**Breeding Pierce’s disease resistant winegrapes.**

**Final Progress Report for CDFA Agreement 12-0117-SA**

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**Reporting period:** The results reported here are from work conducted July 2012 to July 2015

**ABSTRACT**

We continue to make rapid progress breeding Pierce’s disease (PD) resistant winegrapes. Aggressive vine training and selection for precocious flowering have allowed us to reduce the seed-to-seed cycle to 2 years. We are also using marker-assisted selection (MAS) for the PD resistance gene, *PdR1* (see companion report) to select resistant progeny as soon as seeds germinate. These two practices have greatly accelerated the breeding program and allowed us to produce four backcross generations with elite *V. vinifera* wine grape cultivars in 10 years. We have screened through about 2,000 progeny from the 2009, 2010 and 2011 crosses that are 97% *vinifera* with the *PdR1b* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to crop and others are advanced to greenhouse testing. We select for fruit and vine quality and then move the best to greenhouse testing, where only those with the highest resistance to *Xylella fastidiosa (Xf)*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis, and other test sites. The best of these will be advanced to 100 vine commercial wine testing the first of which was planted in Napa in June 2013. We advanced 2 additional selections to Foundation Plant Services (FPS) this winter to begin the certification and release process. Three PD resistant rootstocks were previously advanced to FPS for certification. Other forms of *Vitis arizonica* are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Stacking of *PdR1b* with b42-26 PD resistance has been advanced to the 92% *vinifera* level by combining MAS to confirm the presence of *PdR1b* and greenhouse screening to verify higher than usual levels of PD resistance. PD resistance from *V. shuttleworthii* and BD5-117 are also being pursued but progress is limited by their multigenic resistance and the absence of corresponding genetic markers. Very small scale wines from 94% and 97% *vinifera PdR1b* selections have been very good and have been received well at pubic tastings in Sacramento (CAWG) and Santa Rosa (Sonoma Winegrape Commission), Napa Valley (Napa Valley Grape Growers and Winemakers Associations), Temecula (Temecula Valley Winegrape Growers and Vintners) and Healdsburg (Dry Creek Valley and Sonoma Grape Growers and Winemakers).

**LAYPERSON SUMMARY**

One of the most reliable and sustainable solutions to plant pathogen problems is to create resistant plants. We use a traditional plant breeding technique called backcrossing to bring resistance to PD from wild grape species into a diverse selection of elite winegrape backgrounds. We identified the genomic region that carries a very strong source of PD resistance from a grape species native to Mexico and the southwestern United States (*V. arizonica*). Because we were able to locate this resistance gene/region – *PdR1* (Krivanek et al. 2006), we have able to use marker-assisted selection (MAS) for markers associated with *PdR1* allowing us to select resistant progeny shortly after seeds germinate. MAS and aggressive growing of the selected seedling vines have allowed us to produce new PD resistant high quality winegrape selections that are more that 97% *V. vinifera* in only 10 years. We have evaluated thousands of resistant seedlings for horticultural traits and fruit quality. The best of these are advanced to greenhouse testing, where only those with the highest resistance *to Xf*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and at PD hot spots around California. The best of these are advanced to 100 vine plots for commercial wine testing. We have sent 15 advanced selections to Foundation Plant Services over the past three winters to begin the certification and release process. Three PD resistant rootstocks were also sent to FPS for certification. Other wild grape species are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable PD resistance. Very small-scale wines made from our advanced *PdR1* selections have been very good, and have been received well at professional tastings throughout California.

**INTRODUCTION**

The Walker lab is uniquely poised to undertake this important breeding effort, having developed rapid screening techniques for *Xf* resistance (Buzkan et al. 2003, Buzkan et al. 2005, Krivanek et al. 2005a 2005b, Krivanek and Walker 2005, Baumgartel 2009), and having unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southwestern grape species, which allows the introduction of extremely high levels of *Xf* resistance into commercial grapes. We genetically mapped and identified what seems to be a single dominant gene for *Xf* resistance and named it *PdR1*, which was found in *V. arizonica*/*candicans* b43-17. This resistance has been backcrossed through four generations to elite *V. vinifera* cultivars (BC4) and we now have 97% *vinifera* PD resistant material to select from. Individuals with the best fruit and vine characteristics are then tested for resistance to *Xf* under our greenhouse screen. Only those with the highest levels of resistance are advanced to small-scale winemaking trials by grafting them onto resistant rootstocks and planting 6 to 8 vines sets on commercial spacing and trellising at PD hot spots around California where they continue to thrive (right side of Figure 1). We have made wine from vines that are 94% *vinifera* level from the same resistance background for 6 years and from the 97% *vinifera* level for 4 years. They have been very good and don’t have the hybrid flaws (blue purple color and herbaceous aromas and taste) that were prevalent in red wines from the 87% *vinifera* level. There are two forms of *PdR1* that descend from sibling progeny of b43-17 and they have different alleles of *PdR1* designated *PdR1a* and *PdR1b*. Screening results reported previously showed no significant difference in resistance level in genotypes with either one or both alleles. We have narrowed our focus to *PdR1b* but retain a number of selections at various BC levels with *PdR1a* in the event that there is an as yet unknown *Xf* strain-related resistance associated with the *PdR1*alleles. We also identified a PD resistance locus *PdR1c* from *V. arizonica* b40-14 that maps to the same region of LG14 as *PdR1* from b43-17. In the absence of an understanding of gene function and given the significant geographic separation of the origins of the b43-17 and b40-14 resistance sources, differences in preliminary DNA sequence data and PD phenotypic symptom differences with *PdR1b* containing genotypes we have continued to advance the *PdR1c* line as a future breeding resource. Gene characterization is being explored in our companion report. Resistance from southeastern United States (SEUS) species is being advanced in other lines. However, the resistance in these latter lines is complex and markers have not yet been developed to expedite breeding. The breeding effort with alternative resistance sources and the complexing of these resistances is being done to broaden *Xf* resistance and address *Xf’s* potential to overcome resistance.



Figure 1. Yountville field trial 7-17-2015: At left a block of Riesling vines dying from PD while immediately adjacent blocks of UCD *PdR1b* based selections continue to thrive after 10 years (at right).

**OBJECTIVES**

1. Identify additional unique sources of *Xf* resistance; develop breeding populations and phenotype them with our greenhouse screen to characterize their inheritance of resistance.
2. Develop ~97% *vinifera*-based PD resistant lines of winegrapes utilizing diverse sources of resistance to *Xf*, and conduct fruit and wine evaluations.
3. Utilize marker-assisted selection to allow stacking of resistance loci, screen for resistant genotypes, and develop backcross generations by crossing resistant selections to elite *vinifera* varieties in order to produce high quality and PD resistant winegrapes.
4. Develop and maintain new and existing genetic mapping populations to assist companion mapping/genetics project; begin the mapping of fruit quality traits such as color, tannin content, flavor, and productivity in PDresistant backgrounds.

**RESULTS AND DISCUSSION**

Our PD resistance breeding activities over the last 3 years are quantified and summarized in Table 1. We reached the 97% *vinifera* level in the *PdR1b* line in 2009 and finished planting out additional crosses at that level in 2011. A total of 2,911 genotypes were planted in the 2010-12 period. In the absence of markers for another PD resistance source, our marker testing (Table 1a) has expanded to include testing of crosses without known resistance markers for trueness-to-type and our plantings in (Table 1b) include potential new resistance source F1 and BC1 populations. Fruit evaluations (Table 1c) include new mapping crosses and stacked crosses but doesn't include spring evaluations for horticultural traits, flower sex or productivity. Fruit evaluation activity peaked in 2013 as we evaluated the large number of 97% *PdR1b* genotypes planted in the 2010-12 period. As we continue to advance the backcross level of various lines especially in the absence of resistance markers for sources other than *PdR1* our greenhouse screening has increased significantly over the level of activity in the previous 3 years. In addition to scion genotypes, Table 1d includes rootstock breeding, mapping and germplasm testing but not any spacing or *Xf* strain trials, the testing of bio-control vine genotypes or our testing done in collaboration with the USDA table and raisin grape breeding program that ended in 2013. As we identify particularly resistant individuals we test them multiple times (Table 1e) to properly assess their level of resistance and insure that only the most resistant individuals are advanced. These tests are in addition to those listed in the Table 1d immediately above. All our selections sent to FPS for certification have occurred in this 3-year period and the numbers in Table 1f includes the 3 rootstock selections sent in 2013.

Table 1. 2013-15 PD breeding activity summary. Estimates for the number of genotypes to be greenhouse tested in 2015 is a projection based on any trials in process that will come down before the end of the year and are in italics.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Activity** | **Calendar Year** | | | **2013- 2015 Total** |
| **2013** | **2014** | **2015** |
| 1a. # Genotypes MAS Tested | 899 | 1188 | 1915 | 4002 |
| 1b. # Genotypes Planted to Field | 777 | 876 | 1176 | 2829 |
| 1c. # Genotypes Evaluated for Fruit | 1950 | 330 | *550* | 2830 |
| 1d. # Genotypes Tested in GH | 1058 | 1239 | *899* | 3196 |
| 1e. # Genotypes Tested Multiple Times | 107 | 102 | *88* | 297 |
| 1f. # Advanced Selections sent to FPS | 13 | 3 | 2 | 18 |

To date over 296 wild accessions have been tested for PD resistance with the greenhouse screen, mostly from the southwestern US and Mexico. Our goal is to identify the accessions with potential for having the most unique PD resistance mechanisms. To do so we use LG14 (where *PdR1* resides) and haplotype markers to ensure that we are choosing diverse resistance sources for population development and greenhouse screening efforts. Table 2 lists the 14 accessions chosen for investigation over this period, their resistance ratios of their F1 progeny from crosses to *V. vinifera* and the nature of the genotype frequency distribution of bacterial titer.

Table 2. New southwest US PD resistance source F1 populations tested in the 2013-15 period with our greenhouse screen to identify promising new sources of resistance for the development of markers. The greenhouse testing of the 2014 crosses is scheduled to end on 9/3/2015.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Cross Year** | **R Source** | **# genotypes tested** | **R/S** | **Genotype Frequency Distribution** |
| 2012 | ANU5 | 27 | 0.8 | not normal |
| 2012 | b40-29 | 29 | 6.3 | normal |
| 2012 | b46-43 | 60 | All R | not normal |
| 2013 | b41-13 | 47 | 0.1 | not normal |
| 2013 | b43-57 | 20 & 44 | 1.0 & 2.1 | normal, normal |
| 2013 | b47-32 | 20 & 31 | 0.2 & 6.8 | normal, not normal |
| 2013 | SC36 | 35 | 0.7 | not normal |
| 2013 | T03-16 | 62 | 0.1 | not normal |
| 2014 | A14 | 30 | - | in process |
| 2014 | A28 | 42 | - | in process |
| 2014 | ANU67 | 31 | - | in process |
| 2014 | ANU71 | 31 | - | in process |
| 2014 | C23-94 | 45 | - | in process |
| 2014 | DVIT 2236.2 | 30 | - | in process |
| 2014 | SAZ 7 | 52 | - | in process |

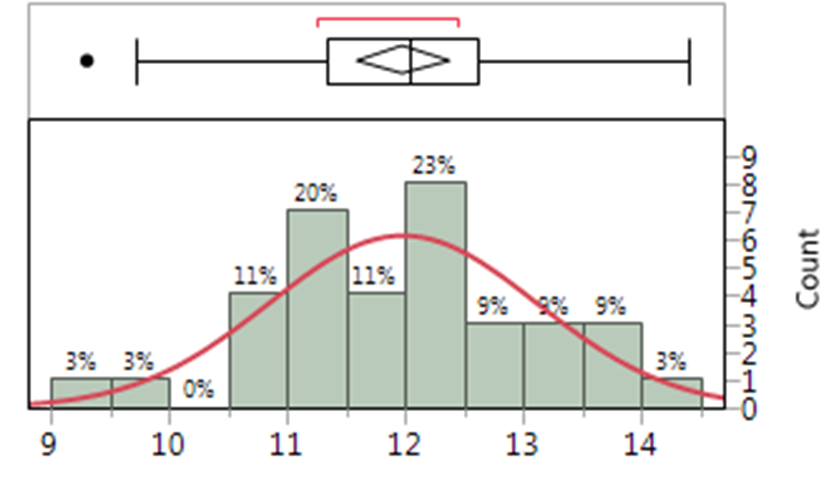
Among the accessions detailed in Table 2, b46-43 from the Big Bend, TX had the strongest resistance to *Xf* of any species accession yet tested. All its F1 progeny were resistant by both ELISA and phenotype evaluation. Although our focus for mapping is now on the b46-43 line we have developed small breeding populations using ANU5 and b40-29 (made in 2014 and screening is underway) and SC36 where crosses were made in 2015 utilizing the most resistant individuals identified in their respective F1 populations. Given this extensive collection of resistance sources, with the exception of crosses to *V. caribaea* in 2015, we have suspended the development of additional F1 populations following the 2014 season.

Since all the F1 progeny in the b46-43 line (cross 12-305) were resistant in our greenhouse screen, we couldn’t use that generation to map *Xf* resistance. We therefore developed several BC1 populations based on three 12-305 progeny to see if the resistance varied in the BC1. In Table 3 we present the first disease phenotype scores from the BC1 (75% *vinifera*) from a very severe greenhouse test. Additionally, the results of two BC1 crosses in the b40-29 line are also reported. The Cane Maturity Shoot Stunting Index (CMSSI) is a 0-8 point scale evaluating the ability of a genotype to normally lignify canes, resist cane necrosis and grow vigorously in our greenhouse test following *Xf* inoculation. For comparison, the highly susceptible *vinifera* cultivar Chardonnay had a mean CMSSI score of 5.8 (SD=0.8, n=13) and our most resistant 88% *vinifera* control genotype U0505-01 had a mean of 1.5 (SD=1.2, n=11). ELISA results are pending.

Table 3. Pierce’s disease phenotype scores for BC1 populations.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Female Name** | **Male Name** | **PDR Type** | **# Genotypes** | **Cross CMSSI Mean** | **Cross CMSSI StDev** |
| Rosa Minna | 12305-55 | b46-43 | 38 | 2.9 | 1.0 |
| Rosa Minna | 12305-56 | b46-43 | 14 | 3.4 | 1.0 |
| F2-35 | 12305-56 | b46-43 | 29 | 1.6 | 1.2 |
| F2-35 | 12305-83 | b46-43 | 32 | 1.6 | 1.3 |
| F2-35 | 12340-13 | b40-29 | 25 | 2.2 | 1.3 |
| F2-35 | 12340-14 | b40-29 | 24 | 1.5 | 1.3 |

Over the last decade, we have devoted a great deal of time and effort attempting to map PD resistance in *V. arizonica/girdiana* b42-26 from Monterrey. To date we have mapped two QTLs that only explain 8-10 % of the variation in disease resistance in the F1 population. However by utilizing our greenhouse screen our breeding program has been able to continue to advance this line to the BC2 generation and still recover plants (although not many) with significant PD resistance. Noteworthy in screens that are particularly severe, b42-26 line genotypes can perform better (lower ELISA and CMSSI scores) than *PdR1b* line genotypes typically characterized as more resistant in less severe greenhouse screens. For example, a comparison of the highly resistant b42-26 line genotype 07344A-35 vs the most resistant standard bio-control *PdR1b* genotype U0505-01 over 5 high to severe rated greenhouse screens had a ln mean cfu/ml of 11.2 for the former and 12.4 for the latter. For the same trials the two had average R-rating of 8.2 for the former vs 3.2 for the latter. This scale factors disease phenotype expression plus ELISA. A score of 10 is the highest possible resistant level and -1 is equivalent to a susceptible *vinifera* cultivar. One of our recent efforts to characterize the nature of inheritance of resistance in this line was to intercross two highly resistant individuals and greenhouse test the resulting progeny (Figure 2). The distribution is normal indicating that PDR in this line is most likely qualitative. Most interesting is that none of the progeny are as resistant as the b42-26 source indicating that not all the important resistance factors present in b42-26 are present in 07344A-11 and 07344A-35, the parents of this intercross.

  
Figure 2. The 13-309 population resulted from the intercross of two of the most highly resistant 75% *vinifera* progeny in b42-26 PDR line. The outlier represented by the black dot above the left-most bar is the original resistance source b42-26. The red line indicates a normal distribution and this distribution is normal. n=34 progeny.

Given the different nature of both the inheritance and the observed manifestation of PD resistance between the *PdR1b* and b42-26 resistance sources, we began stacking them together with crosses beginning in 2011 (Figure 3). Analyzed using JMP12.1.0, the top and bottom of each green diamond represent the 95% confidence interval for each group while the horizontal size of the diamond is proportional to the sample size for the R and S *PdR1b* MAS groups. The center green line indicates the mean and the higher and lower parallel green lines are the overlap lines (here showing no overlap). The comparison circles at the right show the lack of intersection in the t-test results. Those progeny with the *PdR1b* markers (R) have significantly lower means than those without (S). Results are shown from two separate greenhouse trials of the same two crosses. Although the severity of the greenhouse screen was higher in the first trial, there was no genotype-test date interaction validating the relative resistances of the various genotypes analyzed in both screens and extending the ranking results to genotypes only tested in one of the trials. Most importantly however the stacking of the two resistance sources produce a number of exceptionally resistant individuals. The resistance solely from the b42-26 source can be seen in those genotypes without the *PdR1* marker (S) some genotypes of which manifest significant resistance as indicated by the lower dots above the S in both sides of Figure 3. In 2014, we employed this strategy to create 9 stacked lines totaling 314 individuals at the ~92% *vinifera* level all possessing *PdR1b*. These were planted in the vineyard in spring 2015 and a subset of 248 has just been propagated for greenhouse testing. The most resistant progeny identified by our greenhouse screen having resistance from both *PdR1* and b42-26 will be used to backcross in a final generation to pure *vinifera* and will result in cultivars at about the 96% *vinifera* level*.*

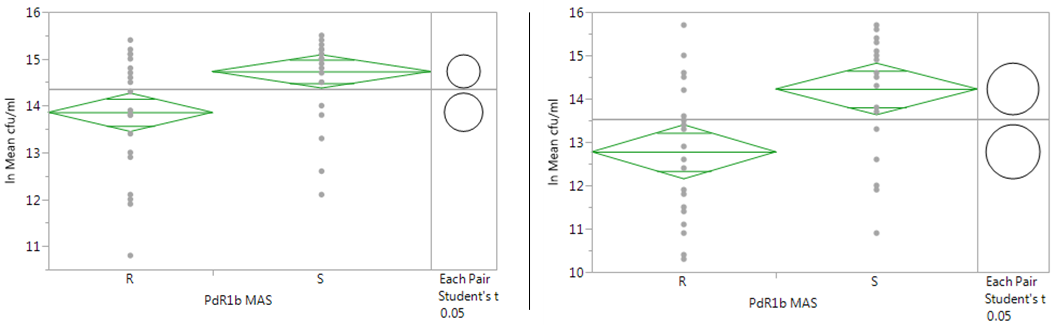


Figure 3. ELISA results for progeny ~86% *vinifera* of crosses between a *PdR1b* line and a b42-26 PDR line. The trial represented at left ended 2/28/13 (n=56 genotypes) while that at right ended 6/19/14 (n=44 genotypes).

In 2015, we made F1 crosses to various *V. caribaea* seedling selections: a cross to expand the b46-43 BC1 mapping population (F2-35 x 12305-55); and crosses to advance the SC36 to the BC1 level. An estimated 440, 550, and 375 seeds respectively are expected this Fall. The *V. caribaea* crosses were made to explore this resistance source from a novel region with high PD pressure. Our Greenhouse screening of a 2011 BC1 cross searching for minor PD genes in the b43-17 background absent *PdR1* revealed a single BC1 progeny that was highly resistant to PD. A BC2 cross made this year is anticipated to produce approximately 50 seeds.

Our other more extensive breeding efforts from 2015 are summarized in Table 4. Our two main PD breeding objectives in 2015 were to advance stacked *PdR1b*/b42-26 lines and stack PD resistance with one or more PM resistance sources. PD resistant cultivars with resistance to powdery mildew would greatly enhance the desirability of new wine grape varieties. Note that all resulting progeny in Table 4 are above 90% *vinifera*, have an elite *vinifera* cultivar in their backgrounds and advance the goal of creating highly resistant breeding lines stacked with multiple resistances to cross one last time to a final elite *vinifera* cultivar resulting in progeny between 96-98% *vinifera*. This year we took advantage of crosses made in recent years that had resulted in breeding parents homozygeous resistant at one or more resistance locus and that had previously stacked *PdR1b* and b42-26 PD resistance sources. Since all progeny of homozygeous crosses will carry that resistance source we only have to screen for the integrity of the cross and any other resistance sources heterozygous in the R-parent. Consequently more progeny will pass through MAS. The powdery mildew resistance sources in Table 4 include *Ren1* found on linkage group (LG) 13 in a number of pure *vinifera* cultivars from Central Asia; *Ren4* a strong and unique powdery mildew resistance locus on LG18 originally discovered in the Chinese species *V. romanetii*; and *Run1* a strong source on LG12 from *M. rotundifolia*.

Table 4. Estimated number seeds produced from PD crosses made in 2015. *PdR1b* (F8909-08) is from Monterrey *V. arizonica/candicans* PD resistance b43-17; b42-26 is Baja *V. arizonica/girdiana* PD resistance source. RR indicates one of the parents is homozygeous resistant at the referenced resistance locus.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Resistances** | **Recent *vinifera* parents in background** | **Progeny Percent *vinifera*** | | | | **Total Resist-ant** |
| **92%** | **93%** | **95%** | **96%** |
| 4a. PD - RR at *PdR1b* with b42-26 | Airen, Sauvignonasse, Marsanne, Valdiguie |  | 1225 |  |  | 1225 |
| 4b. PD - RR at *PdR1b* with b42-26. PM - *Ren4* | Alicante, Colombard, F2-35, Zinfandel |  |  | 1100 |  | 1100 |
| 4c. PD - *PdR1b* with b42-26. PM - *Ren4* with either *Ren1* or *Run1* | Cab Sauvignon, Carignane, Grenache, Petite Sirah, Zinfandel | 75 |  | 875 |  | 950 |
| 4d. PD - RR at *PdR1b*. PM -RR at Ren4 | Airen, Cab Sauvignon, Carignane, Grenache blanc, Touriga nacional, Valdiguie |  |  |  | 1599 | 1599 |
| 4e. PD - RR at *PdR1b*. PM - RR at *Ren4* with either *Ren1* or *Run1* | Cab Sauvignon, Carignane, Grenache, Petite Sirah, Zinfandel |  | 1250 |  | 1850 | 3100 |

Table 5 provides a list of the PD greenhouse screens analyzed, initiated and/or completed over the last year. We are making every effort in new lines to bring minor genes along with those for which we have markers. In group A we tested individuals from the *PdR1b* x b42-26 stacked line (results shown in Figure 2, right) as well as 25 PD rootstock genotypes from 4 crosses made in 2011 – advanced *PdR1b* rootstocks were crossed to Ramsey, 420A or Schwarzmann. Five of the progeny of the crosses to Ramsey are particularly promising. In group B and E we tested BC2 progeny in the b42-26 multigenic resistance source and identified two particularly promising genotypes to advance. New southwestern US species were tested in groups A, B, D, and E to facilitate PD resistance gene discovery work being done in our companion PD mapping project. As noted previously we have now tested 293 different accessions, the most resistant among them multiple times. In groups C and D we tested the 2013 southwest US F1 crosses (results shown in Table 2 under cross year 2013). BC2 progeny in the b40-14 line from crosses made in 2012 were also tested in group D. Along with the aforementioned subgroups in Group E we tested crosses and intercrosses in the Haines City and BD5-117 lines. We only identified one highly resistant genotype out of 13 genotypes tested and this is still at the 75% *vinifera* level. We initiated test group G to confirm previous results in the b40-14 line and to test multiple backcross levels in the same trial. In groups F and H we continued testing of *PdR1b* selections at the 97-98% *vinifera* level. The special focus of these trials was on white-fruited selections and those that descend from Nero d’Avola. Results from these and earlier screens have helped us decide on the selection of the most resistant genotypes to advance to field trials and to Foundation Plant Services for certification. Group H also includes the b42-26 intercross shown in Figure 2 as well as 2015 cross parents used for the crosses in Table 4. Disease resistance phenotype results from Group I were shown in Table 3 and group J was discussed in the context of the Figure 3 above.

Table 5. 2014-15 greenhouse screens

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Group** | **Test Groups** | **No. of Genotypes** | **Inoculation Date** | **ELISA Sample Date** | **PD Resistance Source(s)** |
| A | *PdR1b* x b42-26 stacked, new PD rootstocks, *PdR1a* advanced, SWUS species | 165 | 3/20/2014 | 6/19/2014 | F8909-08, *PdR1a*, b42-26, Ramsey, *V. species* |
| B | b42-26 BC2 & SWUS Species | 48 | 4/8/2014 | 7/8/2014 | b42-26, *V. species* |
| C | 2013 SWUS F1 Cross Seedlings | 192 | 4/24/2014 | 7/29/2014 | b41-13, b43-57, SC36, T03-16 |
| D | 88% b40-14, Additional 2013 SWUS Cross Seedlings | 145 | 5/15/2015 | 8/14/2014 | b40-14, b43-57, b47-32 |
| E | SEUS Xs, SWUS Species, *PdR1a*, b42-26 BC2 | 115 | 9/11/2014 | 12/11/2014 | b40-14, Haines City, BD5-117 |
| F | SWUS Species & Promising Genotypes | 229 | 10/2/2014 | 12/18/2014 | *V. species*, F8909-08, Ramsey |
| G | b40-14 F1, BC1, BC2; 2014 Cross Parents | 170 | 9/23/2014 | 1/8/2015 | F8909-08, b40-14, b42-26 |
| H | *PdR1b*, 2012 PD RS, 2013 PD stacked & PD x PM stacked | 117 | 3/17/2015 | 6/16/2015 | F8909-08, b42-26 |
| I | 2014 Cross SWUS BC1 Seedlings | 171 | 4/28/2015 | 7/28/2015 | b46-43, b40-29 |
| J | 2014 Cross SWUS F1 Seedlings | 268 | 6/4/2015 | 9/3/2015 | A14, A28, ANU67, ANU71, C23-94, DVIT 2236.2, SAZ 7 |

SWUS = southwest United States; SEUS = southeast US; BC = backcross; F1 = first generation

In our program we test selections with the potential for release multiple times using our greenhouse screen to ensure that only selections with the highest levels of resistance are considered for release. These selections have much better resistance than two selections with long histories of field survival in the southern US – Blanc du Bois and Lenoir. We want to avoid releasing selections that are tolerant to *X. fastidiosa* and therefore act as hosts for disease spread within a vineyard. This process involves passing our severe greenhouse screen multiple times. To make this list, selections must also possess desirable horticultural traits and have potential for high quality wine production. Producing small lot wines from multiple vine field trials in Davis and in PD hot spots around California complete the evaluation process. PD resistant scions need PD resistant rootstocks in case low levels of the bacteria work their way into a susceptible rootstock. Three such rootstock selections were sent to FPS in spring 2013 and another 25 genotypes were tested in 2C as discussed above.

In 2012 we conducted the first industry tastings of our advanced selection in Santa Rosa where we presented the best of our 87 and 94% *vinifera PdR1b* wines to about 200 people as part of a Sonoma County Wine Grape Growers Commission. The first tasting in 2013 was with select growers and wine makers in the Napa Valley at the UCD Oakville Station, a second was July 17th in Temecula to the winegrowers association, and a third in early August at Healdsburg (Dry Creek Valley and Sonoma Grape Growers and Winemakers). These wines were also presented at the Napa Grape Expo on November 14th. In various venues over the last three years we have presented wines made from our favorite red and white 94% *vinifera PdR1b* selections in a format where they were blended with Chardonnay or Merlot or Cabernet Sauvignon respectively to show how these wines would be used as blending grapes to fill in chronic PD hotspots and still stay within the 75/25% varietal wine labeling requirements. In the 2012-2014 vintages we have presented wines made from our advanced 97% *vinifera PdR1b* selections. These wines were well received and many have liked the 97% wines the best. The next step is to make larger scale wines in multi-ton lots and these vines are being planted as noted above. We have one selection that has cleared FPS testing and is working its way through the UC patent process. As others selection clear FPS testing the best selections will be ready for release.

**CONCLUSIONS**

We continue to make rapid progress breeding PD resistant winegrapes through aggressive vine training, MAS and our rapid greenhouse screen procedures. These practices have allowed us to produce four backcross generations with elite *V. vinifera* wine grape cultivars in 10 years. We have screened through thousands of seedlings that are 97% *vinifera* with the *PdR1* resistance gene from *V. arizonica* b43-17. Seedlings from these crosses continue to crop and others are advanced to greenhouse testing. We select for fruit and vine quality and then move the best to greenhouse testing, where only those with the highest resistance to *Xf*, after multiple greenhouse tests, are advanced to multi-vine wine testing at Davis and in PD hot spots around California. The best of these are advanced to 100 vine commercial scale testing with the first selection planted this year. We have sent 15 advanced scion selections to FPS over the past three winters to move through the certification and release process. Three PD resistant rootstocks were also sent to FPS for certification. Other forms of *V. arizonica* and other southwest US species are being studied and the resistance of some will be genetically mapped for future efforts to combine multiple resistance sources and ensure durable resistance. Stacking of *PdR1b* with b42-26 PD resistance has been advanced to the 92% *vinifera* level by combining MAS to confirm the presence of *PdR1b* and greenhouse screening to verify higher than usual levels of PD resistance. PD resistance from *V. shuttleworthii* and BD5-117 are also being pursued, but progress and effort is limited because their resistance is controlled by multiple genes without resistance markers. Very small-scale wines from 94% and 97% *vinifera* *PdR1b* selections have been very good, and have been received well at tastings in the campus winery and at pubic tastings throughout California.

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**Talks at Grower Meetings (Extension/Outreach)**

Advances in scion and rootstock breeding. Coppola Grower Meeting, Healdsburg, CA, July 17, 2012.

Advances in rootstock and scion breeding. Roll Global Meeting, UC Davis, August 16, 2012.

Breeding new rootstocks and fruiting varieties. VEN 290 Seminar, UC Davis, November 2, 2012.

UC Davis grape breeding program. INIA Seminar, Santiago, Chile, December 13, 2012.

Walker grape breeding program. Napa Valley Grape Growers, UC Davis, February 8, 2013.

Grape breeding. Napa Valley Vintners. UC Davis, Feb. 27, 2013.

The vineyard of the future. Wine Executive Program, UC Davis, March 28, 2013.

Breeding PD resistant wine grapes (including new PD rootstocks). Santa Rosa Winegrape Association Meeting, Santa Rosa, CA, April 5, 2013.

Sustainable Viticulture. Haas Business School, DNV Top Tech Program, Mondavi Winery, Oakville, CA, April 20, 2013

Pest and disease threats: Decisions and the future of farming. Napa Valley 2030 – Ahead of the Curve, Napa Valley Grape Growers, Napa, May 7, 2013

Walker grape breeding program. Chilean Winegrowers Meeting, UC Davis, May 7, 2013

Grape Improvement: breeding, genetics, genomics, ‘omics’. International Table Grape Symposium, Ica, Peru June 19, 2013.

Marker-assisted selection to optimize grape breeding. Grape Genetics Research Coordination Network. UC Davis, July 11, 2013.

PD resistant wine tasting. Temecula Wine Association, Temecula, CA, July 17, 2013.

PD resistant wine tasting. Healdsburg/Dry Creek Growers and Wineries, Clos du Bois, Healdsburg, CA, July 31, 2013.

Walker lab grape breeding projects. North American Grape Breeder’s Meeting, Fayetteville, AR, Aug14, 2013

Breeding PD resistant winegrapes and tasting. Napa Valley Wine Expo, November 13, 2013

Breeding PD resistant winegrapes and tasting. Martini / Gallo Winemakers, UC Davis, November 14, 2013

PD resistant winegrapes nearing release. PD/GWSS Board Annual Meeting, Sacramento, CA December 13, 2013

PD resistant winegrapes coming soon. Current Wine and Winegrape Research Conference / Unified Grant Management, UC Davis, February 12. 2014

Breeding resistant grapes. Diageo Central Coast Growers Meeting, Asilomar, CA, July 24, 2014.

Industry show and tell tasting of PD resistant selections at the UCD vineyard. August 28, 2014

Walker grape breeding program. Department Seminar October 3, 2014

Not only do they resist PD, they look like winegrapes, taste like winegrapes, make very good wines, and they are getting ready for release” FPS Annual Meeting November 20, 2014

Not only do they resist PD, they look like winegrapes, taste like winegrapes, make very good wines, and they are getting ready for release”, Current Issues in Vineyard Health, UC Davis, December 2, 2014

Walker breeding program. Gallo Lab Tech Team, UC Davis, December 5, 2014

PD resistant winegrapes nearing release. PD/GWSS Annual Meeting, Sacramento, CA December 16, 2014

Molecular genetics ready to launch a golden age of winegrape breeding. The Conversation. January 7, 2015 https://theconversation.com/molecular-genetics-ready-to-launch-a-golden-age-of-winegrape-breeding-35464

Walker grape breeding program. Flash talk for NGWI, UC Davis, January 26, 2015

Releasing PD resistant winegrapes. Viticulture and Enology Research Conference, UC /Davis February 9

Breeding for powdery mildew resistance using lessons from PD breeding. Napa Valley Grape Growers, March 4, 2015

Tasting of 2014 Vintage PD resistant wines, UC Davis, March 12, 2015

Breeding PD and powdery mildew resistant grapes. Diageo Winemaking Team, UC Davis, April 23, 2015

Breeding new wine varieties ... PD, PM and beyond. PD wine tasting included. Constellation Winery Meeting, Napa, CA April 7, 2015

A Look to the Future. What's in Store for California Vineyards? Anderson Valley Wine Technical Conference, Philo, CA May 15, 2015

Breeding new wine varieties ... PD, PM and beyond. PD wine tasting included. Daniel Roberts Clients Meeting, Santa Rosa, July 10, 2015

Breeding new wine varieties ... PD, PM and beyond. Sonoma County Winegrape Commission, Forestville, CA July 31, 2015

Progress in Developing PD Resistant Winegrapes — Tasting Small Scale Wines. California Alliance of Family Farms, Ramona Valley Winegrape Growers, Valley Center, CA August 7, 2015

**Papers and Presentations at Scientific Meetings**

Walker M.A., Riaz S., Agüero C., Bistue C. 2012 Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*). Poster presentation Pierce’s Disease Research Symposium. Sacramento, 12/13-15/2012

Grape Improvement: breeding, genetics, genomics, ‘omics’. International Table Grape Symposium, Ica, Peru June 19, 2013.

Riaz, S. and Walker, M.A. 2013. Using marker-assisted selection to optimize grape breeding. Grape Research Coordination Network Meeting, UC Davis, Davis, CA, July 11, 2013.

Walker, A. 2013. Walker lab grape breeding. North American Grape Breeders Meeting, Fayetteville, AR, August 15, 2013.

Walker, M.A. 2013. Grape breeding at UC Davis, Seminar at Missouri State University, Springfield, MO, August 10, 2103.

Walker, M.A. 2013. Optimizing grape improvement with molecular tools. University of Missouri, Colombia, MO, August 11, 2013.

Grape breeding at UC Davis. Viticulture and Enology Seminar, UC Davis, Oct. 4, 2013.

Agüero, C., Bistué, C., Riaz, S. and Walker, A. 2013. Molecular characterization of the putative *Xylella fastidiosa* resistance gene(s) from b43-17 (*V. arizonica/candicans*). 2013. Pierce’s Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013. <http://www.cdfa.ca.gov/pdcp/Research.html>

Walker, A., A.C. Tenscher and S. Riaz. 2013. Breeding Pierce’s disease resistant winegrapes. Pierce’s Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013.

Riaz, S., R Hu and M.A. Walker. 2013. Genetic mapping of *Xylella fastidiosa* resistance gene(s) in grape germplasm from the southern United States. Pierce’s Disease Research Progress Reports. Sacramento, CA, December 16-18, 2013.

Walker, A. 2014. Disease resistance in perennial crops: classical and molecular approaches using grape as an example. International Plant Breeding and Genetics Conference, Campos dos Goytacazes, November 4, 2014

Walker, A. 2014. PD resistant winegrapes nearing release. Pierce’s Disease Research Symposium, Sacramento, CA, December 18.

Walker, A. 2015. Molecular genetics ready to launch a golden age of winegrape breeding. The Conversation. January 7, 2015. (<https://theconversation.com/molecular-genetics-ready-to-launch-a-golden-age-of-winegrape-breeding-35464>)

**Abstracts**

Bistue C., Agüero C.B., Riaz S., and Walker M.A. 2013. Testing *Vitis arizonica* candidate genes for Pierce’s disease resistance in *Nicotiana tobacum* /SR-1. ASEV 64th National Conference. Monterey, California.

Riaz, S., Tenscher, A., and Walker, M.A. 2013. Phylogeographic analysis of resistance to Pierce’s disease in North American and Mexican species with SSR markers and identification of novel resistance sources. ASEV 64th National Conference. Monterey, California.

Agüero, C. B., S. Riaz, A. Tenscher, X. Xie and M. A. Walker. 2014. Functional analysis of Pierce’s disease resistance genes from *Vitis arizonica*. 65th ASEV National Meeting, Austin, TX

Riaz, S., R. Hu, A. Tenscher and M. A. Walker. 2014. Comparative sequence analysis of the Pierce’s disease resistance locus *PdR1.* 65th ASEV National Meeting, Austin, TX

Walker, A. , A. Tenscher and S. Riaz. 2014. Breeding Pierce’s disease resistant winegrapes. Proceedings of the 2014 Pierce’s Disease Research Symposium, Sacramento, Dec. 15-17 pp 220- 226

Walker, A., S. Riaz, and C. Agüero. 2014. Map-based identification and positional cloning of *Xylella fastidiosa* resistance genes from known sources of Pierce’s disease resistance in grape. Proceedings of the 2014 Pierce’s Disease Research Symposium, Sacramento, Dec. 15-17 pp 227- 239.

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**Publication**

Sun, Q., Y. Sun, M.A. Walker and J.M. Labavitch. 2013. Vascular occlusions in grapevines with Pierce’s disease make disease symptom development worse. Plant Physiology 161:1529-1541.