

**California Department of Food and Agriculture PD/GWSS  
Progress Report  
March 2012**

**Renewal Progress Report for CDFA Agreement Number 11-0184-SA**

**Project Title:** Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*).

**Renewal Progress report for 2011.**

**Project Title: Breeding Pierce's Disease Resistant Table and Raisin Grapes and the development of markers for additional sources of resistance**

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**Time Period Covered:** September 1, 2010 to January 31, 2012.

**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 cultivars existing in markets today. The sixth generation (BC5) crosses to produce quality table and raisin grapes with *V. arizonica* source of PD resistance were made for the first time this year. These families will have high fruit quality as they consist of 98.5% *V. vinifera*. An example of increased fruit quality is the selection for propagation in first stage production trials of six BC4 table, 8 BC4 and 3 BC3 raisin grape selections. These selections show some commercial potential with one table selections showing enough promise for inclusion in a 25 vine cultural production trial. Crosses to combine PM and PD resistance were also made. The use of molecular markers to select for PD resistance and greenhouse screening to select for powdery mildew (PM) resistance allows selection before planting seedlings to the field. Two table and five raisin grapes with PD plus PM resistance were selected and propagated in first stage production trials. PD resistance of over 38 parents and selections from *V. arizonica* populations have been tested in greenhouse tests to insure the molecular markers for PD resistance continue to function properly. Two hundred and forty-three seedlings of the BD5-117 mapping family, which has PD resistance different than *V. arizonica*, have been greenhouse tested. A preliminary rough molecular map based on 70 SSR primers and 144 seedlings indicates a major QTL for PD resistance on linkage group 2. This means that selected molecular markers from linkage group 2 might be useful as markers to select resistance from BD5-117 source of resistance. 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.

### **Objective and Experiments Conducted to Meet Stated Objectives:**

**Objective 1:** Develop PD resistant table and raisin grape germplasm/cultivars with fruit quality equivalent to present day cultivars.

Twenty-nine of 33 seedless x seedless crosses using *V. arizonica* source of resistance made in 2011 were successful, consisting of 61,611 emasculations, and produced 5,866 berries, 5,430 ovules, and 877 embryos (16.2% embryos/ovules) (Table 1). The embryo culture method developed by Emershad et al. 1989 was used to develop plants from seedless parents. The majority of these were BC4 and the first BC5 crosses. The seedlings obtained from these crosses should have high fruit quality as they now have 97 to 98.5% *V. vinifera* in their background. An additional 18 crosses to pyramid PD (*V. arizonica*) resistance with powdery mildew (PM) (*V. romanetii*) resistance consisting of 40,179 emasculations produced 3,772 berries, 3,571 ovules and 651 (18.2%) embryos (Table 1). Ten and five seeded crosses made in 2011 produced 646 seed for PD resistance and 1,190 seed for PD and PD plus PM resistance respectively (Table 2). Leaves from all 2010 *V. arizonica* PdR1 plants were taken when seedlings were still in test tubes starting in November, 2010. They were tested for resistance with molecular markers for the PdR1 locus on chromosome 14 (method of Krivanek et al. 2006). Results for 11 BC3 and 13 BC4 seedless x seedless families is shown in Table 3.

**Table 1.** 2011 table and raisin grape PD resistant seedless crosses that set fruit and the number of ovules and embryos produced.

Female	Male	Type	No. Emasculations	No. berries Opened	No. Ovules	No. Embryos
<b>89-0908 <i>V. rupestris</i> x <i>V. arizonica</i></b>						
04-5015-5	C 61-123	Table BC2	1201	60	46	21
04-5514-144	Y125- 39--05	Table BC2	1 bag	185	315	36
06-5503-107	Y129-161--05	Table BC4	619	90	92	2
Y152-128--08	08-5001-20	Table BC4	3797	27	47	25
06-5501-229	Y536- 53	Table BC4	2702	408	270	28
05-5501-69	B 26-120	Table BC4	5 bags	197	5	0
06-5503-126	Y537-131--06	Table BC3	694	227	136	30
06-5501-423	05-5502-15	Table BC3	1737	212	257	65
06-5501-423	05-5501-27	Table BC3	3064	80	108	20
06-5503-126	C 61-123	Table BC4	1814	458	650	114
06-5503-107	Y142- 54--09	Table BC4	2029	152	166	2
06-5503-121	06-5503-121	Table BC4	1755	200	63	2
08-5001-41	Y525- 60--07	Table BC4	4243	356	500	86
09-5013-013	Y530- 5--07	Table BC5	5 bags	450	500	122
09-5013-067	Y530- 5--07	Table BC5	3015	21	0	0
A 51- 36	05-5551-116	Raisin BC4	2744	422	341	53
07-5052-033	A 63- 85	Raisin BC4	4202	550	483	57
A 51- 36	07-5061-072	Raisin BC4	2437	340	182	68
09-5057-048	B 82- 43	Raisin BC5	5 bags	455	500	26
09-5064-006	B 82- 43	Raisin BC5	4 bags	3	0	0
A 63- 85	09-5056-012	Raisin BC5	2113	43	81	7
A 63- 85	09-5064-038	Raisin BC5	2502	218	268	46
A 63- 85	09-5072-24	Raisin BC5	2552	102	33	0
B 82- 43	09-5056-012	Raisin BC5	2550	135	0	0
B 82- 43	09-5063-023	Raisin BC5	2561	27	22	1

Y143- 26	09-5063-023	Raisin BC5	2489	53	14	2
Y143- 26	09-5064-016	Raisin BC5	1301	70	11	3
Y143- 26	09-5072-024	Raisin BC5	2315	25	23	4
Y144-132--04	09-5074-002	Raisin BC5	2636	300	317	57
<b>Total</b>			<b>61,611</b>	<b>5,866</b>	<b>5,430</b>	<b>877</b>

<b>PM resistance combined with 89-0908 <i>V. rupestris</i> x <i>V. arizonica</i></b>						
05-5501-69	Y312-187--06	Table BC3	3 bags <sup>z</sup>	143	50	2
05-5501-69	Y313-157--08	Table BC3	4 bags	47	36	1
06-5501-229	Y313-191--08	Table BC3	86	14	7	1
06-5501-238	Y308-311--06	Table BC3	1395	200	75	87
Y315-400--04	05-5501-27	Table BC3	2098	73	122	41
Y520- 71--07	08-5001-20	Table BC3	1957	190	198	20
08-6003-002	Y152- 84--08	Table BC5	3453	191	200	38
07-5052-033	Y308-312--06	Raisin BC4	2723	381	487	72
07-5052-033	Y308-329--06	Raisin BC4	2100	195	205	19
A 49- 82	08-6053-020	Raisin BC4	2500	21	32	2
A 51- 36	08-6053-012	Raisin BC4	4374	189	159	23
A 63- 85	08-6053-020	Raisin BC4	3646	214	116	10
08-6052-003	A 51- 60	Raisin BC4	1998	550	500	71
08-6053-028	A 51- 43	Raisin BC4	2627	304	386	73
08-6053-028	A 63- 85	Raisin BC4	2866	520	500	87
B 82- 43	08-6053-012	Raisin BC4	2571	300	265	62
B 82- 43	08-6053-020	Raisin BC4	2481	200	195	36
09-5013-013	Y308-329--06	Raisin BC5	5 bags	40	38	6
<b>Total</b>			<b>40,179</b>	<b>3,772</b>	<b>3,571</b>	<b>651</b>
<b>PD resistance from BD5-117</b>						
03-5003-103	Y540-193--05	Table BC1	3 bags	241	400	75
03-5003-108	Y140- 54--08	Table BC1	3 bags	225	400	17
03-5003-103	Y534-101--06	Table BC1	2 bags	284	472	86
03-5003-108	Y537-168--06	Table BC1	2 bags	115	156	5
03-5003-090	Y534- 91--06	Table BC1	3 bags	126	90	15
03-5003-110	Y538-181--06	Table BC1	2 bags	27	4	0
03-5003-090	Y537- 32--06	Table BC1	2 bags	180	107	20
03-5003-090	03-5003-082	Table BC1	2 bags	211	104	12
03-5003-103	03-5003-082	Table BC1	3 bags	353	500	74
<b>Total</b>			<b>22 bags</b>	<b>1,762</b>	<b>2,233</b>	<b>304</b>

<sup>z</sup> Clusters bagged because flowers are female and do not need emasculation.

**Table 2.** 2011 table and raisin grape PD resistant seeded crosses and the number of seeds produced.

Female	Male	Type	No. Emas- culations	No. seed
<b>89-0908 <i>V. rupestris</i> x <i>V. arizonica</i></b>				
04-5002-18	Y525- 60--07	Table BC1	388	96
04-5514-144	05-5501-27	Table BC2x3	3 bags <sup>z</sup>	13
05-5501-26	Y127-111--05	Table BC3	8 bags	21
05-5501-06	Y152-128--08	Table BC3	5 bags	235
07-5060-134	Y525- 60--07	Table BC3	2 bags	69
07-5060-134	Y540-193--05	Table BC3	3 bags	153
07-5060-134	05-5501-27	Table BC3x3	3 bags	1
07-5060-134	C 61-123	Table BC3	1 bags	30
07-5060-134	C 75- 4	Table BC3	1 bags	28
<b>Total</b>			<b>388 +29 bags</b>	<b>646</b>
<b>PM resistance combined with PD resistance</b>				
07-5060-134	Y308-148--06	Table BC3	1 bags	315
05-5501-57	06-3551-226	Table BC3	1076	231
Y308- 14	Y520- 73--07	Table BC3	2354	269
Y308- 39	Y520- 73--07	Table BC3	1162	184
07-5052-61	Y308-311--06	Table BC4	2999	191
<b>Total</b>			<b>7,591</b>	<b>1,190</b>
<b>PD resistance from BD5-117</b>				
03-5003-052	Y537-168--06	Table BC1	3 bags	172
03-5003-052	Y139-139--04	Table BC1	3 bags	246
03-5003-052	Y131-181--05	Table BC1	2 bags	108
<b>Total</b>			<b>8 bags</b>	<b>526</b>

<sup>z</sup> Clusters bagged because flowers are female and do not need emasculations.

**Table 3.** Determination of seedling resistance based on PdR1 molecular markers for all 89-0908 families made in 2010.

Family	Type Cross	No. Resistant <sup>a</sup>	No. Susceptible <sup>b</sup>	No. Recombinant <sup>c</sup>	No data <sup>d</sup>	Off Types	Total
10-5004 <sup>e</sup>	Table BC4	3	1				4
10-5005	Table BC3	5	2	1			8
10-5006	Table BC3					1	1
10-5007	Table BC3	3	1	3		2	8
10-5008	Table BC3	1	9	1		21	32
10-5009	Table BC3	1	1				2
10-5013	Table BC4					3	3
10-5015	Table BC4	5	1			1	7
10-5052	Raisin BC4	1					1
10-5054	Raisin BC4	2	2				4
10-5063	Raisin BC4	2				1	3
10-5065	Raisin BC4	4	1		1		6
10-5073	Raisin BC4	2	3				5
10-5074	Raisin BC4	6	4	1			11
10-5076	Raisin BC4	2	14	1			20
10-6001	Table BC3	8	17	1			26
10-6002	Table BC3		3				3
10-6003	Table BC3	2	3				5
10-6005	Table BC3	1					1

10-6007	Table BC4					4	4
10-6051	Raisin BC3	1					1
10-6053	Raisin BC3	22	23	2		5	52
10-6056	Raisin BC4	1				1	2
10-6058	Raisin BC4	1	1			2	4
<b>Total</b>		<b>73</b>	<b>86</b>	<b>10</b>	<b>1</b>	<b>41</b>	<b>213</b>

<sup>a</sup>Resistant = marker on both sides of *PdR1* region.

<sup>b</sup>Susceptible = no *PdR1* markers.

<sup>c</sup>Recombinant= genotypes that amplified with one *PdR1* marker.

<sup>d</sup>No data = genotypes that failed to amplify properly.

<sup>e</sup>10-5004 to 10-5076 are for PD resistance: 10-6001 to 10-6058 are for PD + PM resistance.

A total of 213 individuals were tested with SSR markers and 159 showed markers on both sides of the *PdR1* region. A total of 73 individuals (46% of those showing markers) were resistant and 86 plants had susceptible markers. 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. From the crosses made in 2010 which combined PD resistance from *V. arizonica* with PM resistance from *V. romanetii*, 79 seedlings were screened with molecular markers for PD resistance and in the greenhouse for PM resistance. The segregation ratios are shown in Table 4 and they are similar to the expected ratios for segregation of PD and PM as single dominant genes. Leaves from 2011 seedlings are being processed for marker evaluation. Inoculation of plants with *Xylella* in the Greenhouse (method of Krivanek et al. 2005, Krivanek and Walker 2005) was done to determine resistance of 38 selected individuals from *V. arizonica* (Table 5) of which 14 are resistant to date. These seedlings represent the best table and raisin selections that have been used as parents or planted in production trials. 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 its use will continue to be emphasized.

**Table 4.** Segregation of PD and PM resistance in seedlings from 2010 crosses combining resistance from *V. arizonica* and *V. romanetii*, respectively.

		PD resistance from <i>V. arizonica</i>		
		PD Resistant Obs./Exp.	PD Susceptible Obs./Exp.	Total Obs./Exp.
PM resistance from <i>V. romanetii</i>	PM Resistant Obs./Exp.	18/20	28/20	46/40
	PM Susceptible Obs./Exp.	17/20	16/20	33/40
	Total Obs./Exp.	35/40	44/40	79/80

**Table 5.** Results of *Xylella* greenhouse test for determination of PD reaction in 2011.

Population	Resistance Source	Testing Compete		In greenhouse test
		No. tested	No. resistant	
BD5-117 map	BD5-117	30	3	89
Arizonica	<i>PdR1</i>	22	14	16
Other PD	SEUS	2	0	6
<b>TOTAL</b>		<b>54</b>	<b>17</b>	<b>111</b>

In 2011 five BC3 table grape selections were treated with 20 ppm gibberellic acid (GA) at berry set to determine berry response. In all cases the berries increased in weight, diameter and length (Table 6). GA treated berries from all selections were similar in size or larger than Thompson Seedless produced by standard commercial practices at the ARS Parlier research station. The treatments used for Thompson Seedless were 15ppm GA x 2 bloom sprays; 20ppm GA bump spray; 60ppm GA x 2 size sprays; girdle and tip. The selection 08-5001-34 (Fig. 1A) had larger berries than both samples of Thompson Seedless and had only one application of GA. 08-5001-34 has very acceptable fruit quality. The majority of 800 PD resistant BC3 and BC4 *V. arizonica* seedlings planted in 2009 and 2010 fruited and were evaluated. From these seedlings, the number of selections good enough for propagation into the first stage production trials is: 6 BC4 table selections, 8 BC4 and 3 BC3 raisin selections. Fruiting characteristics of these selections are shown in Table 7. One of the table grape selections, 09-5013-118 (Fig. 1B), is good enough for inclusion in the 25 vine advanced cultural production trial. One table grape selection with PD plus PM resistance was also selected for propagation. In addition the following selections were made for use as parents: 3 BC4, 9 BC3 and 1 BC1 table grape; and 6 BC4 raisin grapes. In 2011, 6 table and 8 raisin PD resistant selections and 2 table and 5 raisin PD plus PM resistant selections were planted in production trials. Half of the planting (four of 8 replications) of 12 selections at the USDA ARS research station, Weslaco, Texas were inoculated with *Xylella* on July 15 with the help of David Appel, Texas A&M University. Each plant was inoculated twice. Petiole samples taken just above the inoculation site were collected 10/25/11. No *Xylella* was detected by PCR or culturing.

**Table 6.** Berry size of PD resistant table grape selections treated with 20ppm gibberellic acid at berry set or no treatment (NT).

Name	Treatment	mean ber. Wt. (g)	mean ber. Dia. (mm)	mean ber. Len. (mm)
08-5001-34	NT	7.5	22.05	24.5
08-5001-34	GA	11.7	23.52	33.32
08-5001-21	NT	4.4	18.13	19.6
08-5001-21	GA	7.1	21.07	24.5
08-5001-38	NT	3.7	16.66	17.64
08-5001-38	GA	6.4	20.58	24.01
08-5001-47	NT	3.6	16.17	17.64
08-5001-47	GA	6.0	20.09	21.56
TS ARS Plot	GA	6.8	19.2	31.2
TS commercial	GA	10.2	22.0	36.0

**Table 7.** New PD resistant grape selections made and evaluated 2011.

Name	gene ration	colo r	trac e	berry size	crop	cluster size	berry set	firm nes s	status	comment
Table PD <i>V. arizonica</i> resistance										
09-5013-070	BC4	W8	10	13	2	2	4	8	prop	P1 GH test
09-5013-075	BC4	R8	10	14	4	4	5	3	prop	P1 GH test
09-5013-118	BC4	R7	7	13	3	5	5	6	prop*	GH test
09-5013-122	BC4	W7	10	12	3	4	4	5	prop	GH test
09-5013-125	BC4	W8	5	12	2	4	6	7	prop	GH test
09-5064-022	BC4	W7	5	14	4	5	5	4	prop	P1 GH test
PD +PM resistance										
08-6002-089	BC3	W6	6	13	2	5	4	2	prop	P1 GH test
Name	gene ration	trac e	berry size	crop	cluster size	berry set	flavor	statu s	Type	commen t
Raisin PD <i>V. arizonica</i> resistance										
09-5066-063	BC4	7	midge t	1	1		neutra l	prop	DOV	GH test
09-5066-097	BC4	8	TS					prop	DOV	P1
09-5068-023	BC4	9	TS	1	1		musca t	prop	DOV	GH test
09-5070- 101/015	BC4	10	TS	6	5	5	neutra l	prop	TD	GH test
09-5070- 015/5071-007	BC4	10	TS	6	5	5	neutra l	prop	TD	GH test
09-5063-018	BC4	7	TS	3	4	5	neutra l	prop	DOV	GH test
09-5063-021	BC4	8	TS	2	4	4	neutra l	prop	DOV	GH test
09-5066-039	BC4	7	midge t	4	3	5	neutra l	prop	DOV	GH test
09-5056-089	BC3	10	TS	6	5	5	neutra l	prop	TD	GH test
08-5056-059	BC3	8	midge t	6	4	5	neutra l	prop	TD	GH test
08-5056-072	BC3	10	midge t	5	4	5	neutra l	prop*	TD	GH test
08-5054-047	BC1	10	TS	4	4	4	neutra l	prop	TD	GH test

Trace: 10=none, 9=Thompson Seedless (TS) size, 7= Fiesta size, 6=Flame Seedless size

Berry size = 1/16" for table grapes and Thompson Seedless (TS) or midget size for raisins.

Crop: 9=v. heavy, 7=heavy, 5=average, 3=light, 1=v. light. Cluster size: 9=>2.5 lb, 7=2 lb, 5=1 lb, 3=0.5 lb, 1=widow size.

Berry set: 9=v. tight, 7=tight, 5=average, 3=loose, 1=v. loose. Firmness: 9=v. firm, 7=firm, 5=average, 3=soft, 1=v. soft.

Prop = propagate in first 2 vine plot; prop\* = propagate table grape in 25 vine or raisin in 7 vine production trial.

DOV = natural dry on the vine type. TD = tray dried. GH test = to test for resistant reaction to *Xylella* in greenhouse.



Figure 1. A. Fruit of BC3 PD resistant table grape selection 08-5001-34 treated with 20ppm size GA. B. Natural fruit of BC4 PD resistant table grape selection 09-5013-118, first crop.

**Division of objectives and experiments.** All crosses were made, seedlings grown, evaluated for fruit quality and propagated for production trials at Parlier in Ramming's laboratory. All screening for molecular markers and greenhouse screening for *Xylella* resistance was done at Davis in Walker's laboratory.

**Objective 2:** Develop molecular markers for Xf/PD resistance in a family (SEUS) other than *V. arizonica*.

The PD resistant grape selection BD5-117 from Florida was hybridized with the seedless table grape selection C33-30 to create the mapping population of over 500 individuals. Fruit samples are being taken from all seedlings to have three years data for berry weight and seed/seed trace weight as an indication of fruit quality. Flower type is also being recorded. Greenhouse testing for PD resistance has been accomplished on 243 individuals, with 112 rated clearly resistant or clearly susceptible. One hundred twenty-two individuals are being evaluated this year (Table 5). The 70 polymorphic markers tested on 144 individuals and greenhouse PD resistance evaluations were run in JoinMap which indicated a major QTL on linkage group 2. Forty additional polymorphic primers have been tested on the 144 seedlings for further refinement of the rough framework map. In 2011, 9 seedless x seedless and 3 seeded by seedless BC1 crosses were made for table grape improvement using resistant F1 selections from BD5-117 (Table 1 and 2). A total of 22 and 8 clusters were pollinated and resulted in 304 embryos and 526 seed from seedless x seedless and seeded x seedless crosses respectively.

**Division of objectives and experiments.** All plants are maintained and evaluated for fruit and plant characteristics at Parlier in Ramming's laboratory. Greenhouse screening of plants for *Xylella* resistance was done at Davis in Walker's laboratory. All molecular primers were evaluated and allele calls made with Gene Mapper 4 for all polymorphic primers at Parlier in Ramming's laboratory. QTL determination of linkage groups was done at Davis in Walker's laboratory.

### Summary of Major Research Accomplishments and Results by Objective.

**Objective 1:** Additional families for the development of PD resistant seedless table and raisin grape cultivars are being produced. Emphasis was placed on making the first BC5 and additional BC4 *V. arizonica* PD resistant families. These families will have high fruit quality as they consist of 97 – 98.5% *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 BC4 and BC3 *V. arizonica* and F1 BD5-117 families. For example, 6 new table and 11 new raisin PD resistant and 1 new table PD plus PM resistant selections were made from BC4 and BC3 *V. arizonica* families and will be propagated for production trials.

**Objective 2:** Two hundred and forty-three 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. Initial mapping indicated a major QTL for resistance on linkage group 2 in the BD5-117 family. The development of PD resistant table and raisin grape cultivars will make it possible to keep the grape industry viable in PD infested areas. Molecular markers will greatly aid the selection of PD resistant individuals from SEUS populations.

#### **References:**

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#### **Outside Presentations of Research:**

Results of this research were presented in poster form at the CDFA PD symposium held in Sacramento (December 13-15, 2011). A corresponding article was published in the PD Symposium 2011 Proceedings (pp. 173-174).

#### **Research Success Statement:**

This project has developed very advanced Pierce's disease resistant raisin and table grape germplasm and advanced selections. The first BC5 generation from *V. arizonica* with 97% *V. vinifera* in its background was created this year and will start flowering in 2013. 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 BC4 and BC3 *V. arizonica* and F1 BD5-117 families. For example, 6 new table and 11 new raisin PD resistant and 1 new table PD plus PM resistant selections were made from BC4 and BC3 *V. arizonica* families and will be propagated for production trials. Resistant varieties will allow growers to produce table and raisin grapes in PD infested areas without concern for the vector. Initial mapping indicated a major QTL for resistance on linkage group 2 in the BD5-117 family. Molecular markers will greatly aid the selection of PD resistant individuals from SEUS populations and development of additional PD resistant table and raisin grape cultivars.

**Funds Status:** Funds have been received and are being spent at the appropriate rate.