified as seedless (small hollow seed traces approaching commercial acceptability). Several others were resistant seeded genotypes with high quality fruit characteristics. Some resistant lines were also powdery mildew resistant. Interestingly, only two of the 16 USDA powdery mildew crosses were resistant to *Xf*. These were both first generation crosses from SEUS *Xf* resistant types, but *Xf* resistance does not seem to be correlated to powdery mildew resistance, which reemphasizes the need pre-screen for *Xf* resistance.

When crossed to *vinifera*, different SEUS resistance sources produce very different ratios of resistant (R) to susceptible (S) progeny. For example, screening data from this year produced the following R:S ratios: Calinda x Daytona 2:3 (n=25), C33-30 x DC1-39 1:3 (n=60), C33-30 x BD5-117 2:1 (n=21). Although n is small, it is clear that resistance is not inherited in the same manner among different parents, which emphasizes the importance of careful progeny testing.

We currently have 178 additional genotypes inoculated in our greenhouse screen. One hundred of these genotypes are progeny from four different resistance sources (*V. champinii*, Roucaneuf, Zehnder, and 0023-019 (D8909-15 x USDA seedless table grape)) backcrossed to USDA advanced seedless types. Early testing is being done so that we can select parents that produce the highest percentage of resistant progeny for next year's crosses. The remaining 55 genotypes in testing are progeny of SEUS-type resistant sources crossed to *vinifera* and that were selected for fruit quality this year. We are also testing 23 genotypes from the Olmo VR (*vinifera* x *rotundifolia*) collection consisting of F1 VR hybrids, VR intercrosses and VR backcrosses to *vinifera*. These have excellent powdery mildew resistance and many are expected to be *Xf* resistant.

This year's increased funding was directed at expanding greenhouse screening efforts as well as increasing the number of crosses, progeny and embryo rescued progeny we generate. It is critical to expand and accelerate screening efforts so that results on seedlings that fruited the year before are available to guide the following year crosses. In addition to the genotypes under testing above, 200 genotypes (split equally from USDA-Parlier and UCD) evaluated for fruit quality are being prepared for testing. Results are due before the breeding season 2004. We plan to test about 1,000 genotypes per year with this increased funding.

At UCD we planted 2070 seedlings from crosses made in 2002. About 32% were second generation BC of the *arizonica* resistance source hybrids to high quality *vinifera*, 17% were intercrosses of first generation resistant types, 35% were first generation *vinifera* crosses to a *champinii* resistance source, and 16% were first generation crosses of *vinifera* to two different SEUS *Xf* resistant selections.

From the crosses made and embryos cultured in 2002 at the USDA, 265 plants were produced from 20 BC1 combinations (D8909-08 resistance source) with table and raisin grape selections (13 table grape crosses = 181 plants in field and 7 raisin crosses = 84 plants in the field). An additional 74 plants were planted in the field from SEUS cultivars and selections crossed with table grape selections. Finally, 70 plants were planted in the field from table grape (1), raisin (1) and wine (2) varieties crossed to a selected VR hybrid. This year a large portion of the seedlings from the 2000 and 2001 seedlings produced fruit at Fresno and were evaluated. It is interesting to note that of the three selections from VR hybrids tested for PD resistance in the greenhouse, one was resistant. An additional 14 seedlings have been selected from VR crosses with improved fruit quality. One mildew resistant selection made from a cross with Suwanee was found to have intermediate *Xf* resistance in greenhouse tests. This selection is a white seedless (med to large trace) selection with 2.6 gram berries and will be used as a parent with table and raisin grapes. In the seedlings from crosses with SEUS cultivars and selections with table and raisin selections, 74 selections were made this year of which 42 were seedless. In crosses from other SEUS breeder's selections by table and raisin selections, 47 selections were made of which 31 were seedless. Many of these selections from SEUS material had seedless berries as large as 13-15/16 inch in diameter with firmness rated 6 (5 = average) and one rated 7 (= firm). The fruit quality in many was also rated good (7).

Twenty different cross combinations were made at UCD in 2003. Eight were second generation BCs of the *arizonica* resistance source to *vinifera*; five involved resistant selections from crosses made in 1999 and 2000; six used two promising SEUS selections (one that has previously shown a high percentage of resistant progeny and another that looks very good in our PD field trial); and one cross with a VR hybrid. One of the 5 crosses with our resistant selections was directed at combining resistance from *shuttleworthii* and *smalliana* with *vinifera*. Four additional crosses were made onto female seedless *vinifera* table grapes involving the same two promising SEUS selections used above. A total of 18 clusters were produced and shipped to USDA-Parlier for embryo rescue.

This year (2003) USDA-Parlier cultured ovules from 8 crosses. Three of these were backcrosses of 0023 seedlings with high quality table and raisin grapes. The rest were SEUS selections crossed to high quality table and raisin grape selections. A total of 2,702 seedless ovules were cultured and resulted in 484 embryos, which are now germinating.

Screening existing Xf resistant wine grape cultivars.
We are currently screening 8 *Xf* resistant wine grape selections from Zehnder, a private breeder in North Carolina. A number of the SEUS field resistant cultivars at our PD field trial in Yountville are wine grapes. As an adjunct to that trial, approximately 90 vines of Blanc du Bois were planted in 2001. The vines were harvested this year for small lot winemaking. USDA-Parlier has crossed Zehnder's selections to Merlot and is evaluating for flavor, pH, and TA. Seedlings of SEUS breeder's selections by wine cultivars were evaluated and 36 selections were made. The cluster sizes ranged from small to large and sugars reached 26°B by the first of October. These selections were also screened for leaf, stem and berry resistance to mildew.

Breeding new Xf resistant wine-types.
In 2000 we made 2 wine grape crosses of the highly *Xf* resistant F8909-08 (*arizonica-champinii* type) onto *vinifera* wine grapes to produce 3,227 seeds. These crosses were repeated this year and about 1,000 of these seedlings will be planted next spring. We also made crosses of SEUS cultivars onto Syrah, producing 2,150 seeds. Marker-assisted selection (see Walker/Krivanek/Riaz project) will be used to pre-screen these seedlings before fruit or *Xf* resistance evaluations.

PD resistant rootstock breeding.
We have about 500 seedlings in the field from crosses of D8909-15 and F8909-8 with 101-14 Mgt and 1616C rootstocks. These crosses were repeated this year to produce about 2,500 seeds. Genetic markers for resistance will be used to pre-screen these plants and their evaluation will be tied to research projects in collaboration with Hong Lin (USDA-Parlier) exploring biochemical resistance mechanisms that might be inducible via a resistant rootstock.

PD Field Trial
We expanded our PD field trail in Yountville this year with assistance from Beringer/Blass. Two additional blocks of plants were added. One contained replicated genotypes of recent crosses with varying degrees of *Xf* resistance as measured in our GH screen as well as some additional SEUS field resistant types. A second block was planted to 18 conventional rootstocks (101-14, 3309C, Schwarzmann, Gravesac, SO4, 5, 420A, 161-49C, St. George, 110R, 1103P, 44-53, 039-16, Riparia Gloire, Fercal, Freedom, Ramsey and Dog Ridge), and is destined for field budding to a field resistant SEUS cultivar to evaluate rootstock vulnerability to PD under California conditions. These grafted plants will be inoculated with *Xf* to evaluate the impact an infected, but resistant, scion has on rootstocks. Both the 2001 and 2003 plantings were inoculated with *Xf* again this year and ELISA and disease symptom expression data were taken in October.

CONCLUSIONS
Collaboration between UCD and the USDA-Parlier is excellent and this program is closely integrated with the other Walker lab projects and those of Lin at the USDA-Parlier. Fruit quality is advancing quickly in many backgrounds (see Figures 1 & 2). Aggressive training is allowing fruit evaluation in year 2 after planting, thus shortening generation times to 3 years. Raisin grape types should be ready for field testing within several generations, as will wine grapes. Table grapes will take longer to achieve large seedless berries with crisp texture, but we are very encouraged with our progress.

FUNDING AGENCIES
Funding for this project was provided by the California Table Grape Commission and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Funding in the past has also been received from the California Raisin Marketing Board and the USDA Animal and Plant Health Inspection Service.

Figure 1. An example of the rapid progress being made breeding *Xf* resistant table grapes. From left to right first generation progeny from *V. vinifera* B90-116 x *V. shuttleworthii* F902: F902, 0070-12, 0070-14, 0070-28 and Redglobe (for size comparison).

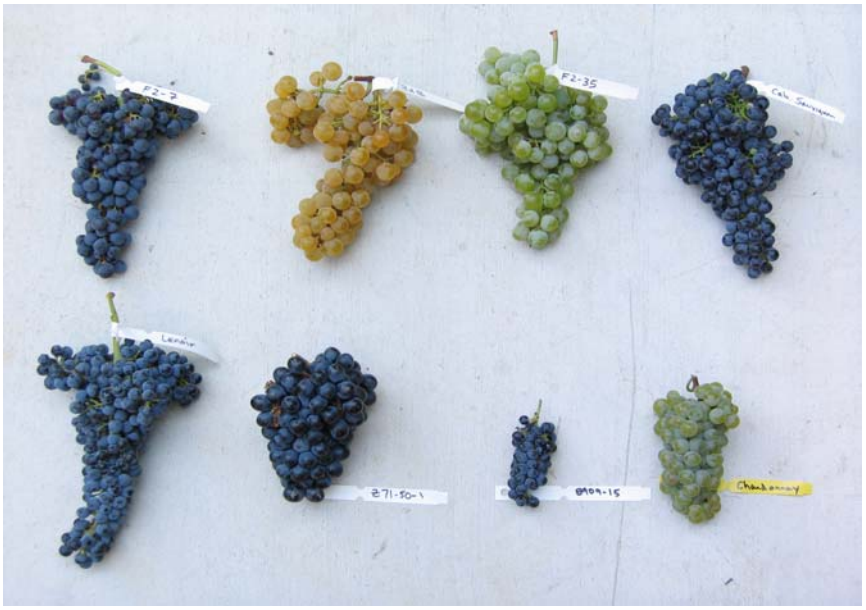

Figure 2. An example of wine grape types being used in crosses. Clockwise from the upper left: F2-7 (Carignane x Cabernet Sauvignon); Blanc du Bois; F2-36 (same cross as F2-7); Cabernet Sauvignon; Chardonnay; D8909-15; Zehnder 71-50-1; Lenoir (Jacquez or Black Spanish).