Project Title: Breeding Pierce's Disease Resistant Table and Raisin Grapes and the Development of Markers for Additional Sources of Resistance.

Principal Investigator:
M Andrew Walker
Department of Viticulture and Enology, University of California, One Shields Ave., Davis, CA 95616-8749, 530-752-0902, awalker@ucdavis.edu

Co-Investigator:
David W. Ramming
Agricultural Research Service, U.S. Dept. Agriculture, Fresno, CA 93727
559-596-2823

Cooperators
Hong Lin
Agricultural Research Service, U.S. Dept. Agriculture, Parlier, CA 93648
559-596-2933 Dr. Lin provides expertise in developing more SSR markers and designing florescent-based multiplex SSR PCR used for marker-assisted breeding and genetic mapping.

This is a collaborative project between the Walker and Ramming labs. Dr. Ramming is primarily in charge of this breeding effort to produce PD resistant table and raisin grapes. The Walker lab's roles in this project are providing marker-assisted selection for his seedling and embryo-rescued progeny allowing us to screen for and eliminate susceptible progeny before planting in the field. We also produce crosses to expand a BD5-117 mapping population that is being used to develop and map an alternative source of resistance to PD. Much of the collaborative breeding effort is contained in Dr. Ramming's report. The reporting of my portion of this breeding effort is detailed below.

List of objectives and description of activities conducted to accomplish each objective:
Objective 1: Develop PD resistant table and raisin grape germplasm/varieties with fruit quality equivalent to standards of present day varieties.

Walker Objectives – Select PD resistant seedlings using molecular markers and greenhouse screen selected progeny for PD resistance.

My lab has developed SSR-based markers for resistance to *Xylella fastidiosa* originating from *Vitis arizonica* b43-17. These markers are tightly linked to a single locus that contains a dominant gene for resistance to PD (*PdR1*) (see references for details of this genetic mapping effort). We are using these markers to select resistant progeny both in our winegrape breeding efforts and in this collaborative effort with Dr. Ramming to breed table and raisin grapes for resistance to PD. We receive tissue during the Winter and Spring from Dr. Ramming that he harvests from his embryo-rescued progeny; the result of crosses between elite seedless table grape selections and *PdR1* containing progeny from later generations of our backcrossing program. Thirty-one seedless crosses were made and embryo rescue procedures were used to recover embryos and establish plants at Parlier. These crosses included BC4 (97% *V. vinifera*) F8909-08 *V. arizonica* families.

Leaf samples from these seedling populations were tested with marker-assisted selection (MAS) beginning in 2009 through Summer 2010. Samples are sent from the Ramming lab and we process them as soon as possible to allow him to discard susceptible embryo-rescued plantlets before transplanting into pots prior to planting in the vineyard. We also evaluate progeny carrying the *PdR1* gene, and progeny from the genetic mapping population based on resistance from the southeast United States (SEUS) hybrid BD5-117, with a well characterized greenhouse screen utilized in the references cited below. Marker-assisted selection techniques and the genetic mapping that resulted in the identification of the *PdR1* locus are also detailed in the references cited below.

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

Walker Objectives - Increase the family size of a BD5-117 family (*V. vinifera* C33-30 x BD5-117 (a complex hybrid with resistance from *V. shuttleworthii*, *V. aestivalis* ssp. *smalliana* and Vidal blanc)) to 500 individuals. Add EST-based SSR markers and others, as they are developed to saturate SSR-based maps. Test PD resistance markers from the BD5-117 map in other selections and germplasm from the southeastern United States.

In order to expand the genetic mapping population based on BD5-117, we crossed C33-30 x BD5-117 in Spring 2008 to produce 14 clusters that were sent to Dr. Ramming. The fertilized ovules of this cross must be embryo-rescued because C33-30 is seedless. The addition of these rescued progeny should increase the size of this population to 500 — large enough for fine-scale mapping and the detection of quantitatively inherited loci. We are also screening this population for resistance to PD using our greenhouse screening process. We are involved in the mapping of this population and will be adding other markers over time and testing markers in other PD resistant varieties and selections from the SEUS when they become available.

References detailing greenhouse-based PD resistance screening, marker development for the PD resistance locus – *PdR1*, and the utilization of SSR-based makers that are tightly linked to *PdR1* for MAS.

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

Krivanek, A.F., T.R. Famula, A. Tenscher and M.A. Walker. 2005. Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* x *Vitis arizonica* hybrid population. Theor. Appl. Genet. 111:110-119.

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

Riaz, S., A.F. Krivanek, K. Xu and M.A. Walker. 2006. Refined mapping of the Pierce's disease resistance locus, *PdR1*, and *Sex* on an extended genetic linkage map of *Vitis rupestris* x *V. arizonica*. Theor. Appl. Genet. 113:1317-1329.

Riaz, S., A.C. Tenscher, J. Rubin, R. Graziani, S.S. Pao and M.A, Walker. 2008. Fine-scale genetic mapping of two Pierce's disease resistance loci and a major segregation distortion region on chromosome 14 of grape. Theor. Appl. Genet. 117:671-681.

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek and M.A. Walker. 2009. Using marker-assisted selection to breed Pierce's disease resistant grapes. Amer. J. Enol. Vitic. 60:199-207.

Summary of major research accomplishments and results for each objective

Objective 1:

Dr. Ramming collected leaf samples from 1,191 seedlings derived from twelve BC3 and two BC2 families that were created from the b43-17 source of resistance in 2007. These samples were sent to us for MAS with SSR markers tightly linked to the *PdR1* locus. Table 1 summarizes the number of seedlings we tested and their source. 559 have been tested or are under test to date and we are expecting a few more this Spring. This table groups the genotypes he sent into 7 groups. Group 1 includes the Ramming mapping population of *V. vinifera* C33-30 x BD5-117 (complex PD resistant hybrid from Mortensen in Florida. Group 2 includes table and raisin grape types from various backcross generations and with *PdR1* from *V. arizonica*. Group 3 includes an array of genotypes that we are waiting for instructions on how to proceed. Group 4 are the 2007 additions to the BD5-117 mapping population that need greenhouse screening data for mapping; Group 5 includes *PdR1* containing selections that had equivocal results from previous greenhouse testing; Group 6 contains genotypes that did not get tested because they were too weak and had to be re-propagated before testing; and Group 7 included genotypes from both the BD5-117 and *PdR1* sources that were hot water dipped to prevent the movement of vine mealy bug from Parlier to UCD.

Although more tests are underway the results to date show our successful use of the *V. arizonica* b43-17 PdR1 resistance source and the high percentage of resistant individuals (Table 2). Table 2 also lists the number of genotypes from the BD5-117 mapping population (*V. vinifera* C33-30 x BD5-117) that have been tested so far. As expected relatively few are resistant from this source of resistance that is quantitatively inherited.

These tests are run with a number of biocontrols so that ELISA values over time can be compared. We separate the resistant from susceptible plants by comparing their mean ELISA values with Dunnett's test using the known resistant *PdR1* containing U0505-01. To date marker-assisted selection using *PdR1* markers worked has agreed with 23 of the 28 tested plants and we will be retesting the 5 non-agreeing plants and verifying the are true to type and not occasional selfs. These plants all descend from the same parental population A81. There were 90% of the *PdR1* plants that were positive by marker and greenhouse screen that were 87.5% *V. vinifera*.

Objective 2:

To date we have established a field planting of 154 individuals for the C33-30 x BD5-117 mapping population and will be adding more from this year's tested plants. One hundred and thirty-eight of these individuals have been evaluated for cluster size, berry size, % soluble solids and seed/aborted seed size. We are also contributing advise on marker use and mapping procedures to the Ramming group as they map this population.

Table 1. Genotypes from David Ramming's PD resistant table and raisin grape breeding program that were or will be tested at UCD in 2009-10. See the text for descriptions of the Groups.

Test Group	# genotypes in group received	# genotypes successfully propagated for testing	# genotypes successfully tested	Date Propagated	Inoculation date (est in italics)	Sample date (est in italics)
1	86	58	48	4/24/09	9/29/09	1/14/10
2	38	35	28	4/24/09	9/29/09	1/14/10
2.5	69	26	26	6/23/09	10/27/09	2/11/10
3	97	waiting for instructions				
4	79	74	pending	<9/1/09	1/5/10	*4/13/10*
5	12	6	pending	<9/1/09	1/5/10	*4/13/10*
6	54	pending	pending	1/29/10	*5/23/10*	*8/29/10*
7	124	pending	pending	2/2/10	*5/25/10*	*8/31/10*

Table 2. Summary of 132 genotypes tested for PD resistance in the green house at UC Davis.

Population	Resistance Source	Testing Complete	
		# Tested	# Resistant
C33-30 x BD5-117 Mapping	BD5-117	48	19
b43-17 *V. arizonica* Breeding (all but two are 88% vinifera)	all with *PdR1*	28	23
Total		76	42

Publications or reports resulting from the project

Riaz, S., A.C. Tenscher, R. Graziani, A.F. Krivanek, D.W. Ramming and M.A. Walker. 2009. Using marker-assisted selection to breed Pierce's disease resistant grapes. American Journal of Enology and Viticulture 60:199-207.

Presentations on research

Impact of invasive species: breeding for resistance to PD. CSREES Review, UC Davis, Jan. 13, 2009.

Current issues in grapevine pests and diseases. UCD Wine Executive Short Course, Mar. 10, 2009.

Crape breeding with an emphasis on flavor. Recent Advances in Viticulture and Enology, Mar. 19, 2009.

PD resistant winegrapes coming soon. Temecula Grape Day, Temecula CA, April 2, 2009.

Grape breeding at UCD. International Grape Research Coordination Network for Grape Functional Genomics, Granlibaken, Lake Tahoe, CA, May 16, 2009.

Twenty years of grape breeding at UC Davis. Honorary Research Lecture, ASEV 60th Annual Meeting, Napa, CA June 24, 2009.

Breeding PD resistant winegrapes. National Grape Breeders Conference, Tallahassee, FL, Aug. 6, 2009.

Breeding grapes with resistance to Pierce's disease. Current Issues in Plant Health, FPS/UCD Extension, Davis, CA, Nov. 19, 2009.

Will you be ready for PD resistant wine grapes? Dept. Viticulture and Enology Seminar, UC Davis, CA, Nov. 20, 2009.

Breeding PD resistant grapevines. CDFA PD/GWSS Meeting, Sacramento, CA, Dec. 10, 2009.

Breeding PD resistant grapevines. Texas Pierce's Disease Symposium, Marble Falls, TX, Mar. 2, 2010.

Research relevance statement

This collaborative project has developed advanced (97% *V. vinifera*) PD resistant raisin and table grape germplasm (BC4 generation with the *PdR1* resistance source). Greenhouse screening verified the resistance of parents used to make the 172 BC3 seedlings the best of which were used to make the BC4 generation (97% *V. vinifera*) in 2009 and these seeds and embryos are now germinating. These PD resistant varieties will allow table and raisin grape vineyards to be planted in PD infested areas.

Lay summary of current year's results

Progress in the development of high quality table and raisin grapes with PD resistance continues through this collaborative project between the Ramming and Walker labs. We have used the backcross three (BC3) generation to produce BC4 (97% *V. vinifera*) table and raisin grape seed populations that will be planted in 2010. This backcross breeding program has been a success because of our use of genetic markers linked to a PD resistance gene – *PdR1* that originates from *V. arizonica* – in a process called marker-assisted selection (MAS). Using this process we maintain high PD resistance in each generation of backcrossing while increasing the percentage of *V. vinifera* from high quality table and raisin grapes. The greenhouse screen is used to verify the PD resistance of seedling populations and selections with *PdR1*, and has been highly accurate. We will test over 600 seedlings with our greenhouse assay and most of these will also be tested for *PdR1* with markers. We are also testing members of a PD resistant table grape population with resistance from B5-117 in the greenhouse and assisting with genetic mapping efforts to develop markers for this marker source.

Status of funds

Funds have been expended.

Summary and status of intellectual property produced during this research project

None produced to date.