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

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Reporting Period: The results reported here are from work conducted September 2006 to September 2007.

ABSTRACT
Fifteen BC3 and two BC2 crosses between the *V. arizonica* source of Pierce's disease (PD) resistance and seedless table and raisin grape selections were made, seedless ovules were cultured and produced 3,396 berries, 4,459 ovules and 1,840 embryos. Two additional seedless crosses from Southeast United States (SEUS) and *V. tiliifolia* were also made. Two seeded BC1 crosses based on a SEUS PD resistance source were made, resulting in 349 seed. Ten 2006 BC2 families *(V. arizonica* source of resistance) consisting of 765 individuals were screened at the seedling stage in the greenhouse with SSR markers for resistance. A total of 262 were resistant and planted in the field. Resistance and susceptibility segregated in a 1:1 ratio in all but one family. Greenhouse screening of the BC2 raisin family 04-5554 confirmed that 17 of 18 individuals with resistant markers were resistant. Four of the 13 resistant fruiting plants had small aborted seed and they will be useful as parents for continued backcrossing. Progress in developing fruit quality improved as rapidly in resistant progeny as in susceptible progeny. An additional 33 plants and 241 embryos have been produced to increase the C33-30 x BD5-117 family size for maker development. This year 424 SSR primers were screened against the parents and resistant and susceptible bulks. From these 30 were confirmed to be polymorphic.

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 varieties 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 varieties 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
Objective 1 This year the majority of seedless embryo culture crosses concentrated on using the *V. arizonica* source of resistance. Fifteen BC3 and 2 BC2 crosses were made and produced 3,396 berries, 4,459 ovules and 1,840 embryos (41% embryos/ovules) (Table 1). The percent embryos recovered is twice the amount normally recovered from seedless embryo rescue. One BC1 cross from SEUS source of resistance and one cross to increase the population from a unique source of resistance from *V. tiliifolia* were also made (Table 1). Two seeded BC1 crosses from SEUS source of resistance were made (Table 2). Fruit has been harvested and 349 seeds extracted for germination in January.

Ten BC2 families (89-0908 *V.* arizonica source of resistance) produced in 2006 and growing in the greenhouse as small plants were tested for molecular markers associated with the PdR1 locus on chromosome 14 (Table 3). A total of 765 individuals were tested with SSR markers and 680 showed markers on both sides of the PdR1 region as expected. Eighty-two percent had either resistant or susceptible bands making selection for resistance effective. A total of 262 individuals (39% of those showing markers) were resistant and planted to the field from the greenhouse. The susceptible individuals were discarded. All families segregated in a 1:1 ratio except for family 06-5552 which had a higher percent of resistant individuals. Greenhouse testing of selected individuals from the BC2 *V. arizonica* raisin family 04-5554 was completed.

Twenty-nine individuals were tested in the greenhouse. Four of the susceptible individuals based on molecular markers were tested and had > $6x10^6$ cfu/ml bacteria and >5 PD symptoms, indicating that they were susceptible. Seventeen of the 18 individuals identified as resistant with markers fell in the resistant class based on the greenhouse test (<250,000 cfu/ml bacteria and <2.8 PD symptom rating). This gives good confidence in the use of molecular markers for the *V. arizonica* (89-0908) source of resistance. Plants from this family fruited in the field for the first time this year. Thirteen were resistant and consisted of seven seeded, two with large aborted seeds, and four with small aborted seeds. This was comparable to the fourteen susceptible which consisted of 10 seeded, one with large aborted seeds, and three with small aborted seeds. The mean berry weight for the resistant individuals was 2.11 g compared to 1.96 g for the susceptible individuals. This shows that progress can be made as fast in developing resistant types with high fruit quality as in susceptible types, and that seedless resistant genotypes can be produced. This family is 87% *V. vinifera* and most of the seedlings are neutral in flavor without off flavors. Seedlings from 2005 crosses have also started to fruit. In the raisin family 05-5551 (seeded x seedless), all resistant seedlings produced seedless fruit, with 13 having large aborted seeds and 10 with small aborted seed traces. The susceptible individuals have not yet been analyzed for their seed content. The 23 resistant individuals were composed of 12 with white fruit and 11 with colored fruit which fits the expected ratio. The average fruit size between the resistant and susceptible individuals was comparable at 1.45 g and 1.48 g respectively. This also indicates that progress is being made equally in the resistant compared to the susceptible types based on berry size. Additional families are still being evaluated for their fruit quality. All PD selections from the California State University, Fresno have been propagated and moved to the USDA/ARS research center at Parlier, California.

Table 1. 2007 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*							
Scarlet Royal	04-5012-2	Table	BC3	2,687	219	297	192
C45-25	04-5012-3	Table	BC3	2,997	107	117	34
A38-7	05-5551-39	Table	BC3	1,530	169	299	99
C49-96	05-5551-62	Table	BC3	942	0	0	0
C49-96	04-5012-3	Table	BC3	1,018	2	0	0
B48-17	05-5551-26	Table	BC3	1,421	94	19	3
A49-82	05-5551-110	Raisin	BC3	4 bags[a]	252	367	78
04-5554-08	A63-85	Raisin	BC3	4 bags	415	657	283
A57-27	05-5551-116	Raisin	BC3	3,365	74	2	0
A50-85	05-5551-109	Raisin	BC3	3,496	300	335	63
A61-97	04-5554-01	Raisin	BC3	2,097	61	63	14
A51-21	04-5554-01	Raisin	BC3	2,404	82	94	15
B82-43	05-5551-30	Raisin	BC3	1,827	251	344	59
B82-43	05-5551-62	Raisin	BC3	2,029	275	350	147
04-5554-08	A50-33	Raisin	BC3	4 bags	492	590	221
A81-138	Summer Muscat	Raisin	BC2	7 bags	265	450	309
A81-138	Selma Pete	Raisin	BC2	8 bags	338	475	323
Total				**25,831**	**3,396**	**4,459**	**1,840**
V. tiliifolia source of resistance							
C33-30	IAC572	Genetic family		5 bags	641	850	261
SEUS source of resistance							
C33-30	BD5-117	Genetic family		10 bags	588	724	241
01-5026-20	C56-11	Table BC1		7 bags	190	450	87

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

Table 2. 2007 table and raisin grape PD resistant seeded x seedless crosses and the number of seeds produced.

Female	Male	Type		No. Emasculations	No. Seeds
SEUS BD5-117 source of resistance					
01-5026-15	C56-11	Table	BC1	1099	25
01-5026-21	B49-128	Table	BC1	6 bags[a]	324

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

Table 3. Determination of seedling resistance based on molecular markers for 89-0908 BC2 families made in 2006.

Family	Type Cross	No. Resistant[a]	No. Susceptible	No. Recombinants[b]	No data[c]	Total
06-5501	Table	40	56	21	7	124
06-5502	Table	24	22	21	9	76
06-5503	Table	30	42	18	14	104
06-5504	Table	27	33	9	8	77
06-5551	Raisin	37	37	4	5	83
06-5552	Raisin	37	20	29	9	95
06-5553	Raisin	50	59	15	13	137
06-5554	Raisin	1	1	0	0	2
06-5555	Raisin	0	0	0	2	2
06-5556	Raisin	16	24	7	18	65
Total		**262**	**294**	**124**	**85**	**765**

[a]Resistant = marker on both sides of *PdR1* region.
[b]Susceptible = no markers.
[c]No data = genotypes that amplified with one marker, off types and that failed with both markers

Objective 2 The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 and a family with 154 individuals was produced. Additional plants are being made to increase the family size. In 2006, 33 plants were produced. In 2007, 724 ovules were cultured and produced 241 embryos that have been sub-cultured on fresh medium for growth into plants. Fruit from the 154 individuals has been collected and is being evaluated for berry size, seed/trace weight and fruit characteristics. This year 424 SSR primers were screened against the parents, a resistant bulk of five individuals and a susceptible bulk of five individuals. Of these SSR primers, 30 showed polymorphism amongst the resistant and susceptible parents and bulks. These polymorphic SSR primers have been retested against the parents and individuals from the bulks and continue to show polymorphism. The next step is to test all 154 individuals and parents with these 30 polymorphic SSR markers.

CONCLUSIONS

Families for the development of PD resistant seedless table and raisin grape cultivars continue to be produced and the first BC3 crosses in the *V. arizonica* source of resistance were made this year. This generation is 93% *V. vinifera*. The use of molecular markers has simplified and sped up the identification of PD resistant individuals from *V. arizonica.* Resistance based on molecular markers continues to segregate in a 1:1 ratio in *V. arizonica* BC2 families. Seedless table and raisin grape selections with PD resistance and improved fruit quality have been made. Thirty 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.*

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program, and the Consolidated Central Valley Table Grape Pest and Disease Control District.