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

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ABSTRACT
Twenty-two seedless x seedless crosses to develop additional BC2 and BC3 *V. arizonica* and BC1 SEUS BD5-117 families were made in 2008. Powdery mildew resistance was included in five of these crosses. These crosses produced 5,148 berries, 8,824 ovules and 1,841 embryos. Nine seeded BC1 crosses based on *V. arizonica* and SEUS PD resistance sources were made, resulting in 1,393 seed. Two BC2 and 12 BC3 families *(V. arizonica* source of resistance) consisting of 1,191 individuals were screened at the seedling stage in the greenhouse with SSR markers for resistance. A total of 363 were resistant and planted in the field. In November, seedlings from 12 crosses made in 2008 were tested with molecular markers and 159 resistant plants identified from 319 individuals. Greenhouse screening was completed on 150 selections and 63 of the 64 resistant individuals were from *V. arizonica*. Twelve resistant selections have been planted in the field at Weslaco, Texas to determine their field resistance. An additional 89 plants and 692 embryos have been produced to increase the size of the C33-30 x BD5-117 family for molecular maker development. A total of 105 SSR primers are polymorphic between the parents and screening of the first 154 individuals to develop a framework map has started. Greenhouse testing of 125 individuals was completed with 25 being resistant.

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. The first crosses to make the BC4 generation of table and raisin grapes with *V. arizonica* source of PD resistance were made this year. These families will have high fruit quality as they consist of 97% *V. vinifera*. An example of increased fruit quality would be this year's selection of five raisin grapes made from BC3 *V. arizonica* families which will be propagated for production trials. An additional fifteen 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. Three hundred twenty-seven resistant seedlings were selected from 885 seedlings this year, thereby making the program more efficient. Advanced selections are screened in the greenhouse to verify PD resistance. Powdery mildew (PM) resistance is being combined with PD resistance and this year 54 of 97 PD resistant seedlings showed PM resistance after greenhouse screening. A family from BD5-117 source of resistance that is different from *V. arizonica* has been made to develop molecular markers for this source of resistance. To date, 154 seedlings have been tested with 70 fluorescent labeled SSR markers. The PD resistance of 125 of these seedlings has been tested in the greenhouse. 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 might 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* in these families 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 help introgress the seedless trait with PD resistance quickly (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 those from *V. arizonica.*

RESULTS AND DISCUSSION

Objective 1

Fifty-one crosses using *V. arizonica* and SEUS (BD5-117 and Zehnder) sources of resistance were made in 2009 and produced 5,918 berries, 6,661 ovules, and 1,719 embryos (25% embryos/ovules) (**Table 1**). The first BC4 crosses (97% *Vitis vinifera*) was made this year and consisted of 31 crosses of which 19 produced 3,931 berries, 5,000 ovules and 1,372 embryos. These crosses were made before observing fruit on the 18 month old seedlings and the size of the seed/aborted seed was unknown at bloom. The seed traces were too small to culture from six of these crosses. The seedlings obtained from these crosses should have high fruit quality as they now have 97% *V. vinifera* in their background. In additional to the BC4 crosses, 17 BC3 crosses were made and consisted of 1,313 berries, 987 ovules and 312 embryos. Five and three crosses combined *V. arizonica* and SEUS PD resistance respectively with powdery mildew resistance. No seeded crosses were made in 2009.

Leaves were taken from seedlings in test tubes in November from 2008 crosses and tested for resistance with molecular markers for the PdR1 locus on chromosome 14. Results for three BC1 and ten BC3 seedless x seedless families (89-0908 *V. arizonica* source of resistance) is shown in **Table 2**. A total of 885 individuals were tested with SSR markers and 812 showed markers on both sides of the PdR1 region as expected. A total of 327 individuals (43% of those showing markers) were resistant and planted to soil in cups for growth in the greenhouse before planting to the field in April, 2009. Thirty-nine percent of the plants showing markers were susceptible. This is very similar to the percent 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. A total of 172 seeded by seedless BC3 seedlings were also screened with molecular markers and 34% and 44% were resistant and susceptible respectively. Only the resistant plants were planted in the field. Four BC3 crosses made in 2008 combined PD resistance from *V. arizonica* with powdery mildew (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 97 PD resistant seedlings screened to date, 54 were resistant, which is as expected. Inoculation of plants with *Xylella* in the Greenhouse (method by Krivanek et al. 2005, Krivanek and Walker 2005) was done to determine resistance of 105 selected individuals from BC2 *V. arizonica* and F1, BC1 SEUS (Table 3). Sixty-three resistant individuals were from *V. arizonica* and only one was from SEUS source of resistance. This shows that a high level of resistance is being passed on by *V. arizonica*. 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 be emphasized.

Fifty percent of the 400 resistant BC2 and BC3 *V. arizonica* seedlings planted in 2008 produced fruit. From these seedlings, 3 BC2 and 3 BC3 tray dried raisin, and 2 natural DOV raisin selections were made that are good enough for propagation into advanced production trials. An additional 15 tray dried raisin, 4 natural DOV raisin, 10 table grape and 2 wine selections were kept for use as parents and for additional evaluations. Raisin samples were dried from 41 PD resistant seedlings from 2 BC2 raisin families. Nine seedlings were selected for propagation in production trials and as parents. One selection had a rating of 52 which was higher than Selma Pete (50), Fiesta (48) and DOVine (47) and equal to Diamond Muscat (52). Nine table grape selections from BC2 *V. arizonica* have been selected for further observation and as parents. Four have been tested in the greenhouse for *Xylella* infection and were resistant. Five table grapes and nine raisin grapes were also selected from 227 BC2 *V. arizonica* seedlings that were planted in 2007 and fruited for the first time in 2009. Three of the raisin selections will be propagated for yield trials. Twelve advanced selections, with a range of PD resistant in greenhouse tests, have been planted in a replicated plot at the USDA ARS research station, Weslaco, Texas. Samples were taken in September, 2009 to determine if any plants have become infected after one year's growth

Objective 2

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 and 300 individuals are fruiting. Fruit samples were taken from all seedlings for cluster weight, berry weight and seed/seed trace weight as an indication of fruit quality. Greenhouse testing for PD resistance is complete on 125 individuals, with 25 being resistant (**Table 3**). Three hundred additional plants were planted this year to increase the family size to over 500 individuals. Of the 105 SSR polymorphic primers identified last year, 70 have been labeled with fluorescent dyes and run on all 154 individuals plus the parents. The additional 35 labeled polymorphic primers are being tested on the 154 seedlings.

Table 1. Successful 2009 table and raisin grape PD resistant seedless crosses 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*						
B71-60	07-5054-12	Table BC4	2,823	229	408	234
Scarlet Royal	07-5054-12	Table BC4	3,115	11	20	6
07-5061-04	A63-85	Raisin BC4	2 bags[a]	252	285	68
07-5061-04	A50-33	Raisin BC4	3 bags[a]	391	411	148
07-5061-14	Y143-39	Raisin BC4	3 bags[a]	256	460	69
07-5061-14	B82-43	Raisin BC4	4 bags[a]	643	1,168	97
07-5061-14	Y144-157	Raisin BC4	2 bags[a]	298	441	239
A49-82	07-5061-34	Raisin BC4	3,000	74	50	24
A50-39	07-5061-34	Raisin BC4	3,315	402	394	100
A50-91	07-5051-28	Raisin BC4	1,500	108	117	21
A50-91	07-5061-146	Raisin BC4	1,830	160	178	44
A61-79	07-5058-16	Raisin BC4	2,800	142	144	37
B82-43	07-5052-43	Raisin BC4	2,606	223	250	64
Y142-76	07-5061-34	Raisin BC4	496	42	45	14
Y142-76	07-5061-72	Raisin BC4	1,492	88	84	13
Y142-76	07-5061-106	Raisin BC4	1,156	38	31	7
Y143-161	07-5053-33	Raisin BC4	3,946	207	229	28
Y144-132	07-5061-34	Raisin BC4	2,390	32	50	10
05-5501-27	Y129-176	Table BC3	5 bags[a]	36	3	1
05-5501-27	05-5501-28	Table BC3	4 bags[a]	170	16	2
05-5501-28	Y133-191	Table BC3	1,564	21	23	4
05-5502-25	Y129-176	Table BC3	2,631	163	162	30
05-5501-68	C57-60	Table BC3	5 bags[a]	240	287	99
05-5501-68	C45-64	Table BC3	5 bags[a]	48	13	2
05-5501-40	Y129-161	Table BC3	5 bags[a]	51	52	7
05-5502-15	04-5514-2443	Table BC3	2,708	382	290	39
05-5551-19	A63-58	Raisin BC3	650	77	94	18
04-5514-28	C61-123	Table BC2	1,375	75	93	23
07-5061-14	Y308-344	PM Raisin BC4	3 bags[a]	210	235	149
Total			**39,397**	**44,256**	**6,033**	**1,597(26%)**
SEUS source of resistance (BD5-117 or Zehnder)						
03-5003-10	C45-64	Table BC1	5 bags[a]	2	2	1
03-5003-10	Y308-314	PM Table BC1	1,554	69	88	20
Y305-58	C61-123	PM Table BC1	3,097	308	436	101
Total			**5,285**	**3,291**	**4,836**	**1,179(23%)**

[a]Parents with female flowers were not emasculated, only bagged and pollinated.

Table 2. Determination of seedling resistance based on molecular markers for 89-0908 BC2 families made in 2008.

Family	Type Cross	No. Resistant[a]	No. Susceptible[b]	No. Recombinant[c]	No data[d]	Off Types	Total
08-5001	Table BC3	46	17	13	1	19	96
08-5002	Table BC1	40	35	29	9	14	127
08-5003	Table BC3	6	6	2	1	0	15
08-5054	Raisin BC1	38	37	13	5	0	93
08-5055	Raisin BC1	29	33	18	8	1	89
08-5056	Raisin BC3	27	45	14	16	0	102
08-5057	Raisin BC3	29	28	6	2	2	67
08-5058	Raisin BC3	6	2	0	3	4	15
08-6002	Table BC3 PM	35	28	18	9	3	93
08-6003	Table BC3 PM	2	2	0	2	0	6
08-6052	Raisin BC3 PM	5	3	1	6	2	17
08-6053	Raisin BC3 PM	62	59	18	11	10	160
08-6054	Raisin BC3 PM	2	2	1	0	0	5
Total		**327 (43%[e])**	**297 (39% [e])**	**133 (18% [e])**	**73**	**55**	**885**
	Seeded x Sdlss						
08-5504	Table BC3	7	8	3	4	0	22
08-5505	Table BC3	1	0	1	0	0	2
08-5552	Raisin BC3	47	66	16	6	0	135
08-6501	Table BC3 PM	3	2	5	2	0	12
08-6502	Table BC3 PM	1	0	0	0	0	1
Total		**59 (37% [e])**	**76 (48% [e])**	**25 (16% [e])**	**12**	**0**	**172**

[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 markers properly.

Table 3. Results of greenhouse test for determination of PD reaction.

Population	Resistance Source	Total sent	Testing Compete	
			No. tested	No. resistant
BD5-117 map	BD5-117	154	125	25
Arizonica	PdR1	113	105	63
Other PD	SEUS	65	45	1
Total		**332**	**275**	**109**

CONCLUSIONS

Families for the development of PD resistant seedless table and raisin grape cultivars continue to be produced. Emphasis was placed on *V. arizonica* BC3 crosses (93% *V. vinifera*) and BC1 crosses of BD5-117. 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 BC2 *V. arizonica* and F1 BD5-117 families. One hundred five polymorphic SSR primers have been identified in the BD5-117 family in the search for molecular markers from sources of resistance other than *V. arizonica*. SSR primers are now being tested on all 154 individuals from the BD5-117 family to develop a frame work map. 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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