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

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ABSTRACT
This year six crosses for Pierce's disease (PD) resistant table and raisin grapes using seedless parents were made, resulting in 2,398 ovules and 747 embryos. These crosses were based on *Muscadinia* and *V. tiliifolia* sources of resistance. Nineteen seeded crosses consisting of 3,950 emasculations and 49 bagged clusters were made. These crosses were based on *V. arizonica, Muscadinia,* and southeastern United States (SEUS) sources of resistance. Over 120 selections have been made based on fruit quality and are ready for greenhouse screening for resistance to PD. Two BC2 families from 89-0908 *V. arizonica* source of resistance segregated in a 1:1 ration for resistance:susceptibility, based on molecular markers associated with the *PdR1* locus. A smaller family from the same source of resistance had an unexpectedly small number of resistant seedlings. DNA samples have been collected from 154 seedlings from the C33-30 x BD5-117 family and are ready for SSR marker analysis. Additional seedlings are being produced to increase probability of identifying markers associated with this source of resistance.

INTRODUCTION
Pierce's disease (PD) has existed in California at least since the late 1800s when it caused an epidemic in Anaheim. A number of vectors for PD already exist in California causing its spread. The introduction of the glassy-winged sharpshooter (GWSS) to California in the 1990's increased the spread and damage caused by PD. Other vectors exist outside California and are always a threat. All of California's table and raisin grape cultivars grown commercially 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 does not match the *Vitis 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. 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 *Xylella fastidiosa (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 the *Muscadinia* source of resistance and a unique source of resistance from *V. tiliifolia*. Six crosses were made for a total of 2,398 ovules cultured (Table 1). A total of 748 (31%) embryos were extracted and transplanted on fresh medium for growth into plants. Nineteen seeded crosses were made for PD resistance (Table 2). For five of the crosses, 3,950 emasculations were made and 49 clusters were bagged for 14 additional crosses which had female flowered parents. Fruit has been harvested and seeds are being extracted for germination in January. The number of seeds produced from each resistant source was: 1,881 from *V. arizonica*; 1,643 from *Muscadinia,* and 2,071 from SEUS with an additional 184 from BD5-117 BC1. Over 120 selections have been made based on fruit quality from populations made for PD resistance. These families are from resistant sources different than the *V. arizonica* source of resistance. These selections are in line for PD testing in the greenhouse. Three families (89-0908 *V. arizonica* source of resistance) produced in 2005 were tested for molecular markers associated with PdR1 locus on chromosome 14

(Table 3). Families 05-5551 and 05-5501 segregate in a 1:1 ratio. Family 05-5502 does not fit the same segregation ratio having only 16.7% resistant plants.

Table 1. 2006 table and raisin grape PD resistant seedless x seedless crosses and the number of ovules and embryos produced.

Female	Male	Type		No. berries Picked	No. berries Opened	No. Ovules	No. Embryos
Muscadinia source of resistance							
A90-45	Scarlet Royal	Table	BC1	171	171	319	86
A90-45	A85-40	Table	BC1	353	353	433	114
A90-45	Diamond Muscat	Raisin	BC1	260	247	275	76
A90-45	B82-43	Raisin	BC1	404	390	350	85
V. tiliifolia source of resistance							
C33-30	IAC572	Table	F1	593	536	610	118
B82-43	IAC572	Raisin	F1	315	315	411	158
SEUS source of resistance							
C33-30	BD5-117	Genetic family		460	430	631	118

Table 2. 2006 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*					
A81-17	B28-126	Table	BC2	4 bags (a)	446
A81-17	C45-64	Table	BC2	4 bags	390
A81-17	Y150-14	Table	BC2	3 bags	396
A81-17	Y315-54	Table	BC2	4 bags	96
A81-138	A95-21	Raisin	BC2	3 bags	106
A81-138	B82-43	Raisin	BC2	3 bags	124
A81-138	C81-116	Raisin	BC2	3 bags	193
A81-151	B82-43	Raisin	BC2	3 bags	9
A81-151	C81-116	Raisin	BC2	3 bags	8
A81-151	A95-21	Raisin	BC2	3 bags	113
Muscadinia source of resistance					
A90-37	C45-64	Table	BC1	5 bags	787
Bloodworth	A51-50	Raisin	F1	5 bags	856
SEUS source of resistance					
Z74-26-1	Autumn Royal	Table	F1	1005	375
Z74-26-1	A63-85	Raisin	F1	997	820
Z74-26-1	B82-43	Raisin	F1	1072	800
Z70-8-1	C57-94	Table	F1	3 bags	76
Z70-8-1	B82-43	Raisin	F1	3 bags	-
SEUS BD5-117 source of resistance					
A104-29	B28-126	Table	BC1	451	103
A104-29	C45-64	Table	BC1	455	81

(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.

Family	Type Cross	No. Resistant (a)	No. Resistant? (b)	No. Susceptible (c)	Off type
05-5551	Raisin	40	13	45	8
05-5501	Table	28	12	26	0
05-5502	Table	4	8	20	0

(a) Resistant = marker on both sides of *PdR1* region.
(b) Resistant? = marker on one side of *PdR1* region.
(c) Susceptible = no 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 produced. Initially, 20 plants were evaluated in the greenhouse for resistance to PD and 10 were found resistant with very low bacteria counts and PD symptoms. One hundred fifty-four DNA samples have been extracted and are ready for screening against SSR primers. Fruit from these individuals has been collected and is being evaluated for berry size, seed/trace weight and fruit characteristics. The cross was repeated this year to increase the number of individuals in the family (Table 1). A total of 631 ovules from the seedless parent C33-30 were cultured and 118 embryos have been extracted and sub-cultured to be grown into plants.

CONCLUSIONS

Populations for the development of PD resistant seedless table and raisin grape cultivars continue to be produced from seedless and seeded parents. Sources of resistance in addition to *V. arizonica* are being used. Over 120 selections have been made based on fruit quality and are ready for greenhouse testing for resistance to PD. Two BC2 families from 89-0908 *V. arizonica* show a 1:1 segregation ratio for resistance:susceptibility resulting in a total of 68 resistant seedlings from these two families.

REFERENCES

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.

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MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*

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ABSTRACT
This progress report shows that open pathways likely exist for *Xylella fastidiosa* (*Xf*) movement across grafts in grape stems via xylem pathways. Studies thus far have been conducted on grafted and non-grafted three-year-old *Vitis vinifera* cv. Chardonnay plants. The movement of air was used to determine if open xylem conduits were present through grafts into canes, and the length of these pathways was measured. It was determined that connections via xylem vessels are generally about twice as long in non-grafted plants (450 mm) compared with grafted plants (225 mm). Current investigations are underway with dilute use latex paint and tagged *Xf* to understand the pathways for bacterial movement across grafts.

INTRODUCTION
Grapes are one of the important crop plants in which the shoots of one variety are grafted to root stocks of another to generate plants with the desired characteristics of both. Reports have clearly shown the presence of long, open xylem conduits that connect stems to leaves in chardonnay (Thorne et al., 2006; Chatelet et al, in press). Anatomical studies have also indicated that *Xylella fastidiosa (Xf)* appears to be primarily restricted to xylem vessels in canes, however little is known about the vessels, and subsequently the movement of *Xf* across grafts. The capacity for *Xf* to move in plants differs among species ranging from generally unrestricted throughout the major organs, to only a few centimeters from the original inoculation point. The objectives of this study are to examine the connection of vessels from canes into stems through grafts, and determine if it is possible for *Xf* movement to occur freely across these grafts. To meet these objectives a strategy of air and latex paint are being implemented to study open anatomical systems, and most importantly the use of *Xf* to examine movement across grafts.

OBJECTIVES
1. Conduct a study of connections in grafted *Vitis vinifera* cv. Chardonnay, and determine if open vessel systems allow movement of *Xf* across grafts via air pressure.
2. Conduct an anatomical study of connections in grafted *Vitis vinifera* cv. Chardonnay, and determine if open vessel systems allow movement of *Xf* across grafts with latex paint.
3. Use PCR to determine the presence of *Xf* across graft unions after inoculation at known positions relative to the graft.

RESULTS
Following inoculation in grapevine, *Xf* moves in the nutrient poor xylem vessels and eventually causes disease symptoms that result plant death by unknown mechanisms. Previously, reports from our labs have indicated that bacteria can move freely in canes and from petioles into leaves during a systemic infection process. It is of interest to determine the movement across grafts to clarify movement into stems and possibly into root systems. Our preliminary results indicate that the graft unions of *Vitis vinifera* cv. Chardonnay do indeed contain continuous vessels; however, the open system length into canes is about ½ of that when compared with non-grafted plants of the same cultivar (Figure 1A). Measurements collected of cane length and associated open conduits appeared significantly different between grafted and non-grafted plants (Figure 1B). Differences were not found to be significant in stem length between grafted and non-grafted plants (Figure 1C). These results indicate that graft unions would not be an impediment to bacterial movement, and that *Xf* would be able to move further distances across in non-grafted areas of the plant because of the presence of continuous vessels. This study of the xylem structure will be further evaluated with current studies to determine the connective pathway of air movement by latex paint, and confirm that *Xf* can be moved through the vessels in the presumed transpiration stream.

CONCLUSIONS
From our preliminary results, graft unions in stems do not appear to restrict the movement of *Xf* in *Vitis vinifera* cv. Chardonnay. Although the length of open vessels is reduced by about ½, open vessels cross the graft union as determined by air movement. However, the numbers of vessels that cross the graft are less than 10% in distribution when compared with non-grafted plants (data not shown). In order for *Xf* to move from a cane or leaf across a graft it would need to be inoculated into a vessel that happens to extend through the graft union, or the bacteria would need to degrade membranes to move into adjacent vessels through bordered pits. Current studies with paint and PCR detection of *Xf* will confirm these results.