BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

Project Leaders:
Andrew Walker and Alan Tenscher
Dept. of Viticulture and Enology
University of California
Davis, CA 95616-8749

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ABSTRACT
Strong and continued progress is being made breeding Pierce's disease (PD) resistant winegrapes. We have been able to markedly improve fruit quality while maintaining high levels of PD resistance. At this point we have third generation backcrosses of our 8909-08 (*Vitis arizonica/candicans*) resistance source onto *V. vinifera* grapes. This will be the first time that PD resistant selections with so great a percentage (87.5%) of *V. vinifera* have been produced. We continue to make many crosses, produce thousands of seeds, and plant about 2,000 plants in the field each year. The greenhouse screening system continues to be refined and we have moved to smaller pots and more rapid evaluation to increase the number of seedlings and high fruit quality selections we test. This screening is very severe, but material that passes the screen is reliably resistant and dramatically restricts *Xylella fastidiosa* (*Xf*) movement. We are also co-screening for powdery mildew resistance. Strong progress on the development of *Xf* resistance markers has allowed us to use our *PdR1* markers for marker-assisted selection. This year's crosses will produce up to 14,000 seeds – many with >75% *V. vinifera* parentage. We have high expectations for strong resistance and excellent wine quality in this and the next generation of seedlings.

INTRODUCTION
The PD threat in California has greatly increased with the establishment and spread of the glassy-winged sharpshooter (GWSS). All of California's winegrapes are susceptible to PD and no effective prevention or cure currently exists. Under severe PD pressure, culture of *V. vinifera* grapes is not possible and new PD resistant cultivars are needed. PD resistance exists in a number of *Vitis* species and in the related genus, *Muscadinia*. Many resistant cultivars, which derive their resistance from these sources exist, but they lack *V. vinifera* fruit quality and have very complex resistance genetics. This complex genetics greatly limits the number of resistant progeny they produce when crossed to *V. vinifera* cultivars, which dramatically slows breeding progress.

At UC Davis, we are uniquely poised to undertake this important breeding effort. We have developed rapid screening techniques for *Xf* resistance and have optimized ELISA and PCR detection of *Xf* (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005). We have unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, that offer the introduction of extremely high levels of *Xf* resistance into commercial grapes. We also have several years' worth of seedlings in the ground that need evaluation as winegrape types. We are now breeding in a broad range of PD resistant backgrounds with most of our activity directed at resistance from *V. arizonica/candicans* b43-17, for which we have located a resistance locus that maps as a single dominant gene (*PdR1* – detailed in the companion report "Map-based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce's disease resistance in grape"). We have seed that is 87.5% *V. vinifera*, from winegrape cultivars, with resistance from the b43-17 resistance source and progress has been dramatically improved with marker-assisted selection.

OBJECTIVES
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.
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.

RESULTS
Objecitve 1
2005 Crosses – We made a wide range of crosses this year detailed in Table 1. Thus far in 2005, we have extracted 2,308 seeds and expect to extract another 11,725. These crosses were made in five groups. The first group (Table 1a) utilizes the b43-17 *V. arizonica/candicans* resistance source in a third generation backcross to produce progeny with 87.5% *V. vinifera* parentage. These plants have great potential and will contain more *V. vinifera* parentage than has been produced in past PD winegrape breeding programs. We will be testing this population with our *PdR1* genetic markers. This population will contain not only dramatically increased fruit quality, but also verify the utility of the *PdR1* markers in later generations. The second group (Table 1b) also utilizes the b43-17 resistance source and its progeny will contain 75% *V. vinifera*. The 03188 and 0062 selections used in these crosses are 50% F2-7 or F2-35 both female flowered selections from a Carignane x Cabernet Sauvignon cross. We have used a variety of "classic" winegrape cultivars in these crosses to promote productivity

and fruit quality traits (Airen, Alicante Bouschet, Barbera, Cabernet franc, Cabernet Sauvignon, Chardonnay, Sauvignon blanc, Syrah, Tempranillo and Viognier).

The third group of crosses (Table 1c) utilized the b42-26 *V. arizonica* PD resistance source. This genotype is the strong *Xf* resistance source for the 8909-15 parent in the 9621 genetic mapping progeny (detailed in the companion report – "Map-based identification and positional cloning of *X. fastidiosa* resistance genes from known sources of Pierce's disease resistance in grape"). This group of crosses will include progeny with 50% *V. vinifera* and 75% *V. vinifera* parentage. This source of resistance is multi-genic and we are attempting to establish quantitative trait loci (QTL) markers for its resistance in our mapping program. Although this resistance source is not as amenable to marker-assisted selection, it does produce progeny with very high levels of resistance. It may also be more valuable given its multi-gene resistance, making it more difficult for *Xf* to overcome its resistance mechanisms. Our current plan is to advance both the b43-17 and b42-26 sources of resistance and then intercross advanced resistant selections to broaden, and thus increase the durability, of PD resistance in their offspring. The use of selections from the 03188 (this cross was made in 2003) was only possible with our accelerated growing and screening conditions and techniques).

The fourth group (Table 1d) continues our efforts to use a broad range of southeastern US (SEUS) PD resistant cultivars. None of these sources has proven to be simple genetically and they produce widely ranging percentages of resistant progeny when crossed to *V. vinifera* cultivars. This inconsistent and low inheritance of PD resistance has greatly impeded the progress of past PD resistance breeding programs because very few resistant progeny are produced making it very difficult to get the numbers of seedlings required for selection of resistance in combination with high fruit quality. We are placing less emphasis on using SEUS parents, but continue with several to ensure that the base of our resistance is not too narrow. We also made crosses with two very promising VR (*vinifera* x *rotundifolia*) hybrids that have had strong resistance in our screens. The fifth group (Table 1e) are crosses we made to support mapping effort and increase the number of individuals in two specific mapping populations (further detailed in our report on fine-scale mapping).

Table 2 presents the number of progeny from the 2004 crosses that went to the field for evaluation of fruit traits and for *Xf* resistance screening. The populations with resistance from 8909-17 and 8909-08 were screened for the presence of the *PdR1 Xf* resistance marker and segregated in the expected 1 resistant: 1 susceptible ratio. The b43-36 and b43-56 *V. arizonica* selections performed very well in a resistance screen and were chosen as parents. Testing for the presence of PdR1 in these plants is under way. The crosses to the *V. smalliana*, *simponii* and Midsouth resistance sources were made to address breadth of resistance issues as noted in regard to Table 1d above. These plants will be evaluated for fruit quality and then tested for *Xf* resistance. Only 25 progeny from the *M. rotundifolia* resistance sources were planted in the field, displaying the difficulty in making these crosses and their low fertility and viability.

Objective 2

We optimized our *X*f screening system using smaller pots and a shortened period before ELISA and symptom evaluation. These efficiencies are allowing us to test more seedlings, selections, and genotypes for the mapping and gene characterization project. We are also testing a wide range of seed germination techniques to not only hasten germination, but to also increase the rate and make germination more uniform. We tested about 300 seedlings from a wide range of *Xf* resistance backgrounds including *V. champinii, M. rotundifolia, V. shuttleworthii, V. simpsonii, V. smalliana*, and a variety of more complex SEUS cultivars. The only seedling populations with predictable segregation ratios of resistant to susceptible plants were those from *V. arizonica/candicans* b43-17. This result has concentrated our efforts on this resistance source, while fewer plants from other resistance sources are being evaluated as noted above.

Table 3 presents the groups of genotypes currently under *Xf* resistance screening. The 0023 group testing completes *Xf* resistance testing of this mapping population (*V. vinifera* x *V. arizonica* b42-26). This group is currently under study for mapping of QTLs for *Xf* resistance. The 03300/5 group is a cross of 101-14Mgt x F8909-08. This group has been screened for PdR1 and it segregated 1:1 (n=30), confirming the use of these markers in a non-winegrape background. It will also produce PD resistant rootstocks on which PD resistant winegrapes will have to be grafted. The 04190 population is also under testing to help refine *PdR1* markers and for winegrape production. We are testing a number of 89 series seedlings that are crosses of *V. rupestris* to *V. arizonica* and *V. arizonica/candicans*. Additional sources of strong *Xf* resistance sources might be discovered from these results and the results should clarify the extent of resistance from other selections of these species. We have 32 new SEUS and *V. arizonica* type genotypes under test to evaluate potential parental germplasm.

We have many seedlings going to the field in Spring 2006 that will be 87.5% *V. vinifera* (Table 1a) and many more that will be 75% *V. vinifera* (Table 1b). These plants will begin fruiting in summer 2007. *PdR1* testing will identify *Xf* resistant individuals by early spring 2006. We take potted greenhouse plants of resistant selection and convert their tendrils to clusters with cytokinins. Pollen from these plants will be crossed onto *V. vinifera* winegrapes to produce seeds with 93.75% *V. vinifera*. This process will be combined with evaluation for the *PdR1* marker and decrease the traditional breeding cycle by several years.

As a prelude to much larger scale fruit quality evaluations, we tested the juice quality of 42 genotypes this year. This group included the *V. vinifera* parents we used in the 2005 crosses, the *Xf* resistant parents, and selections from a number of our populations with clusters that appeared to have high wine quality potential. Table 4 presents examples of the juice from *Xf* resistant selections from the 0058 Midsouth (*V. champinii*) resistance source and from the 03188 b43-17 resistance source; both sets of selections are 50% *V. vinifera*.

CONCLUSIONS

This project is developing PD resistant winegrapes, evaluating novel and known sources of PD resistance, and providing testing and support for our genetic mapping efforts. New winegrape selections will likely be available for wine and field-testing in about two years and will continue to be refined. The first phase of winegrape releases is aimed at use for planting in PD hot spots to act as buffers and have their fruit blended with traditional wine varieties.

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Table 1. 2005 PD breeding program crosses and the number of seeds collected or expected (in italics).

Female	Male	# Seeds	Comments
1a. Monterrey *V. arizonica/candicans* resistance source to produce progeny with 87.5% *V. vinifera* parentage.			
A81-138	Cab Sav, Chard, SB, Syrah	307	Highly resistant table grape selection by classic wine grape cultivars
1b. Monterrey *V. arizonica/candicans* resistance source (8909-08) to produce progeny with 75% *V. vinifera* parentage.			
03188-06	Airen, Barb, Chard, Temp, Viog,	419	03188 population is 50% *V. vinifera* with resistance from 8909-08 and contains the *PdR1* locus.
03188-07	Barb, Syrah, Viog, Zin	472	" "
03188-12	Alic Bousch, Barb, CF, Chard, Syrah, Temp, Viog	664	" "
03188-32	Airen, Syrah, Viog	331	" "
CS, F2-7, -35	03188-25	*1,250*	" "
F2-7, F2-35	03188-01	*2,000*	" "
F2-35	03188-30	*500*	" "
F2-7, F2-35	0062-81	*1,250*	" "
F2-7	03188-30	*1250*	" "
Sauv. blanc	03188-25	69	" "
Sauv. blanc	0062-81	46	" "
1c. Baja California *V. arizonica* resistance sources.			
D8909-15	Airen	*500*	Potential mapping population
D8909-15	Barbara	*25*	Winegrape breeding
F2-7	0023-019	*350*	75% *V. vinifera* breeding population
F2-35	0023-019	*250*	75% *V. vinifera* breeding population
F2-35	b42-26	*600*	Eliminates *V. rupestris* from resistance
1d. Other resistance sources.			
0028-44	0028-35, 0058-09, 0058-23, 0078-01	*275*	Midsouth resistance source and >50% *V. vinifera*
F2-35	b59-45	*50*	*M. rotundifolia* resistance and >75% *V. vinfera*
NC-11J	Cabernet Sauvignon	*25*	*M. rotundifolia* resistance and >75% *V. vinifera*
1e. Miscellaneous wine crosses with PD resistance sources.			
F2-7	F8909-08	*2400*	Remake 04190 mapping population
F2-35	F8909-08	*1000*	Remake 03188 as mapping population

Table 2. 2004 progeny that went to UCD breeding blocks for evaluation.

Female Parent	Male Parent	Resistance Source	Seeds	Seedlings to field
BO2SG	Cabernet Sauvignon	*V. smalliana*	376	25
BO2SG	Carignane	*V. smalliana*	196	25
BO2SG	Sauvignon blanc	*V. smalliana*	404	40
BO3SG	Chambourcin	*V. smalliana/simpsonii*	412	20
BO3SG	Petite Sirah	*V. smalliana/simpsonii*	419	20
BO3SG	Cabernet Sauvignon	*V. smalliana/simpsonii*	371	20
BO3SG	Carignane	*V. smalliana/simpsonii*	350	40
BO3SG	Sauvignon blanc	*V. smalliana/simpsonii*	223	25
F2-7	Midsouth	*V. champinii*	522	50
F2-7	F8909-08	*V. arizonica/candicans*	4,500	220
F2-7	F8909-17	*V. arizonica/candicans*	300	107
F2-35	b43-17	*V. arizonica/candicans*	323	65
F2-35	b43-36	*V. arizonica*	141	65
F2-35	b43-56	*V. arizonica*	56	25
F2-35	Midsouth	*V. champinii*	522	25
NC-11J	0124-01	*M. rotundifolia* x SEUS complex	175	21
0110-050	0124-01	SEUS complex x SEUS complex	750	65
Midsouth	Midsouth	*V. champinii*	500	10
NC6-15	Sauvignon blanc	*M. rotundifolia*	50	4
Total			10,590	872

Table 3. Seedling populations currently under *Xf* resistance testing. Five replicates of each genotype are being tested and results are expected between mid Oct and January.

Group Name	Resistance source	Genotypes tested	Comments
0023	D8909-15	75	b42-26 mapping population
03305	b43-17	20	Production of PD resistant rootstock
03188	b43-17	33	Resistant winegrape breeding and also verifies *PdR1* markers
04190	b43-17	114	Resistant winegrape breeding and mapping to refine *PdR1* markers
89 series untested	*V. rupestris* x *V. arizonica /candicans* types	56	Completes *Xf* resistance survey
Misc. types	SEUS or *V. arizonica*	32	types for wine breeding

Table 4. Juice quality data from *Xf* resistant selections and three *V. vinifera* cultivars. Absorbance readings were made in 1 cm cuvettes.

Genotype	Sample Date	Brix	pH	TA (g/l)	Juice Hue	Juice Intensity	Absorbance 420nm	Absorbance 520nm
0058-03	27-Sep	23.3	3.95	6.8	1.04	9.64	4.905	4.730
0058-09	27-Sep	21.0	3.53	5.8	2.01	1.67	1.113	0.554
0058-23	27-Sep	23.4	3.96	4.5	1.24	6.44	3.566	2.876
0028-35	27-Sep	21.8	3.74	5.3	1.38	2.89	1.673	1.215
03188-02	27-Sep	26.9	3.23	10.5	0.73	3.24	1.368	1.869
03188-05	2-Sep	24.3	3.36	11.4	1.17	10.44	5.635	4.805
03188-06	24-Aug	22.0	3.25	10.2	1.20	8.51	4.645	3.860
03188-07	16-Sep	27.0	3.20	15.2	0.96	13.38	6.550	6.825
03188-09	16-Sep	24.2	3.50	8.6	1.21	8.26	4.530	3.730
03188-17	2-Sep	25.0	3.44	12.2	1.03	10.91	5.540	5.365
03188-32	24-Aug	24.5	3.34	11.9	1.20	13.76	7.510	6.245
F2-35 (*V. vinifera*)	27-Sep	26.2	3.46	5.6	1.75	1.23	0.781	0.446
Cabernet Sauvignon	16-Sep	24.4	3.81	6.6	1.38	8.81	5.105	3.700
Chardonnay	27-Sep	26.7	3.51	7.2	1.72	4.24	2.684	1.558