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ORIGINAL PAPER

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Identification and molecular mapping of *PdR1*, a primary resistance gene to Pierce's disease in *Vitis*

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Abstract A major quantitative trait locus (QTL) controlling resistance to Pierce's disease (PD) of grape, caused by the bacterium *Xylella fastidiosa* (Xf), was identified on a *Vitis* linkage map and denoted as 'Pierce's disease resistance 1' (*PdR1*). Placement of the locus was accomplished by evaluating a family of full-sib progeny from a cross of two PD-resistant interspecific hybrids with resistance inherited from *Vitis arizonica*. Resistance was measured under greenhouse conditions by direct quantification of Xf numbers in stem tissues as well as by evaluation of disease symptoms based on leaf scorch and a cane maturation index (CMI). A large QTL (LOD 17.2) accounting for 72% of the phenotypic variance in bacterial numbers was localized to linkage group 14 of the male parent F8909-17. The approximate 95% confidence interval around the QTL peak extended 5.7 cM when using composite interval mapping. The other disease evaluation methods (leaf scorch and CMI, respectively) placed the resistance QTL to the same region on linkage group 14, although at wider 95% confidence intervals (6.0 and 7.5 cM), lower peak LOD scores (11.9 and 7.7) and accounting for less phenotypic variance (59 and 42%). This is the first report of an Xf resistance QTL mapped in any crop species. The relevance of the markers located in the region spanning the QTL will be discussed, addressing their usefulness for the development of PD-resistant grape cultivars.

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Introduction

Pierce's disease (PD) of grapevines, caused by the bacterial pathogen *Xylella fastidiosa* (Xf), is a severe problem for grape cultivation throughout the southern areas of North America (Hewitt 1958; Loomis 1958; Perry et al. 1974; Goheen et al. 1979; Jimenez 1985; Halbrooks and Mortensen 1989). The European bunch grape *Vitis vinifera*, from which the majority of wine, table grape and raisin production is derived, is highly susceptible to PD, and its cultivars succumb to the disease within 1–5 years after infection.

Over a dozen American *Vitis* species are native to the regions where PD is endemic, and resistant genotypes from these wild species have been utilized in the parentage of many grape cultivars grown in the southeastern United States (Mortensen et al. 1977; Overcash et al. 1981; Mortensen 1988; Halbrooks and Mortensen 1989). Although different strains of Xf have been identified and defined by the species and agricultural crops that they differentially infect (Hopkins and Purcell 2002), there is no published evidence of grape-specific strains that can infect some resistant cultivars but not others, indicating that resistance in these American *Vitis* species sources appears to be relatively durable. Although there are readily available and reliable sources of resistance, the acreage planted with these PD-resistant *V. vinifera* × *Vitis* spp. hybrids has remained relatively small and their acceptance has been limited by their reduced fruit quality in comparison to pure *V. vinifera*. The development of high-quality PD-resistant cultivars will be facilitated by increased understanding of the genes controlling resistance, allowing a more precise introgression of the trait into domesticated backgrounds.

Two studies have focused on the genetics of resistance to PD. In the first study, resistance derived from *V. aestivalis* ssp. *smalliana*, *V. simpsonii* and *V. shuttleworthii* was evaluated under field conditions and native disease pressure (Mortensen 1968). Leaf scorch symptoms, plant vigor and longevity measured over a 5-year

period determined whether a genotype was resistant or susceptible. Segregation ratios of progeny derived from controlled crosses were used to analyze the inheritance of PD resistance. Mortensen concluded that resistance was dominant to susceptibility and suggested that complementary gene action among three independent genes could best explain the results.

In a second study, we investigated the inheritance of PD resistance from the southwestern species *V. arizonica* by evaluating a 4×4 factorial mating design under greenhouse conditions (Krivanek et al. 2005b). Tests of simple Mendelian segregation ratios and complex segregation analysis (CSA) strongly indicated the existence of a major gene with a dominant allele controlling the PD resistance inherited from a *V. arizonica*/*V. candicans* genotype collected near Monterrey in north-central Mexico. Another source of resistance, inherited from a *V. arizonica* accession from Baja California, did not fully fit the single gene/dominant allele model and results suggested that this resistance was potentially inherited in a polygenic manner.

The objective of this investigation was to conduct a quantitative trait locus (QTL) analysis to place the *V. arizonica*/*V. candicans* 'Monterrey' major resistance locus on a molecular linkage map. In addition, mapping of the quantitative loci from the *V. arizonica* 'Baja California' and potential minor loci inherited from *V. arizonica*/*V. candicans* 'Monterrey' was also attempted. Heritability estimates for three diseases resistance evaluations were calculated and compared. Our research fits within the broader objective of discovering resistance-linked markers for use in a marker-assisted breeding program for accelerating the development of high-quality PD-resistant wine, table and raisin cultivars.

Materials and methods

Plant material

The female parent of the mapping family D8909-15 is a PD-resistant selection from a cross of *V. rupestris* 'A. de Serres' × b42-26 (a *V. arizonica* from Baja California, Mexico) (Krivanek et al. 2005a, b; Krivanek and Walker 2005). The pollen parent F8909-17 is a PD-resistant selection from a cross of *V. rupestris* 'A. de Serres' × b43-17 (a *V. arizonica*/*V. candicans* genotype from Monterrey, Mexico) with resistance equivalent to that of D8909-15. The grandparent 'A. de Serres' is highly susceptible to PD. The progeny derived from D8909-15 × F8909-17 was designated as the 9621 family consisting of about 200 individuals, from which a subset of 116 progeny was previously used to construct a linkage map of *Vitis* (Doucleff et al. 2004). Of these progeny, 73 were still available for further disease evaluation while the reminder were not strong enough for propagation or had died in the field (which was under no PD pressure). This study used a second randomly selected subset of 137 genotypes from the 9621 family, including the 73 progeny that had already been genotyped. Segregation and heritability analyses of PD resistance utilized all 137 genotypes, whereas the QTL analysis was performed on 73 progeny for which both marker data and PD resistance information were available.

The genotypes of the 9621 family along with the susceptible grandparent 'A. de Serres' were propagated as herbaceous cuttings from grapevines in the University of California, Davis, CA, vineyards and prepared for inoculation as outlined previously (Krivanek et al. 2005a). Briefly, rooted plants were grown for 6 weeks, pruned to the basal two buds and allowed to re-grow so as to ensure uniform shoot development. Actively growing plants were watered twice per day for 5 min using 1.9 l/h emitters and fertilized with a 25% Hoagland's solution (Sigma-Aldrich Ltd, St. Louis, MO) via the drip system. Once the main shoot reached 60 cm, lateral and apical shoot tips were removed every 3 weeks on average to facilitate light penetration and air circulation.

Experimental design

Plants were Xf inoculated and the number of genotypes with four, three and two inoculated replicates was 116, 15 and 6 genotypes, respectively. The individual potted plants of each genotype served as the experimental unit and they were distributed randomly on greenhouse benches in a randomized complete block design. Blocks were chosen based on the greenhouse bench position and, in the case of bacteria quantification, on sample runs. Each block included a water-inoculated negative control. Due to the unequal number of replications, the statistical design was treated as unbalanced.

Disease evaluation

The 'Stags Leap' isolate of Xf was prepared and plants were inoculated, as previously described (Krivanek et al. 2005a), 6 weeks after the plants were cut back. Control plants were inoculated with ddH_2O in a similar manner. Three methods of quantitatively measuring the extent of disease progression were employed at 16 weeks post-inoculation. The mean percentage area of scorch on four leaves above and nearest to the point of inoculation (POI) was recorded. The degree of cane maturation and necrosis development, designated as the cane maturation index (CMI; 0–6 scale), was also measured as previously described (Krivanek et al. 2005a). The CMI scores were evaluated on the stem from the POI to 40 cm above the POI. Finally, Xf populations were measured with a previously described, quantitative ELISA procedure (Krivanek and Walker 2005). Absorbance readings were measured from extracts of stem tissue samples taken 10 cm above the POI and the values were converted to

cells/ml concentrations using a standard calibration curve derived from a dilution series of bacteria added to healthy stem extract and included on each microtiter plate. All predicted values were reported as cells/ml; however, since the buffer volume to sample weight ratio was 10:1, the cells/ml concentrations equate to one-tenth the cells/g of sample.

Statistical analyses of disease resistance data

The bacteria number, CMI and leaf scorch datasets were evaluated using the statistical procedure MIXED of the software package SAS version 8 (SAS Institute, Cary, NC, USA), treating the variables genotype and block of the experimental design as fixed factors. Due to the unbalanced nature of the design the Satterwaith method was used for estimating degrees of freedom for all analyses. In order to evaluate the quality of the disease resistance data for statistical analysis, the residuals (or data remaining after removal of this model) were assessed for the assumption of normal distribution and homogeneous variances. First, residuals were analyzed for homogeneous variances by running the ANOVA procedure with genotype as the sole class factor and selecting Brown and Forsythe with the 'hovtest' option. For visual evaluation, residuals were plotted with the GPLOT procedure. To evaluate the residuals for normal distribution the MIXED procedure was run with genotype and block as fixed factors, creating an output file of the residuals from the model and then using the Shapiro–Wilk test from the 'normal' option within the UNIVARIATE procedure. Visual assessment of residual distribution was also assessed using the 'histogram' and 'QQplot' options. After running these quality tests, bacteria numbers were natural log transformed and outlying data points from the datasets removed. Finally, as the data were unbalanced, block-adjusted means for each genotype were calculated using the 'lsmeans' option of the MIXED procedure.

Prior to estimating variance components, which are required for heritability calculations, the negative controls and susceptible control were removed from each of the data sets in order to base the estimates strictly on the 9621 genotypes. Estimates were made under a random model in the MIXED procedure by treating genotype and block as random factors. The 'covtest' option of the MIXED procedure was used to report the genetic variance (σ_g^2) and experimental error variance (σ_e^2) and respective error terms as listed under the 'Covariance Parameter Estimates'.

Broad-sense heritability (H^2) estimates were calculated on a single-plant basis via the equation: $H^2_{(\text{plant basis})} = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$ (Fehr 1991) and on a genotype mean (entry mean) basis via the equation: $H^2_{(\text{mean basis})} = \sigma_g^2/(\sigma_g^2 + (\sigma_e^2/r))$ (Hallauer and Miranda 1988; Fehr 1991), where the term r refers to the average number of replicates for each genotype in the unbalanced design. Approximate standard errors of the H^2 estimates were calculated using $\text{S.E.}(H^2_{(\text{plant basis})}) = \text{S.E.}(\sigma_g^2)/(\sigma_g^2 + \sigma_e^2)$ and $\text{S.E.}(H_{(\text{mean basis})}{}^2) = \text{S.E.}(\sigma_g^2)/(\sigma_g^2 + (\sigma_e^2/r))$, respectively, where the term $\text{S.E.}(\sigma_g^2)$ refers to the square root of the variance of the genetic variance estimate (Hallauer and Miranda 1988; Nyquist 1991).

QTL analysis

A reference genetic map was constructed based on 116 individuals using only the framework markers segregating in a 1:1 manner in a previously published map (Doucleff et al. 2004). A total of 105 markers from the female map and 111 markers from the male map were selected. The original parental framework maps, constructed with an average confidence level of 93 and 96%, had map lengths of 756 and 1,082 cM and mean marker spacing of 8.7 and 11.7 cM for the female and male maps, respectively. No gaps between markers of larger than 30 cM exist on the female map. On the male map, three gaps of larger than 30 cM were present. After the first QTL analysis run, three additional simple sequence repeat (SSR) markers, VMC6e1, VMC5b3 and VMC6c10, were added to the linkage map. Those markers were developed by the Vitis Microsatellite Consortium, and linkage information from an additional map (Riaz et al. 2004) indicated that they were localized on the same linkage group where a significant QTL was identified. Segregation data for these markers were obtained in the same manner as the original markers (Doucleff et al. 2004) for a subset of 87 progeny. Marker order and map distances were calculated using the two-way pseudo-test cross strategy (Grattapaglia and Sederoff 1994) in concert with the computer program JoinMap 3.0 (Van Ooijen and Voorrips 2001) using the Kosambi function.

Initial QTL analyses were performed using the interval mapping option (Lander and Botstein 1989) within the computer package MAPQTL 4.0 (Van Ooijen et al. 2000). The quantitative measurements of PD resistance: leaf scorch, CMI scores and stem Xf populations based on the SAS calculated lsmeans for 73 individuals were used for three separate analyses. The criterion for detecting a significant QTL was based on a logarithm of the odds ratio (LOD) threshold of 3.0 (Van Ooijen 1999).

Upon identification of a region with a significant QTL effect, further analysis was conducted using composite interval mapping with the 'MQM' option within MAPQTL. The marker located closest to the peak of the major QTL in each analysis was used as a cofactor in order to localize potential QTL elsewhere in the genome and to more precisely define the position of the QTL identified as significant with interval mapping (Zeng 1994). Confidence intervals for the location of significant QTL were estimated at the 95% level by identifying boundaries to either side of the composite interval mapped QTL at which the LOD score was 2 less than the peak (Lander and Botstein 1989).

Results

Statistical analyses of PD resistance data

To achieve homogeneous variances and normally distributed residuals in the ELISA data set, the cells/ml concentrations were natural log transformed and 13 outliers out of 563 data points (2.3%) were removed. From the CMI dataset, 12 outliers out of 563 data points (2.1%) were removed. No outliers were identified in the leaf scorch data set. The Brown and Forsythe (BF) variance homogeneity test resulted in P values of: $P = 0.34$, $P < 0.01$ and $P = 0.07$ for the leaf scorch, CMI and Xf population data sets, respectively. The significant test on the CMI data set was due to the high proportion of genotypes (45) with scores of 0, without which the BF test was not significant ($P = 0.08$). The Shapiro–Wilk test was run for normally distributed residuals and the resulting W statistic for leaf scorch and the Xf population data sets were not significant ($W = 1.0$, $P = 0.40$ and $W = 0.99$, $P = 0.09$, respectively), indicating normally distributed residuals. While the W statistic for the CMI residuals was significant ($P < 0.01$), visual assessment of the distribution and the high W statistic itself ($W = 0.97$) showed no dramatic deviation from normality. The effects of genotype and block when treated as fixed factors were found to have highly significant ($P < 0.0001$) effects on Xf bacteria numbers, CMI and leaf scorch scores.

Broad-sense heritability, a direct measure of environmental effects on phenotypic variance, was calculated for PD resistance using 137 genotypes and estimated individually for each of the three PD evaluation methods. On a genotype mean basis, the estimates were relatively high ranging from 0.80 to 0.95, with the Xf population measurements producing the highest heritability (Table 1). Broad-sense heritability on a single-plant basis was considerably lower for each of the evaluation methods, ranging from 0.51 to 0.83 (Table 1).

Frequency distributions for the 137 genotype subset of the 9621 family for each disease evaluation method are presented in Fig. 1a–c. The frequency distributions of the subset used for QTL analysis are also included in the respective figures. For reference, the susceptible grandparent 'A. de Serres' was evaluated in this study and had values of 13.3 natural log (cells/ml), 6.0 and 100% for mean stem Xf populations, CMI and leaf scorch scores, respectively. The resistant female parent D8909-15 was evaluated previously for CMI and leaf scorch (Krivanek et al. 2005a) and for stem bacteria numbers (Krivanek and Walker 2005) under similar screening conditions and had scores of 10.2, 0.4 and 34%, for respective disease evaluations. Respective scores for the resistant male parent F8909-17 in a separate unpublished study were 10.1, 0.0 and 60%. The segregation pattern of the 9621 progeny (Fig. 1) with distinctive bimodal or trimodal patterns of distribution, skewing towards resistance relative to that expected with a normal distribution and presence of susceptible genotypes in a family derived from a resistant by resistant cross suggest that the resistance is controlled by a dominant allele at a major locus with some phenotype modification due to environmental or minor gene effects.

Table 1 Broad-sense heritability (H^2) of PD resistance and respective standard errors based on three evaluation methods

Disease evaluation method	H^2 genotype mean basis	H^2 single-plant basis
Xf populations	0.95 ± 0.12	0.83 ± 0.11
Cane maturation index	0.90 ± 0.12	0.72 ± 0.10
Leaf scorch	0.80 ± 0.12	0.51 ± 0.08

QTL analysis

A significant QTL controlling PD resistance was identified at the middle-upper end of linkage group 14 of the male parent F8909-17. The linkage group was denoted according to the reference map of the International Grape Genome Program (Riaz et al. 2004). Analysis based on stem bacteria numbers, leaf scorch and CMI scores gave maximum LOD scores for the QTL of 17.2, 11.9 and 7.7 and markers at the peak of the QTL explained 72, 59 and 42% of the total phenotypic variance for the three disease evaluation methods, respectively.

This QTL localized at overlapping locations when using any of the three disease evaluation methods and in each case placed between the SSR markers VMC6e1 and VMC5b3 (Fig. 2). SSR marker order was consistent with that reported by Riaz et al. (2004). Using interval mapping, approximate 95% confidence intervals for the QTL extended 10.5, 11.0 and 21.5 cM, respectively, for the three disease evaluation methods, around the peak of the QTL. The marker closest to the peak was chosen as a cofactor and further evaluation with composite interval mapping narrowed the confidence interval to 5.7, 6.0 and 7.5 cM, respectively (Fig. 2). Further analysis with composite interval mapping gave no clear indication of there being any other QTL, and therefore the locus at this position was denoted as 'Pierce's disease resistance 1' (*PdR1*).

Other than the *PdR*1 locus on linkage group 14, no additional loci with significant LOD scores could be identified within the male parent linkage map. In addition, no significant QTL was identified on linkage group 14 of the female parent D8909-15 or on any other linkage group of the female genome when using any of the three disease evaluation methods.

Discussion

We have placed a major Pierce's disease resistance locus, which accounts for 72% of the total phenotypic variance as measured by bacterial quantification, to a specific region on a *Vitis* genetic linkage map. The locus was designated *PdR1*, and to our knowledge this

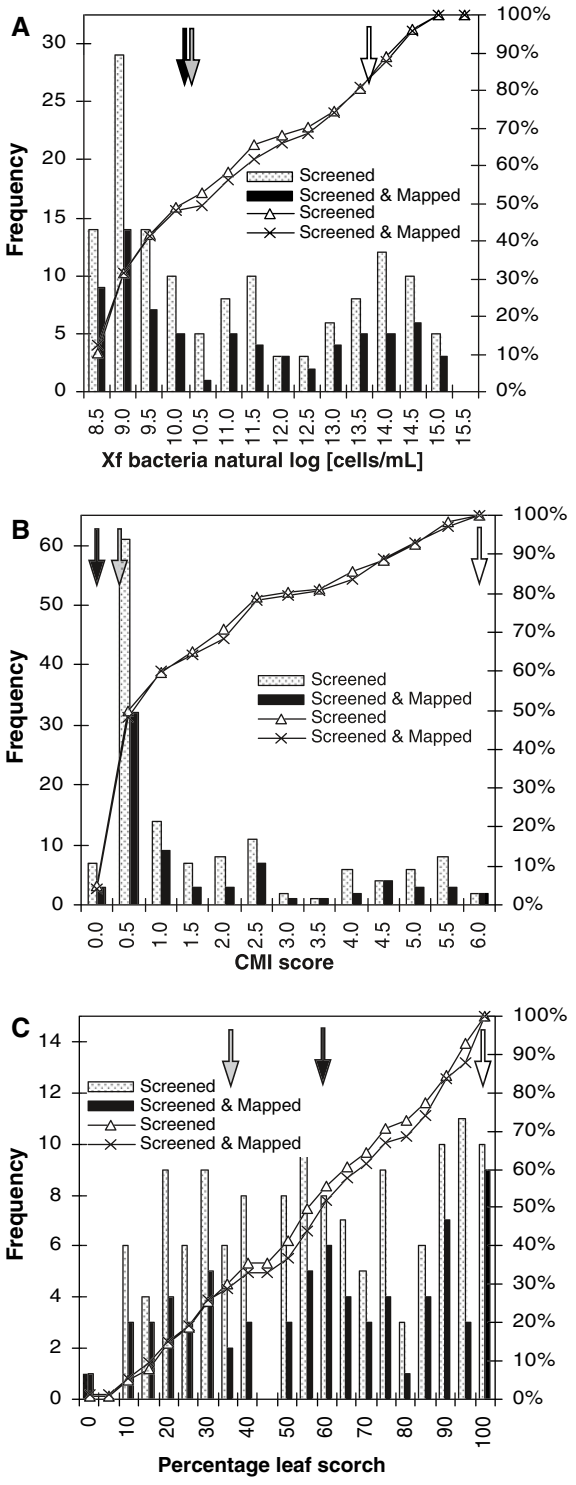

Fig. 1 Frequency distributions based on least squares adjusted means of: Xf populations in stem tissue extract as determined by ELISA (**a**), cane maturation index (CMI) scores (**b**) and leaf scorch scores (**c**) for the 9621 mapping family. *Hatched bars* indicate the 137 genotypes screened in the greenhouse evaluation, *solid black bars* indicate a subset of 73 genotypes utilized in the QTL analysis. The *open arrow* indicates mean scores for the susceptible grandparent as evaluated in this study. The *black arrow* indicates mean scores for the male parent F8909-17 and the *gray arrow* indicates mean scores for the female parent D9009-15, both as measured in previous studies. *Plotted line* is the cumulative percentage (*right vertical axis*) of the genotypes in the family

is the first report of markers linked to an Xf resistance gene in any crop species. These results are in agreement with our previous study of the inheritance of PD resistance within a related genetic population (Krivanek et al. 2005b), which concluded that resistance-derived *V. arizonica*/*V. candicans* genotypes collected from Monterrey, Mexico, is controlled by a dominant allele at a major locus. The dominance of the resistance to susceptibility is also in agreement with the study focused on *V. aestivalis* and *V. shuttleworthii* populations (Mortensen 1968). The genetic model proposed by Mortensen to explain PD resistance is, however, different from our *V. arizonica*/*V. candicans* 'Monterrey' resistance. The disparity is likely reflective of different PD resistance mechanisms or genetic systems across distinct *Vitis* species. Indeed, the *Vitis* species studied by Mortensen are phenotypically different and geographically isolated from *V. arizonica*.

The use of ELISA in this study for quantification of Xf numbers in stem tissues proved to be an effective tool. The method was more powerful at mapping PD resistance than were leaf scorch or CMI scores as judged by an increase in the LOD measurement, smaller 95% confidence interval and greater percentage of the phenotypic variance explained when bacterial quantification was compared with the other two methods. ELISA has been effectively utilized in other studies for quantitative measurements of fungal, bacteria and virus populations (Adlerlieste and Van Eeuwijk 1992; Ikuta et al. 2000; Meyer et al. 2000) and in one study the technique was used to assess virus accumulation in plant tissues in order to map a QTL responsible for virus resistance in rice (Albar et al. 1998). We have also utilized ELISA to accurately distinguish previously characterized genotypes of variable field resistance (Krivanek and Walker 2005).

The use of clonal replication for mean disease evaluation measurements allowed for environmental and genetic effects to be distinguished and effectively raised the broad-sense heritability of resistance relative to the estimate based on single plants when using any of the three evaluation methods. Broad-sense heritability estimates based on genotype means of Xf populations in stem tissue were estimated at 0.95 in this study, indicating that approximately 5% of the total phenotypic variance was due to environmental variation. An additional 72% of the phenotypic variation was accounted for by the effects of the *PdR1* locus. Therefore an approximate estimation of the effects of minor secondary or modifying genes is estimated at 23%.

The inability to detect any major QTL in the female parental genotype D8909-15 in this study or to fit the inheritance of resistance to a single gene/dominant allele model in a previous study (Krivanek et al. 2005b) leads us to hypothesize that resistance in the female parent (as inherited from the Baja California *V. arizonica* b42-26) is controlled by minor genes in a genetic model distinctive from the *V. arizonica*/*V. candicans* 'Monterrey' resistance source.

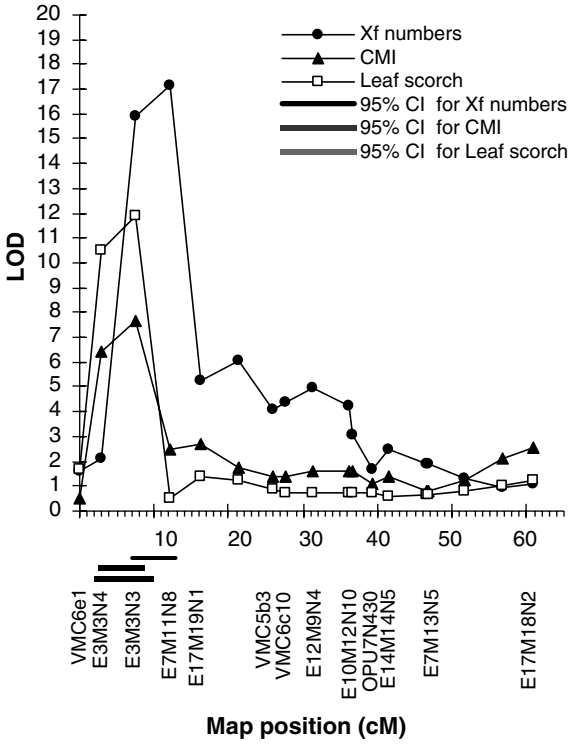

Fig. 2 Placement of the quantitative trait locus designated Pierce's disease resistance 1 (*PdR1*) on linkage group 14 of the male parent F8909-17. The locus profiles are based on the logarithm of the odds ratio (LOD) scores determined via composite interval mapping using three independent disease evaluation methods: Xf bacteria numbers in stem tissue, cane maturation index (CMI) scores and leaf scorch percentages. Map positions are given in centimorgans (cM). The *bars* indicate approximate 95% confidence intervals (CI)

The inability to detect minor resistance loci in either parent in this study may be due to various possibilities. First, the modest family size utilized for the analysis would inhibit detection of minor resistance QTL and in future studies a larger family should prove more useful in the detection of the smaller effect QTL. Another potential reason for the lack of detection of other loci with significant effects on PD resistance may be due to gaps within the genetic linkage maps utilized in the QTL analysis. Three gaps of larger than 30 cM are present in the male map and while no distances between markers larger than 30 cM exist on the female map, three gaps of greater than 20 cM exist. If an important QTL existed within these areas, such distances would potentially effect their detection, particularly when using a small mapping family. In future studies, increased marker density should mitigate such potential deficiencies for QTL detection.

Finally, the inability to detect minor resistance QTL may be due to the low information content of loci-controlling resistance given the dominance and segregation of resistance in both parents. The dominant nature of markers (or loci-controlling traits of interest) is not a problem under the pseudo-test cross strategy when only one parent is heterozygous, and it is similar to a backcross scenario where markers in progeny (whether dominant or co-dominant) are fully informative with respect to meiosis in the heterozygous parent (Mather 1951). The problem arises when a dominant locus or marker is heterozygous in both parents. This mapping situation is similar to that under an F2 scenario whereby progeny have a maximum information content of only 25% with respect to meiosis in either heterozygous parent (Mather 1951). Three of the four resulting F2 genotypes with the dominant allele will have the same phenotype and these progeny are useless for identifying meiotic recombinations. Progeny homozygous for the null or susceptible allele are useful for mapping, but this occurs in only one quarter of the family, thus explaining the reduced information content. In future evaluations of mapping families derived from a resistant by resistant cross, a more extensive or sole use of co-dominant markers would be advisable.

Molecular markers identified in the region surrounding the QTL peak of *PdR1* will be validated by a bulk segregant analysis and incorporated into an active grape-breeding program held at UC Davis. Backcross introgression via molecular markers to more precisely introgress resistance alleles into more agriculturally acceptable backgrounds has been accomplished successfully in other crops (Young and Tanksley 1989) by effectively breaking linkage drag or negative associations of undesirable traits to the trait of interest. The same strategy may not be directly employed in a species such as *Vitis* in which the cultivars are highly heterozygous and often susceptible to inbreeding depression. However, the technique should be very effective at precisely introducing the *PdR1* resistance allele into a quality *V. vinifera* background by backcrossing at each generation to different high-quality *V. vinifera* cultivars. Molecular markers linked to the resistance allele will also serve to preferentially remove susceptible genotypes from breeding populations so that larger numbers of resistant progeny can be evaluated for fruit traits in the field, thus increasing the effectiveness of selection in traditional breeding programs. Marker-assisted selection using additional markers identified in the region surrounding the QTL peak of *PdR1* has already begun to produce immediate benefits toward accelerating the development of PD-resistant wine, table and raisin cultivars of high fruit quality.

The major gene nature of the *PdR1* locus makes it an excellent candidate for further characterization via map-based cloning and eventual gene sequencing. Sequence analysis of the grapevine resistance gene and potential Xf avirulence genes will provide a wealth of information on the interaction of xylem-limited pathogens with their host crop plants. The nature of the interaction between the host and a bacterial pathogen that does not contact living cells suggests that the resistance mechanism is not due to a typical hypersensitive response and therefore is of particular research interest. A cloned PD resistance allele would also be useful for genetic engineering strategies at combating the disease in many agricultural crops, and efforts at map-based cloning the *PdR1* locus are currently under way.

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References

Adlerlieste MFJ, Van Eeuwijk FA (1992) Assessment of concentrations of beet necrotic yellow vein virus by enzyme-linked immunosorbent assay. J Virol Methods 37:163–176

Albar L, Lorieux M, Ahmadi N, Rimbault I, Pinel A, Sy AA, Fargette D et al (1998) Genetic basis and mapping of the resistance to rice yellow mottle virus: I. QTLs identification and relationship between resistance and plant morphology. Theor Appl Genet 97:1145–1154

Doucleff M, Jin Y, Gao F, Riaz S, Krivanek AF, Walker MA (2004) A genetic linkage map of grape utilizing *Vitis rupestris* and *Vitis arizonica*. Theor Appl Genet 109:1178–1187

Fehr WR (1991) Principles of cultivar development—theory and technique, vol 1. Iowa State University

Goheen AC, Raju BC, Lowe SK, Nyland G (1979) Pierce's disease of grapevines in Central America. Plant Dis Rep 63:788–792

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudotestcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Halbrooks MC, Mortensen JA (1989) Origin and significance of Florida hybrid bunch grapes and rootstocks. Hortscience 24:546–550

Hallauer AR, Miranda JB (1988) Quantitative genetics in maize breeding, 2nd ed. Iowa State University Press, Ames

Hewitt WB (1958) The probable home of Pierce's disease virus. Plant Dis Rep 42:211–214

Hopkins DL, Purcell AH (2002) *Xylella fastidiosa*: cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis 86:1056–1066

Ikuta H, Noda N, Ebie Y, Hirata A, Tsuneda S, Matsumura M, Inamori Y (2000) The rapid quantification and detection of nitrifying bacteria by using monoclonal antibody method. Water Sci Technol 42:1–7

Jimenez LG (1985) Pierce's disease of grape in Venezuela immunological evidence. Phytopathology 75:1175

Krivanek AF, Walker MA (2005) *Vitis* resistance to Pierce's disease is characterized by differential *Xylella fastidiosa* populations in stems and leaves. Phytopathology 95:44–52

Krivanek AF, Stevenson JF, Walker MA (2005a) Development and comparison of symptom indices for quantifying grapevine resistance to Pierce's disease. Phytopathology 95:36–43

Krivanek AF, Famula TR, Tenscher A, Walker MA (2005b) Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* × *Vitis arizonica* hybrid population. Theor Appl Genet 111:110–119

Lander E, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Loomis NH (1958) Performance of *Vitis* species in the south as an indication of their relative resistance to Pierce's disease. Plant Dis Rep 42:833–836

Mather K (1951) The measurement of linkage in heredity, 2nd ed. Wiley, New York

Meyer UM, Spotts RA, Dewey FM (2000) Detection and quantification of Botrytis cinerea by ELISA in pear stems during cold storage. Plant Dis 84:1099–1103

Mortensen JA (1968) The inheritance of resistance to Pierce's disease in *Vitis*. J Am Soc Hortic Sci 92:331–337

Mortensen JA (1988) Blanc Du Bois grape. Hortscience 23:418–419

Mortensen JA, Stover LH, Balerdi CF (1977) Sources of resistance to Pierce's disease in *Vitis*. J Am Soc Hortic Sci 102:695–697

Nyquist WE (1991) Estimation of heritability and prediction of selection response in plant populations. Crit Rev Plant Sci 10:235–322

Overcash J, Hegwood C, Stojanovic B (1981) 'Midsouth' and 'Missblue'—two new bunch grape cultivars. Mississippi Agricultural and Forestry Experiment Station Research Report, vol 6, issue 18

Perry RL, Mollenhauer HH, Bowen HH (1974) Electron photomicroscopy verification of Pierce's disease on grape plants from Texas. Plant Dis Rep 58:780–782

Riaz S, Dangl B, Edwards KJ, Meredith CP (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor Appl Genet 108:864–872

Van Ooijen J (1999) LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624

Van Ooijen J, Boer M, Jansen R, Maliepaard C (2000) MAPQTL version 4.0: software for the calculation of QTL positions on genetic maps. Plant Research International, Wageningen

Van Ooijen J, Voorrips RE (2001) JoinMap 3.0, software for the calculation of genetic linkage maps. Plant Reasearch International, Wageningen

Young ND, Tanksley SD (1989) RFLP analysis of the size of chromosomal segments retained around the *Tm-2* locus of tomato during backcross breeding. Theor Appl Genet 77:353–359

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468