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

Principal Investigator:
David W. Ramming
Crop Dis., Pests, & Gen. Res. Unit
USDA, ARS
Parlier, CA 93648
david.ramming@ars.usda.gov

Co-Principal Investigator:
Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis, CA 95616
awalker@ucdavis.edu

Cooperator:
Hong Lin
Crop Dis., Pests, & Gen. Res. Unit
USDA, ARS
Parlier, CA 93648
hong.lin@ars.usda.gov

Reporting Period: The results reported here are from work conducted September 2007 to September 2008.

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 1,861 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. Greenhouse screening was completed on 57 selections and 20 of the 21 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 75 individuals was completed with 21 being resistant. An additional 49 are currently in greenhouse tests.

INTRODUCTION
Pierce's disease (PD) has existed in California since the late 1800's 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
Objective 1
This year the seedless embryo culture crosses concentrated on using *V. arizonica* and SEUS (BD5-117) sources of resistance. Twenty-two crosses were made and produced 5,184 berries, 8,824 ovules and 1,861 embryos (21% embryos/ovules) (**Table 1**). Five of the seedless crosses combined *V. arizonica* PD resistance with powdery mildew resistance. Nine seeded crosses from *V. arizonica* and SEUS sources of resistance were made (**Table 2**). Fruit has been harvested and 1,393 seeds extracted for germination in January.

Two BC2 and twelve BC3 families (89-0908 *V.* arizonica source of resistance) produced in 2007 and growing in the greenhouse as small plants this spring were tested for molecular markers associated with the PdR1 locus on chromosome 14 (**Table 3**). A total of 1,191 individuals were tested with SSR markers and 1,121 showed markers on both sides of the PdR1 region as expected. Eighty-seven percent had either resistant or susceptible bands, similar to 2007 results, which makes selection for resistance effective. A total of 363 individuals (32% of those showing markers) were resistant and planted to the field from the greenhouse. The susceptible individuals were discarded making use of field space much more efficient. Greenhouse testing of 57 selected individuals from BC2 *V. arizonica* and SEUS populations was completed and 21 identified as resistant. All resistant individuals were from *V. arizonica* except one from SEUS, showing the high level of resistance

passed on by *V. arizonica*. Another 70 selections are in greenhouse tests that will be completed this fall. Greenhouse testing is absolutely necessary to make the final decision about resistance of individual selections. Because the highest levels of resistance are being obtained from *V. arizonica* and BD5-117, we have and will continue to emphasize their use over other sources of resistance in the breeding program. Fruit samples have been taken from four populations observed last year to continue comparing fruit quality between resistant and susceptible seedlings to verify that fruit quality is segregating independently from PD resistance. Raisins are being produced from the resistant *V. arizonica* with small aborted seeds. Evaluation of over 2,000 fruiting seedlings in the field is being conducted this year to determine those with fruit quality good enough to consider as parents or potential cultivars. Twelve advanced selections that were rated as resistant in the greenhouse have been planted in a replicated plot at the USDA ARS research station, Weslaco, Texas. This is an area with high levels of PD and glassy-winged sharpshooters and is being used to determine field resistance of these selections.

Table 1. 2008 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*						
05-5502-05	A 85- 40	Table BC3	2,691	452	650	147
Scarlet Royal	05-5502-42	Table BC3	3,860	92	127	19
04-5554-8	A63-85	Raisin BC3	7 bags[a]	296	650	173
05-5555-108	A56-92	Raisin BC3	2,054	306	525	114
05-5551-49	Selma Pete	Raisin BC3	3,897	487	556	49
05-5502-05	Y315-400	PM Table BC3	2,529	443	795	72
Y314-360	05-5502-05	PM Table BC3	2,310	59	54	11
04-5554-19	Y313-137	PM Raisin BC3	800	94	136	26
04-5554-8	Y313-137	PM Raisin BC3	7 bags	440	882	230
05-5551-108	Y313-137	PM Raisin BC3	2,435	129	154	8
Total			**20,576**	**2,798**	**4,529**	**849(18.7%)**
B43-17 *V. rupestris* x *V. arizonica*						
04-5002-42	Y129-161	Table BC1	2,902	264	675	189
04-5051-14	A56-92	Raisin BC1	4,168	345	700	177
04-5051-4	A63-85	Raisin BC3	6 bags	333	434	159
Total			**7,070**	**942**	**1,809**	**525(29.9%)**
SEUS source of resistance BD5-117						
C33-30	BD5-117	Genetic family	10 bags	1,847	2,350	692
01-5026-20	B28-128	Table BC1	7 bags	165	350	31
01-5026-28	B28-126	Table BC1	7 bags	285	328	73
01-5026-31	C62-109	Table BC1	7 bags	149	350	126
01-5026-20	01-5026-8	Table F1 sib	7 bags	152	207	7
01-5026-20	01-5026-33	Table F1 sib	7 bags	206	375	52
01-5026-10	A56-66	Raisin BC1	2,602	188	350	91
01-5026-20	A56-92	Raisin BC1	7 bags	140	351	51
01-5026-20	A51-55	Raisin BC1	7 bags	16	11	0
01-5026-10	Y313-137	PM Raisin BC1	2,683	143	164	56
Total			**5,285**	**3,291**	**4,836**	**1,179(24.4%)**

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

Table 2. 2008 table and raisin grape PD resistant seeded x seedless crosses and the number of seeds produced.

Female	Male	Type	No. Emasculations	No. Seeds
89-0908 *V. rupestris* x *V. arizonica*				
05-5501-26	C45-64	Table BC3	3 bags[a]	41
05-5501-26	A40-93	Table BC3	3 bags	12
04-5554-1	A56-94	Raisin BC3	800	385
05-5501-26	Y315-400	PM Raisin BC3	3 bags	29
05-5501-26	Y314-360	PM Raisin BC3	3 bags	10
B43-17 *V. rupestris x V. ariconica*				
04-5001-11	C58-37	Table BC1	3 bags	334
04-5001-8	C61-123	Table BC1	5 bags	191
SEUS BD5-117 source of resistance				
01-5026-11	C45-64	Table BC1	3 bags	15
01-5026-21	A50-33	Raisin BC1	3 bags	376
Total			**800 + 26 bags**	**1,393**

[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 2007.

Family	Type Cross	No. Resistant[a]	No. Susceptible[b]	No. Recombinants	No data[c]	Total
07-5001	Table BC3	1	78	39	2	120
07-5002	Table BC3	0	15	4	0	19
07-5003	Table BC3	3	58	5	0	66
07-5006	Table BC3	0	1	0	0	1
07-5051	Raisin BC3	13	23	1	1	38
07-5052	Raisin BC3	72	90	14	10	186
07-5054	Raisin BC3	5	20	2	1	28
07-5055	Raisin BC3	1	7	1	2	11
07-5056	Raisin BC3	3	2	4	0	9
07-5057	Raisin BC3	14	17	5	1	37
07-5058	Raisin BC3	27	49	8	5	89
07-5059	Raisin BC2	111	108	25	12	256
07-5060	Raisin BC2	60	95	21	2	178
07-5061	Raisin BC3	53	48	18	34	153
Total		**363**	**611**	**147**	**70**	**1,191**

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

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

Population	Resistance Source	Total sent	Testing Compete		Test pending No. tested	Total Tested	No. to repropagate[a]
			No. tested	No. resistant			
BD5-117 map	BD5-117	154	75	21	49	124	30
Arizonica	PdR1	113	28	20	70	98	24
Other PD	SEUS	65	29	1	16	45	20
Total		**332**	**112**	**42**	**135**	**267**	**74**

[a] Not all selections had enough replications in greenhouse for definitive results.

Objective 2

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 and 154 individuals have been produced that are fruiting. BD5-117 has given the highest number of resistant offspring of any of the SEUS resistant selections to date and makes an excellent choice for study for molecular markers. The fruit of these seedlings is being evaluated for berry size, fruit quality and seed/aborted seed size. Cuttings of all 154 were made for

evaluation of PD resistance in the greenhouse. Testing is complete on 75 individuals, with 21 being resistant. An additional 49 are currently being tested and 37 need to be repropagated for testing (**Table 4**). Additional plants are being made to increase the family size to at least 500 individuals. In 2006, 65 plants were produced. In 2007, an additional 89 plants were produced and planted in the field. This year 1,847 berries were produced for this cross from which 2,350 ovules were cultured and 692 embryos produced. In 2008 SSR primers that have shown polymophism are being tested on all 154 individuals to develop a framework map. A total of 105S polymorphic markers have been identified to date.

CONCLUSIONS

Families for the development of PD resistant seedless table and raisin grape cultivars continue to be produced. Emphasis was placed on making additional *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.

REFERENCES CITED

Emershad, R. L., D. W. Ramming and M. D. Serpe. 1989. In-ovulo embryo development and plant formation from stenospermic genotypes of *Vitis vinifera*. Am. J. Bot. 76:397-402.

Krivanek, A.F. and Walker, M.A. 2005. *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44-52.

Krivanek, A.F., Stevenson, J. F. and Walker, M.A. 2005. Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology 95:36-43.

Krivanek, A. F., S. Riaz, and M. A. Walker. 2006. Identification and molecular mapping of *PdR1*, a primary resistance gene to Pierce's disease in *Vitis*. Theor. Appl. Genet. 112:1125-1131.

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.