BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

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ABSTRACT
Strong and continued progress is being made in breeding Pierce's disease (PD) resistant grapes. Fruit quality has markedly improved while maintaining high levels of PD resistance. We continue to make many crosses, produce thousands of seeds, and plant about two 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 (Xf)* 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 the past few years and have tested hundreds of potential parents before we need to make breeding decisions the following year.

INTRODUCTION
Renewed and intensified PD outbreaks in historic PD zones in wine regions around the state and the introduction of GWSS into the southern San Joaquin Valley demonstrate the vulnerability of *V. vinifera* wine grape culture in California. All of California's wine grapes are susceptible to PD and no effective prevention or cure currently exists. Under severe PD pressure, culture of *V. vinifera* grapes is not possible. We are currently breeding PD-resistant wine grape cultivars for localized use in traditional PD "hot-spots" that are common in the North Coast, and it is likely that acceptable white and red wine grapes for these areas can be produced in two generations of crosses with our current *Xf* resistant selections. To further improve the utility of these *Xf* resistant cultivars, we are co-selecting for high levels of powdery mildew resistance. Unlike wine varieties for widespread use where the need for "pure *V. vinifera*" cultivars is enforced by marketing, given adequate quality (neutrality, color, season, cultural characteristics) varieties for localized use should prove useful to industry as blenders and by keeping "hot-spot" vineyard acreage in production. Our concurrent efforts to identify *Xf* resistance genes (see companion proposal – Walker and Riaz) will make it possible in the future to transform wine grapes with grape-derived resistance genes. Using grape genes to transform grapes should help overcome public reluctance about GM grapes and provide durable PD resistance.

PD resistance exists in a number of *Vitis* species and in the related genus, *Muscadinia*. Resistant cultivars have been developed in public and private breeding programs across the southeastern United States (SEUS). These cultivars have high PD resistance, but relatively low fruit quality relative to *V. vinifera* grapes. In the southeastern US, they must also resist downy and powdery mildew, black rot and anthracnose, which have as great an effect on viticulture in the southeast as PD does. Most of these diseases are not found in California, allowing breeders to incorporate more high quality *V. vin*ifera into their breeding efforts and enabling the production of much higher quality PD resistant cultivars in a shorter time span. We have characterized (see past reports) and employed a wide range of PD resistant germplasm from the collections at the National Clonal Germplasm Repository, Davis; selections obtained from breeders in the southeastern U.S.; from *V. rupestris* x *V. arizonica* selections that have exceptional PD resistance; and from several *V. vinifera* x *M. rotundifolia* hybrid winegrape types that have some fertility. These breeding efforts have already resulted in relatively high quality selections with excellent PD resistance.

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. 2004, Krivanek et al. 2004, Krivanek and Walker 2004). 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.

OBJECTIVES
The objectives of our PD breeding project are divided into two primary parts. The first is the breeding of *Xf* resistant wine grapes through backcross techniques using *V. vinifera* wine grapes and *Xf* resistant selections and sources characterized from our previous breeding efforts. The second is the continuing 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. These efforts support both the breeding program and the genetic mapping program.

Completion of these objectives is tied to the speed with which seedlings can be produced, fruited and evaluated and subsequent generations produced.

- Develop multiple lines of *Xf* resistant wine grapes using 8909 (*V. rupestris* x *V. arizonica* selections; *Xf* resistant breeder selections (DC1-39, Zehnder selections, etc); and southern grape species (*V. arizonica*, *V. champinii*, *V. shuttleworthii*, *V. simpsonii*, *M. rotundifolia*, and others).
- Continue backcross generations with 8909-08, DC1-39, and other lines to advanced *vinifera* selections and select for high quality wine grape characteristics.
- Continue to identify and characterize additional sources of *Xf* resistance with high levels of powdery mildew resistance.
- Maintain current and produce additional populations for genetic mapping efforts aimed at characterizing *Xf* resistance genes, and identifying and mapping fruit quality traits such as color, tannin content, flavor, production, etc. in *Xf* resistant backgrounds.
- Study the inheritance of *Xf* resistance from a broad range of resistance sources.

RESULTS AND CONCLUSIONS

Shift From Table Grape Breeding to Wine Types

Because the California Table Grape Commission's decision to not fund the breeding of PD resistant grapes, as of May 2004 we are now solely breeding PD resistant wine grapes. This year we evaluated 4,042 seedlings from 39 different crosses made in the last three years for use as wine grapes. From this number, four subgroups based on different resistance source were identified as particularly promising (Table 1). Promise was based on resistance to *Xf* and powdery mildew, fruit quality parameters, and viticultural characteristics such as yield and growth habit.

Evaluation of Fruit Quality

Within a cross we observed useful segregation of wine grape quality factors such as quality and quantity of color, acidity, pH, flavor, and skin and seed tannin. Table 2A and 2B present data for typical genotypes from three of the four resistance groups. These were harvested on August 26, 2004. Figure 1 displays clusters from two of the four promising *Xf* resistance subgroups listed in Table 1. Their morphology is becoming very vinifera-like in the first generation. Figure 2 displays juice extracted from some of the *Xf* resistant crosses in comparison with the juices from Cabernet Sauvignon and Pinot noir. There are a wide variety of colors that should allow matching enological needs with our selection process.

Planting of 2003 Crosses

Table 3 summarizes the field planting of wine crosses made in 2003. We did not germinate the 2,150 seeds of the cross of a SEUS cultivar by Syrah since our GH screening of progeny from the same SEUS female by pure *V. vinifera* indicated only 1 in 12 of the seedlings was likely to be resistant. Crosses made in Spring 2003 contained efforts directed at table and raisin grape production. This year's crosses were entirely devoted to wine grape efforts.

Wine Crosses Made in 2004

Table 4 details the wine grape crosses made during Spring 2004. We were able to tailor our choices for PD resistant parents with our previous experiences directed at table grape breeding. The assays of subsets of progeny from crosses with various parental sources found that the expression of PD resistance in progeny varies. *Vitis arizonica/candicans* selections from near Monterey, Mexico (b43-17, b43-36, and b43-56) produced 100% resistant progeny in the testing of the subset and should therefore be homozygous resistant. F8909-08 and F8909-17 were both derived from b43-17. The heritability of selections from Florida varied: BO2SG, BD5-117 and Midsouth produced 50% resistant progeny; while only 20% of the progeny of BO3SG was resistant, so progeny from it will be planted sparingly. NC-11J x UCD0124-01 represents a resistant x resistant cross from two different resistant backgrounds. B55-1 and NC6-15 are opportunities to ingress resistance from *Muscadinia rotundifolia* into wine crosses. We plan to plant between two and three thousand of the most promising seedlings from the crosses detailed above in Spring 2005.

Greenhouse Screen Results

We screened 474 genotypes with our greenhouse screen. The tested genotypes included cultivars and species from the SEUS, many Olmo Vinifera/Rotundifolia (VR) hybrids with potential PD resistance and for use as parents, table and wine grape crosses, and possible *Xf* resistant wine grape selections from a private breeder in North Carolina. Several promising *Xf*-resistant SEUS genotypes were identified. Six of 19 Olmo VR hybrids tested resistant. Two may be promising parents. None of the wine grape selections from North Carolina proved to be adequately resistant.

Table 5 presents the ratio of resistant to susceptible (R:S) progeny from crosses of highly susceptible *V. vinifera* parents crossed with a variety of *Xf* resistance sources. One *V. smalliana* and one *V. champinii* F1 hybrid progeny had R:S ratios of close to 1:1, suggesting that the resistance in these parents was heterozygous and controlled by a single gene. Other parents had ratios ranging from 1:3 through 1:11. Details are summarized in Table 5. We made crosses onto the *V. champinii* hybrid this year and they will be tested to see if the inheritance ratio remains 1:1, as does our F8909-17 resistance source (see Walker-Krivanek report). In other backgrounds, resistance seems to erode with continued backcrossing to *V. vinifera*, thus these stable resistance sources are very valuable and are easily adapted to marker-assisted selection.

Progeny from crosses of field resistant parents, like JS23-416 – judged resistant in Florida (Herb Barrett, personal communication) yet has been susceptible in our greenhouse tests, to *V. vinifera* do not seem to be resistant (<100,000 fu/ml). However, they do produce a broad and relatively even distribution of progeny from 170,000 to almost 6,500,000 cfu/ml. Although we would not consider those at the low end of this scale to be resistant, they have as low or lower bacterial levels than do some of the field resistant genotypes from the SEUS we have tested. We have avoided these progeny and using these parents to prevent release of field resistant cultivars that may survive PD infection, but allow vine-to-vine movement in vineyards.

We are beginning testing of about 200 genotypes with results expected in March 2005. These results will be used to direct backcrossing of the most resistant genotypes to *V. vinifera* wine grapes.

Napa Field Trial

This year we planted another block in our field trial at Beringer Vineyards in Yountville. We expanded the plot by adding 6 vine replicates of 20 different genotypes from 4 different resistant sources. Based on our GH screen results, both highly resistant and highly susceptible genotypes from each resistant source were planted. These will be inoculated with *Xf* next April and ELISA tested in October 2005.

This fall we observed the most pronounced visual PD symptoms to date in the 2001 and 2003 plantings following inoculation with *Xf* early this spring. We used a mixture of 5 different Napa PD strains as inoculum. The 2001 planting consists of known field resistant selections from the SEUS, and the 2003 planting consists of 3 vine reps of some of our early crosses and a few more SEUS field resistant types. On October 8, 2004 we scored these vines for visual symptoms and took samples for ELISA testing from 291 vines in these blocks. Results will be reported in December.

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Table 1. Summary of different crosses within the subgroups and the relative number of genotypes within each group that merit further evaluation.

Resistance Source	*V. vinifera* Parent	Genotypes Evaluated	Genotypes Selected
BO2SG (*V. smalliana*)	C1020	36	10
	Princess	21	9
BO3SG (*V. smalliana-simpsonii*)	C67-129	30	7
	Princess	81	14
AW C52-94 (*V. simpsonii*)	C51-63	353	71
Midsouth	B90-116	39	4
	C67-129	46	1
	Princess	8	1
Total		614	117

Table 2A. Analytical evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Cluster Wt. (g)	Brix	pH	TA (g/L)	Berry Wt. (g)	Est. Yield (gal/ton)
BO2SG	*V. smalliana*	45	24.5	3.28	19.7	0.3	129
BO3SG	*V. smalliana-simpsonii*	66	25.0	3.53	12.1	0.3	90
Cab Sauv	*V. vinifera*	269	23.0	3.52	6.8	1.0	160
Pinot noir	*V. vinifera*	299	25.5	3.72	6.1	1.2	182
J13-09	BO2SG x Melissa	184	24.2	3.16	12.1	1.3	160
J13-13	BO2SG x Melissa	62	25.5	3.22	9.8	1.4	162
J14-09	BO2SG x C1020	90	25.2	3.36	9.1	1.2	176
J14-12	BO2SG x C1020	125	27.0	3.46	8.3	1.0	167
J14-16	BO2SG x C1020	120	26.0	3.38	9.8	1.4	170
J17-3	BO3SG x C67-129	100	25.0	3.32	7.1	1.3	150
J17-06	BO3SG x C67-129	102	25.8	3.53	6.4	1.4	149
J17-08	BO3SG x C67-129	117	26.5	3.43	7.7	1.0	135
J17-14	BO3SG x C67-129	200	27.0	3.68	5.9	0.9	148
J17-24	BO3SG x C67-129	224	26.0	3.62	6.7	1.1	137
J17-25	BO3SG x C67-129	70	27.0	3.65	5.9	1.0	146
J17-36	BO3SG x Melissa	110	26.5	3.76	4.5	0.9	154
J17-39	BO3SG x Melissa	70	25.0	3.33	7.4	0.8	176
J17-50	BO3SG x Melissa	185	24.0	3.32	6.8	1.2	165
J18-18	BO3SG x Melissa	195	23.0	3.14	9.8	1.1	143
J18-24	BO3SG x Melissa	60	26.5	3.54	5.5	1.1	148
J18-35	BO3SG x Melissa	93	26.2	3.55	6.2	0.9	152
J18-37	BO3SG x Melissa	100	23.5	3.14	9.7	0.7	158
J18-38	BO3SG x Melissa	101	25.0	3.23	8.6	1.0	154
J27-03	Midsouth x B90-116	99	23.5	3.85	8.3	1.2	168
J27-06	Midsouth x B90-116	125	25.0	3.76	5.2	1.2	145

Table 2B. Sensory evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Skin Tannin Intensity[a]	Seed Color[b]	Juice Hue	Juice Color Intensity	Juice Flavor
BO2SG	*V. smalliana*	2	4	red	dark	fruity, peppery
BO3SG	*V. smalliana-simpsonii*	1	4	red	dark	fruity, peppery
Cab Sauv	*V. vinifera*	3	2.5	pink	light	slightly vegetal
Pinot noir	*V. vinifera*	1	4	pink	very light	fruity
J13-09	BO2SG x Melissa	2	4	red	medium +	tart, red fruit
J13-13	BO2SG x Melissa	2.5	4	red-purple	medium +	fruity, slight hot pepper
J14-09	BO2SG x C1020	2	4	red	medium	tart, jammy, very slight hot pepper
J14-12	BO2SG x C1020	2	4	pink	light	slightly jammy, broad fruity
J14-16	BO2SG x C1020	2	4	green		green pepper, hot pepper
J17-3	BO3SG x C67-129	1.5	4	red-purple	medium +	slightly fruity, hot pepper
J17-06	BO3SG x C67-129	2	3.5	pink-red	medium	hay, hot pepper
J17-08	BO3SG x C67-129	1.5	4	pink-orange	light +	vinifera-like, acidic, hot pepper
J17-14	BO3SG x C67-129	2	4	red	medium	slightly jammy, fruity
J17-24	BO3SG x C67-129	4	4	red	medium +	fruity, hot pepper
J17-25	BO3SG x C67-129	1.5	4	red	medium	very slightly vegetal-herbal
J17-36	BO3SG x Melissa	2	4	pink	medium -	slight hay, hot pepper
J17-39	BO3SG x Melissa	2	4	red	medium +	tart, raspberry, very slight hot pepper
J17-50	BO3SG x Melissa	2	4	pink-red	medium	simple fruit, berry
J18-18	BO3SG x Melissa	3	4	pink-red	medium -	slight hay, canned
J18-24	BO3SG x Melissa	2	4	red	medium	slight hay, fruity
J18-35	BO3SG x Melissa	2	3.5	pink-red	medium -	hay, hot pepper
J18-37	BO3SG x Melissa	2	4	pink-brown	light	tart berry, slightly buttery
J18-38	BO3SG x Melissa	1	4	red	medium -	berry, slight hot pepper
J27-03	Midsouth x B90-116	1	4	purple	dark	current, vegetal
J27-06	Midsouth x B90-116	1	4	red	medium-	strawberry, herbal

a = (1=low, 4= high); b = (1=green, 4= brown)

Table 3. UC Davis field plantings of wine crosses made in 2003. F2-7 and F2-35 are respectively a black and a white female seedling of the cross Cabernet Sauvignon x Carignane. B34-82 is a USDA cross.

Cross	Resistance Source	Seedlings Planted
F2-7 x F8909-08	*V. arizonica*	10
F2-35 x F8909-08	*V. arizonica*	38
F2-35 x BD5-117	SEUS complex	164
F2-7 x BD5-117	SEUS complex	149
BD5-117 x B34-82	SEUS complex	141
	Total	502

Table 4. Wine grape crosses made at UCD in 2004.

Female Parent	Male Parent	Resistance Source	# Seeds
BO2SG	Cabernet Sauvignon	*V. smalliana*	376
BO2SG	Carignane	*V. smalliana*	196
BO2SG	Sauvignon blanc	*V. smalliana*	404
BO3SG	Chambourcin	*V. smalliana-simpsonii*	412
BO3SG	Petite Sirah	*V. smalliana-simpsonii*	419
BO3SG	Cabernet Sauvignon	*V. smalliana-simpsonii*	371
BO3SG	Carignane	*V. smalliana-simpsonii*	350
BO3SG	Sauvignon blanc	*V. smalliana-simpsonii*	223
F2-7 (CabS x Carig.)	BD5-117	SEUS complex	1131
F2-7	Midsouth	*V. champinii*	522
F2-7	F8909-08	*V. arizonica - candicans*	4,500
F2-7	F8909-17	*V. arizonica - candicans*	300
F2-35 (CabS x Carig.)	B55-1	*M. rotundifolia*	18
F2-35	B43-17	*V. arizonica-candicans*	323
F2-35	B43-36	*V. arizonica*	141
F2-35	B43-56	*V. arizonica*	56
F2-35	BD5-117	SEUS complex	783
F2-35	Midsouth	*V. champinii*	522
NC-11J	UCD0124-01	*M. rotundifolia*-SEUS complex	175
Midsouth	Midsouth	*V. champinii*	500
NC6-15	Sauvignon blanc	*M. rotundifolia*	50
Total			11,772

Table 5. Ratios of *Xf*-resistant: susceptible (R:S) progeny in populations from various resistance sources by *V. vinifera* parents based on a greenhouse screen. Resistance is defined as a mean value less than 100,000 cfu/ml (colony forming ***units per ml).***

Resistant Parent	Resistance Source	Number Resistant	Number Tested	Percent Resistant	Approx: R/S ratio
Midsouth	*V. champinii*	9	17	53%	1:1
BO2SG	*V. smalliana*	11	23	48%	1:1
Cha3-48	*V. champinii*	8	26	31%	1:2
DC1-39	Complex	9	33	27%	1:3
BO3SG	*V. smalliana-simpsonii*	1	6	17%	1:5
F901	*V. shuttleworthii*	1	7	14%	1:6
AW c52-94	*V. simpsoni*	2	15	13%	1:6
Z 71-50-1	Complex	2	25	8%	1/11
AT0023-019	*V. arizonica* (La Paz)	2	29	7%	1/11
F902	*V. shuttleworthii*	0	16	0%	-
Roucaneuf	Complex	0	22	0%	-
Villard blanc	Complex	0	6	0%	-
JS23-416	Susceptible	0	19	0%	-
Total			244		

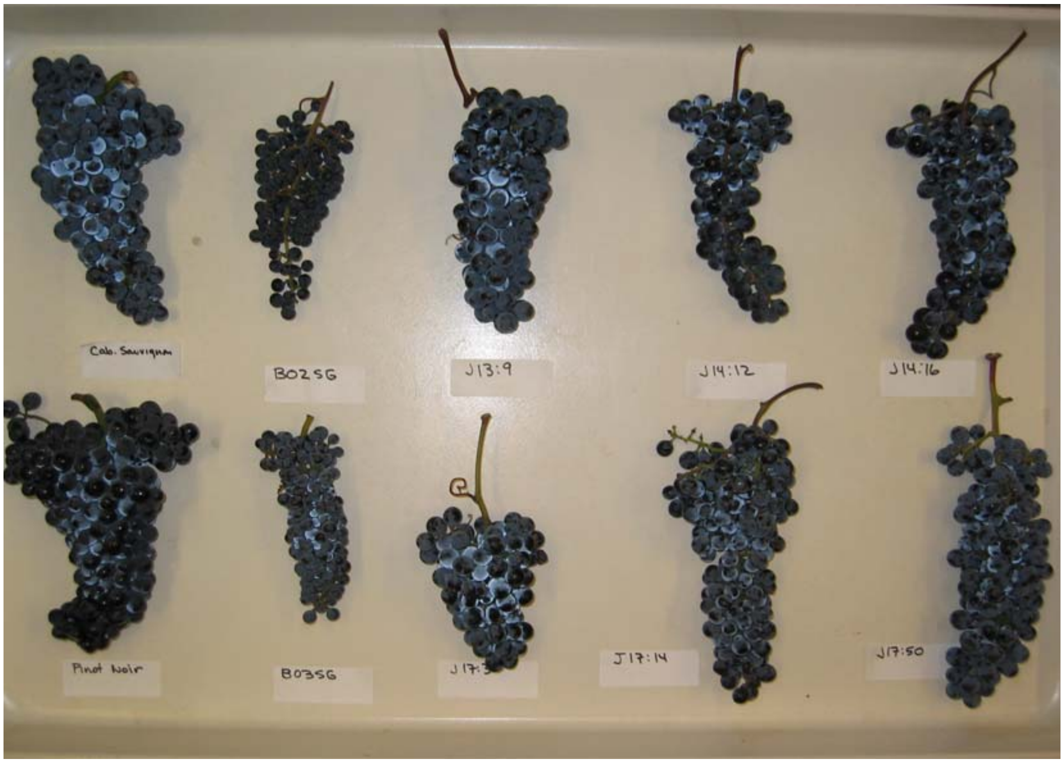

Figure 1. Representative clusters from two promising *Xf* resistance source subgroups. BO2SG and BO3SG are the resistant female parents. Cabernet Sauvignon and Pinot noir are shown for size/shape comparisons. Crosses to BO2SG are in the top row while crosses to BO3SG are in the bottom row. The other clusters are from first generation crosses. Analytical details can be found in Table 2.

Figure 2. Juice extracted from selected clusters of *Xf*-resistant crosses shown in Figure 1 and detailed in Table 2. Note the high quantity of red color and the variation in hue from some of the crosses. This variation allows for tailoring varieties to meet particular enological needs. Juice from Cabernet Sauvignon and Pinot noir are on the left in the first two vials respectively.

Section 2: Vector Biology and Ecology