BREEDING PIERCE'S DISEASE RESISTANT TABLE AND RAISIN GRAPES AND THE DEVELOPMENT OF MARKERS FOR ADDITIONAL SOURCES OF RESISTANCE

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ABSTRACT
The first BC4 *Vitis arizonica* crosses made in 2009 resulted in 283 resistant seedlings with molecular markers for PdR1 that were planted in the field. These seedlings will have high fruit quality as they consist of 97% *V. vinifera*. A total of 998 seedlings from all *V. arizonica* crosses made in 2009 were screened with molecular markers for PdR1 while still in test tubes from embryo culture and 413 (47%) were resistant. The use of molecular markers to select for Pierce's disease (PD) resistance and greenhouse screening to select for powdery mildew(PM) resistance allows the pyramiding of these two types of resistance and 31 plants with both were planted in the field. In 2010, 28 of 41 crosses attempted were successful and produced 1,600 berries, 2,114 ovules and 293 (13.9%) embryos for PD resistance. An additional 13 crosses to pyramid PD and PM resistance produced 1,782 berries, 2,172 ovules and 341 (15.7%) embryos. Eight seeded crosses made in 2010 produced 415 seed for PD resistance. Evaluation of BC3 fruiting seedlings continued with the selection of 19 new raisin seedlings with PdR1 molecular markers for propagation in production trials. The fruit quality of these selections is similar to the most advanced selections in our raisin breeding program. Over 500 seedlings from the BD5-117 family, with PD resistance different than *V. arizonica*, are being evaluated for cluster size, berry size and color, and seed/aborted seed size to determine fruit quality. Two hundred and forty-seven seedlings of this family have been evaluated in the greenhouse for PD resistance. A rough molecular map is being developed from this population to determine additional molecular markers for PD resistance.

LAYPERSON SUMMARY
Although Pierce's disease (PD) has existed in California since the late 1800s, the introduction of the glassy-winged sharpshooter to California in the late 1990's significantly increased the spread and damage caused by PD. A collaborative breeding program was started in 2000 to develop PD resistant table and raisin grapes with high fruit quality comparable to that existing in markets today. Fifth generation (BC4) crosses for quality table and raisin grapes with *Vitis arizonica* source of PD resistance were made again this year. These families will have high fruit quality as they consist of 97% V. vinifera. An example of increased fruit quality is the selection this year of 19 new raisin grapes made from BC3 *V. arizonica* families which will be propagated for production trials. Three new BC2 *V. arizonica* raisin selections were made and will be propagated for production trials. The use of molecular markers has allowed the selection of PD resistant seedlings while they are still in test tubes. Four hundred thirteen resistant seedlings were selected from 998 seedlings this year, thereby making the breeding program more efficient. Advanced selections are screened in the greenhouse to verify PD resistance. The use of molecular markers and greenhouse screening has made possible the pyramiding of PD with PM resistance. Forty-five PD resistant plants were selected by markers from 98 plants and 31 of these were resistant to PM in the greenhouse screen. Over 500 seedlings from BD5-117 family, a source of resistance that is different from *V. arizonica*, are being evaluated a second time for fruit characteristics to map with resistance. To date, 247 seedlings have been tested in the greenhouse for resistance to PD. A frame-work molecular map is being made with 65 fluorescent labeled SSR markers. This collaborative research between USDA/ARS, Parlier and University of California, Davis has the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry where PD could restrict the use of conventional table and raisin grape cultivars.

INTRODUCTION
Pierce's disease (PD) has existed in California since the late 1800s when it caused an epidemic in Anaheim. A number of vectors for PD already exist in California, and they account for the spread and occurrence of the disease. The introduction of the glassy-winged sharpshooter to California in the 1990's significantly increased the spread and damage caused by PD. Other vectors exist outside California and are always a threat. All of California's commercially grown table and raisin grape cultivars are susceptible to PD. An effective way to combat PD and its vectors is to develop PD resistant cultivars so that PD epidemics or new vectors can be easily dealt with. PD resistance exists in a number of *Vitis* species and in *Muscadinia*. PD resistance has been introgressed into grape cultivars in the southeastern United States, but fruit quality is inferior to *V. vinifera* table and raisin grape cultivars grown in California. Greenhouse screening techniques have been improved to expedite the selection of resistant individuals (Krivanek et al. 2005, Krivanek and Walker 2005). Molecular markers have also been identified that make selection of PD resistant individuals from *V. arizonica* even quicker (Krivanek et al. 2006). The USDA, ARS grape breeding program at Parlier, CA has developed elite table and raisin grape cultivars and germplasm with high fruit quality. Embryo rescue procedures for culturing seedless grapes are being used to quickly introgress the

seedless trait with PD resistance (Emershad et al. 1989). This collaborative research gives the unique opportunity to develop high quality PD resistant table and raisin grape cultivars for the California grape industry.

OBJECTIVES

1. Develop PD resistant table and raisin grape germplasm/cultivars with fruit quality equivalent to standards of present day cultivars.
2. Develop molecular markers for Xf/PD resistance in a family (SEUS) other than *V. arizonica*.

RESULTS AND DISCUSSION

Objective 1

Twenty-eight of 41 crosses using *V. arizonica* source of resistance made in 2010 were successful and produced 1,601 berries, 1,700 ovules, and 293 embryos (17% embryos/ovules) (**Table 1**). The majority of these crosses were BC4 and BC3 crosses. The seedlings obtained from these crosses should have high fruit quality as they now have 93 to 97% *V. vinifera* in their background. Berry set was very poor this year due to the late spring and poor weather conditions. An additional 13 crosses to pyramid PD (*V. arizonica)* resistance with powdery mildew (PM) (*V. romanetii*) resistance produced 1,782 berries, 2,172 ovules and 341 (15.7%) embryos. Molecular markers should be available soon to select for PM resistance. Eight seeded crosses made in 2010 produced 415 seed for PD resistance (**Table 2**).

The first BC4 crosses (97% *Vitis vinifera*) was made in 2009 and consisted of 31 crosses of which 19 produced 3,931 berries, 5,000 ovules and 1,372 embryos and 846 plants. A total of 283 BC4 PD resistant plants based on PdR1 markers were planted in the field. Leaves from all 2009 *V. arizonica* PdR1 plants were taken when seedlings were still in test tubes starting in November, 2009. They were tested for resistance with molecular markers for the PdR1 locus on chromosome 14. Results for one BC2, six BC3 and 19 BC4 seedless x seedless families (89-0908 *V.* arizonica source of resistance) is shown in **Table 3**. A total of 998 individuals were tested with SSR markers and 877 showed markers on both sides of the PdR1 region as expected. A total of 413 individuals (47% of those showing markers) were resistant and planted to soil in cups for growth in the greenhouse before planting to the field in April, 2010. Forty-six percent of the plants showing markers were susceptible. This is very similar to the ratio of resistant and susceptible plants obtained for over 1,600 F1, BC1, BC2 table and raisin seedlings reported by Riaz et al. 2009. The susceptible and recombinant individuals were discarded, making more efficient use of greenhouse and field space. One BC4 cross made in 2009 which combined PD resistance from *V. arizonica* with PM resistance from *V. romanetii*. Usually resistance from this source of PM resistance segregates in a 1:1 resistant:susceptible ratio. The seedlings that had PD resistant markers were screened in the greenhouse for PM resistance. Of the 44 PD resistant seedlings screened, 31 were PM resistant and planted to the field. Inoculation of plants with *Xylella fastidiosa (Xf)* in the Greenhouse (method of Krivanek et al. 2005, Krivanek and Walker 2005) was done to determine resistance of 63 selected individuals from *V. arizonica* (**Table 4**) of which 49 were resistant. These seedlings represent the best table and raisin selections that have been used as parents or planted in production trials. An additional 95 selections are in the greenhouse tests and will be evaluated for PD resistance before the end of this year. Greenhouse testing is absolutely necessary to make the final decision about resistance of individual selections. The highest level of resistance is being obtained from *V. arizonica* and BD5-117 and their use as parents will continue to be emphasized.

The majority of the 400 resistant BC2 and BC3 *V. arizonica* seedlings planted in 2008 have fruited and have been evaluated. From these seedlings, three BC2 and 19 BC3 tray dried raisin selections were made in 2010 which are good enough for propagation into advanced production trials in 2011. These selections range in berry size from Thompson Seedless down to Zante Currant size. The seedlings planted in 2009 had very little fruit production this year and will be evaluated in 2011. Older seedlings are being evaluated again for fruit quality to determine the best to propagate for production trials or for use as parents. In 2010, 13 raisin selections (11 BC2 and two BC3) were planted in production trials. No *Xf* infections have been found to date in the twelve advanced selections planted in a replicated plot at the USDA ARS research station, Weslaco, Texas.

Objective 2

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 and 500 individuals are now fruiting of the 565 seedlings. Fruit samples were taken from all seedlings for cluster weight, berry weight and seed/seed trace weight as an indication of fruit quality. Two years data for most of the seedlings has now been collected. Greenhouse testing for PD resistance has been accomplished on 210 individuals, with 109 rated clearly resistant or clearly susceptible. One hundred twenty-one of the 210 individuals were evaluated in 2010 (**Table 3**). The 70 polymorphic markers tested on 154 individuals, greenhouse PD resistance evaluations and fruit characteristics are ready to be mapped. Thirty-five additional polymorphic primers have been labeled and are being tested on the 154 seedlings.

CONCLUSIONS

Additional families for the development of PD resistant seedless table and raisin grape cultivars are being produced. Emphasis was placed on making additional BC4 *V. arizonica* PD resistant families. These families will have high fruit quality as they consist of 97% *V. vinifera*. The use of molecular markers has simplified and sped up the identification of PD resistant individuals from *V. arizonica.* Seedless table and raisin grape selections with PD resistance and improved fruit

quality have been made in both BC3 *V. arizonica* and F1 BD5-117 families. For example, 19 new raisin selections were made from BC3 *V. arizonica* families and will be propagated for production trials. Two hundred and forty-seven seedlings from the BD5-117 family to develop a frame-work map for this source of PD resistance have been evaluated in the greenhouse for PD resistance. The development of PD resistant table and raisin grape cultivars will make it possible to keep these grape industries viable in PD infested areas. Molecular markers will greatly aid the selection of PD resistant individuals from SEUS populations.

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Table 1. 2010 table and raisin grape PD resistant seedless crosses that set fruit and the number of ovules and embryos produced.

Female	Male	Type	No. Emas-culations	No. berries Opened	No. Ovules	No. Embryos
89-0908 *V. rupestris* x *V. arizonica*						
07-5052-036	Y538-58	Table BC4	1000	9	14	8
07-5052-036	Y151-58	Table BC4	500	47	54	33
07-5052-71	07-5054-12	Table BC3	1950	28	26	9
06-5501-229	Y129-176	Table BC3	1468	80	42	18
06-5501-229	Y529-4	Table BC3	1411	49	23	2
06-5501-229	Y540-193	Table BC3	1936	86	54	11
06-5501-229	Y537-32	Table BC3	1500	384	416	62
05-5502-15	Y129-161	Table BC3	687	31	38	2
06-5501-423	05-5501-53	Table BC2	2905	6	13	0
07-5052-090	Y143-39	Raisin BC4	1081	9	7	0
07-5058-016	Y124-203	Raisin BC4	2359	29	29	0
07-5058-016	Y144-135	Raisin BC4	2609	24	31	0
07-5058-029	B82-43	Raisin BC4	1400	92	121	12
07-5058-029	Y143-39	Raisin BC4	2100	155	165	39
07-5058-029	A51-60	Raisin BC4	806	27	12	1
B82-43	07-5052-109	Raisin BC4	2500	152	254	55
07-5058-029	05-5551-049	Raisin BC4	2572	79	120	17
07-5052-086	05-5551-049	Raisin BC4	2800	2	2	0
07-5052-086	06-5551-239	Raisin BC4	2910	78	50	7
A63-85	07-5052-61	Raisin BC4	3500	9	8	0
07-5052-52	07-5054-6	Raisin BC4	1600	6	5	1
07-5058-016	07-5052-61	Raisin BC4	2500	3	4	0
07-5061-134	07-5052-182	Raisin BC4	2037	125	128	12
07-5061-86	07-5052-182	Raisin BC4	2384	35	3	1
05-5551-012	05-5551-049	Raisin BC3	2500	7	10	0
05-5551-012	A51-60	Raisin BC3	1500	4	5	1
05-5551-116	Y144-157	Raisin BC3	3056	15	16	0
A49-82	06-5551-239	Raisin BC3	2800	30	50	2
Total			**56,371**	**1,601**	**1,700**	**293**
PM resistance combined with 89-0908 *V. rupestris* x *V. arizonica*						
07-5052-032	B88-69	Table BC4	550	101	2	0
07-5052-036	B88-69	Table BC4	1100	29	40	12
Y314-17--04	05-5502-05	Table BC3	2574	307	474	68
Y314-17--04	05-5501-53	Table BC3	2570	24	67	13
05-5501-06	Y308-289	Table BC3	2590	105	112	44
05-5502-15	Y308-311	Table BC3	436	54	55	2
07-5058-029	Y302-152	Raisin BC4	2568	66	50	3
Y302-178	06-5551-242	Raisin BC3	806	87	118	1
Y309-397	06-5551-242	Raisin BC3	600	13	25	4
Y520-202--08	06-5551-239	Raisin BC3	2647	667	850	115
05-5551-012	Y308-345	Raisin BC3	2500	11	11	3
Y518-112	05-5551-049	Raisin BC3	2100	32	19	0
Y309-397	07-5060-061	Raisin BC3	2306	62	50	10
Total			**23,347**	**1,558**	**1,873**	**275**

Table 2. 2010 table and raisin grape PD resistant seeded crosses and the number of seeds produced.

89-0908 *V. rupestris* x *V. arizonica*				
Female	Male	Type	No. Emas-culations	No. seed
07-5052-061	Y129-161	Table BC4	600	49
07-5052-061	07-5052-36	Table BC4	1200	68
04-5002-18	Y536-1	Table BC2	1069	115
04-5002-23	Y536-1	Table BC2	1127	80
04-5002-23	Y129-161	Table BC2	1145	13
07-5052-168	A51-60	Raisin BC4	1035	9
07-5052-168	Y144-157	Raisin BC4	1177	1
04-5002-18	Y133-206	Raisin BC2	1206	80
Total			8,559	**418**

Table 3. Determination of seedling resistance based on PdR1 molecular markers for all 89-0908 families made in 2009.

Family	Type Cross	No. Resistant[a]	No. Susceptible[b]	No. Recombinant[c]	No data[d]	Off Types	Total
09-5003	Table BC2	7	6			2	15
09-5007	Table BC3	6	9	4		3	22
09-5008	Table BC3	18	17			35	70
09-5009	Table BC3	2	1				3
09-5010	Table BC3	0	2				2
09-5011	Table BC3	12	7	1	1	7	28
09-5013	Table BC4	92	67	12	1	6	178
09-5016	Table BC4	2	1	1			4
09-5055	Raisin BC3	7	3	1		1	12
09-5056	Raisin BC4	4	8	2	2		16
09-5057	Raisin BC4	21	45	8	1	2	77
09-5063	Raisin BC4	17	16	2			35
09-5064	Raisin BC4	21	21	5	2	1	50
09-5066	Raisin BC4	82	89	11	4	1	187
09-5067	Raisin BC4	4	7	2		1	14
09-5068	Raisin BC4	30	21			1	52
09-5069	Raisin BC4	1	2			8	11
09-5070	Raisin BC4	8	6			8	22
09-5071	Raisin BC4	8	6			8	22
09-5072	Raisin BC4	6	5		1	20	32
09-5073	Raisin BC4	8	4		1		13
09-5074	Raisin BC4	6	2				8
09-5075	Raisin BC4	0	3	1		1	5
09-5076	Raisin BC4	7	6			2	15
09-5077	Raisin BC4	0	7				7
09-6054	Raisin BC4	44	46	7	1		98
Total		**413 (47% [e])**	**407 (46% [e])**	**57 (7% [e])**	**14**	**107**	**998**

[a]Resistant = marker on both sides of *PdR1* region.
[b]Susceptible = no *PdR1* markers.
[c]Recombinant= genotypes that amplified with one *PdR1* marker.
[d]No data = genotypes that failed to amplify properly.
[e]%= Number of seedlings in each category / total number of seedlings showing the proper markers.

Table 4. Results of greenhouse test for determination of PD reaction in 2010.

Population	Resistance Source	Testing Compete		In greenhouse test For evaluation by December
		No. tested	No. resistant	
BD5-117 map	BD5-117	121	44	70
Arizonica	PdR1	63	49	74
Other PD	SEUS	0	0	21
Total		**184**	**93**	**165**