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Inheritance of resistance to *Xylella fastidiosa* within a *Vitis rupestris* × *Vitis arizonica* hybrid population

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Abstract The inheritance of resistance to *Xylella fastidiosa* (Xf), the bacterium which causes Pierce's disease (PD) in grapevines, was evaluated within a factorial mating design consisting of 16 full-sib families with resistance derived from *Vitis arizonica* interspecific hybrids. Measurements of disease progression under greenhouse conditions were based on quantitative assessment of Xf populations in stem tissues and on three phenotypic scores: leaf scorch, a cane maturation index (CMI) and an index that incorporated shoot stunting into the cane maturation index (CMSSI). Measurement of bacterial populations yielded the highest broad-sense heritability for resistance on a genotype mean basis (0.97), indicating that this measure of resistance was the least effected by environmental variation. Narrow-sense heritability of PD resistance was moderately high and measured 0.52, 0.60, 0.63 and 0.37 for Xf populations, CMI scores, CMSSI scores and leaf scorch values, respectively. Complex segregation analysis using the computer program Statistical Analysis for Genetic Epidemiology (SAGE) strongly indicated the existence of a major gene for PD resistance, which accounted for 91% of the total genetic variance. Conversion of the quantitative data into qualitative resistance levels and evaluation via a chi-square analysis showed that 15 of the 16 families segregated in accordance with a single gene hypothesis with a dominant allele controlling PD resistance. These data indicate that the trait should be relatively easy to pass on from parents to progeny in a breeding program for the development of PD-resistant grape cultivars, particularly when selection is based on cane maturation scores or stem Xf populations.

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Introduction

The European bunch grape *Vitis vinifera* accounts for more than 92% of the grape production in the United States (National Agricultural Statistics Service; http://www.usda.gov/nass). However, vinifera grapevines are highly susceptible to a disease known as Pierce's disease (PD), which is caused by the bacterium *Xylella fastidiosa* (Xf). In areas where the bacterium is endemic, the disease severely limits vinifera cultivation, particularly in the Gulf Coast states and in several regions of California (Hewitt 1958; Loomis 1958; Halbrooks and Mortensen 1989; Hopkins and Purcell 2002).

Characteristic symptoms of PD include leaf scorching, fruit cluster dehydration, uneven maturation of cork on infected canes (referred to as "green islands"), stunting and eventual plant death (Butler 1910; Hewitt et al. 1942; Esau 1948; Halbrooks and Mortensen 1989; Hopkins 1989; Goodwin and Purcell 1992). Although these symptoms have been well-described, researchers have primarily relied on longevity studies to evaluate grape germplasm for resistance. An alternative method of measuring resistance was presented by Krivanek and Walker (2005) in which field performance and stem bacteria populations measured under greenhouse conditions were shown to be highly correlated.

Resistance to PD exists in American *Vitis* species and has been introgressed into many hybrid cultivars, but very little is known about the genetics of this trait and only one study has investigated the inheritance of PD resistance. Mortensen (1968) evaluated the inheritance PD resistance from *V. aestivalis* ssp. *smalliana*, *V. simpsonii* and *V. shutttleworthii* in Florida under field conditions and

native disease pressure. Plant vigor and longevity measured over a 5-year period were used to determine whether a genotype was resistant or susceptible. Upon evaluation of qualitative segregation ratios of progeny derived from several controlled crosses, Mortensen concluded that resistance was dominant to susceptibility and suggested that complementary gene action among three independent genes could best explain the results. No quantitative measurements of genetic variation or heritability estimates of PD resistance were reported.

Improvement of crops through breeding is facilitated by genetic knowledge of the traits under selection. Such genetic information can be used to calculate heritability estimates, which help breeders select parents for controlled crosses. Heritability estimates are derived from parameters of covariance (degree of resemblance) among relatives. One method of covariance estimation is through factorial sib analysis, which is a mating system that is less biased by environmental covariances than other methods (Fehr 1991; Falconer and Mackay 1996). The Design II mating factorial (Comstock and Robinson 1952; Hallauer and Miranda 1988) consists of a series of males each mated to a series of females. Calculations are simplified relative to other mating designs because the selected females are not mated to each other, the selected males are not mated to each other and there are no reciprocal or selfing crosses. Such a factorial is particularly suited to a dioecious species such as grape.

The objective of this study was to use a Design II factorial to characterize the inheritance of Xf resistance utilizing geographically distinct sources of the grape species *V. arizonica*. The species is native to southern Arizona, Baja California and northern Mexico (Munson 1909; Bailey 1934; Wiggins 1980; Comeaux 1991) where PD is also endemic (Raju et al. 1980; Guevara 1997). We hypothesized that *V. arizonica*'s PD resistance is under genetic control similar to that reported of *V. aestivalis* and *V. shutttleworthii* (Mortensen 1968). Our study tested this hypothesis and expanded upon this earlier work by estimating quantitative genetic parameters and heritability for the trait. Complex segregation analysis (CSA) and tests of qualitative segregation ratios were also used on the mating factorial to determine if the resistance trait is controlled by several minor genes, a major gene or a combination of both, and to determine whether the resistance from the different *V. arizonica* sources has similar or dissimilar modes of inheritance.

Materials and methods

Plant materials

Eight genotypes (four males and four females) were used as parents for the mating design (Table 1). The parents were randomly selected from a population of *Vitis* interspecific hybrids designated as the 8909 population and share *V. rupestris* 'A de Serres' (PD susceptible) as the maternal parent. The 8909 genotypes have different paternal parents: b42-26 is a PD-resistant *V. arizonica* collected near La Paz, Baja California, Mexico; Y14-122 is a *V. arizonica* × *V. vulpina* hybrid (with the *V. arizonica* originating from southern Arizona); b43-17 is a PD-resistant *V. arizonica*/*candicans* type collected near Monterrey, Nuevo Leon, Mexico; Pillans is a PD-susceptible *V. rupestris*.

Table 1 Eight *Vitis* genotypes evaluated and utilized as parents in the 4 × 4 Design II mating design

Genotype	Genetic background
D8909-15[a]	A de Serres (*V. rupestris*) × b42-26 (*V. arizonica*)
J8909-02[a]	A de Serres (*V. rupestris*) × (Y14-122 (*V. arizonica* × *V. vulpina*))
F8909-16[a]	A de Serres (*V. rupestris*) × b43-17 (*V. arizonica*/*candicans* type)
C8909-07[a, b]	A de Serres (*V. rupestris*) × 'Pillans' (*V. rupestris*)
F8909-01[c]	A de Serres (*V. rupestris*) × b43-17 (*V. arizonica*/*candicans* type)
F8909-08[c]	A de Serres (*V. rupestris*) × b43-17 (*V. arizonica*/*candicans* type)
F8909-26[c]	A de Serres (*V. rupestris*) × b43-17 (*V. arizonica*/*candicans* type)
C8909-19[a, b]	A de Serres (*V. rupestris*) × Pillans (*V. rupestris*)

[a]Female parent
[b]Genotype previously identified as PD-susceptible
[c]Male parent

Population development and experimental design

The four males were crossed to the four females in a Design II mating factorial (Comstock and Robinson 1952; Hallauer and Miranda 1988). Flower clusters of both male and female grapevines were enclosed in paper bags prior to anthesis to prevent random out-crossing. When at least 80% of the flowers were open, bags from the female clusters were removed and replaced with bags from the male clusters and shaken to facilitate pollination. Fruit was collected in the fall, and seeds were processed and germinated as previously described (Cousins and Walker 2002). On average, 50 seedlings from each of the 16 crosses were transplanted to the field. In the following year, an average of 33 seedlings (ranging from 19 to 38) from each of the families, along with the eight parent genotypes, were clonally propagated in four replicates, potted and grown under greenhouse conditions as previously described (Krivanek et al. 2005) with the exception that the plants were limited to a height of 60 cm.

Potted plants were randomly distributed on greenhouse benches in four separate blocks in a randomized complete block design. Some replicates were not available for the full screen and as such the design was unbalanced. Plants were inoculated with Xf as previously described (Krivanek et al. 2005), and each block had one to two water-inoculated controls.

Disease evaluation

Four quantitative methods were used to evaluate disease expression 16 weeks post-inoculation as described previously (Krivanek et al. 2005). First, the mean percentage of the leaf area with marginal leaf scorch on four leaves immediately above the inoculation point (scale of 0–100) was measured. Second, the degree of cane maturation and necrosis, utilizing a cane maturation index cane maturation index (CMI with a scale of 0–6) was measured. A modified CMI incorporating the degree of shoot stunting was also used and designated the cane maturation shoot stunting index (CMSSI). The CMSSI (scale of 0–8) was calculated by adding 0 for no stunting, 1 for moderate stunting and 2 for severe stunting to the CMI score. Stunting pertained to the re-growth of shoots at the top of the plant after the main shoot was pruned 3–4 weeks prior to scoring. Finally, Xf populations were measured with a quantitative double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) as described previously (Krivanek and Walker 2005). Briefly, absorbance readings were measured from extracts of stem tissue samples taken 10 cm above the point of inoculation (POI) from all plants. Absorbance values were converted to cells per milliliter concentrations using a standard calibration curve derived from a dilution series of bacteria added to healthy stem extract and included on each microtitre plate. All predicted values were reported as cells per milliliter. However, as the buffer volume to sample weight ratio was 10:1, the cells per milliliter concentrations equate to one-tenth the number of cells per gram of sample.

Statistical analyses of disease resistance data

The CMI, CMSSI, leaf scorch and ELISA cells per milliliter datasets were analyzed for normal distribution visually using the NORMAL, HISTOGRAM and QQPLOT options within UNIVARIATE procedure of SAS Version 8 (SAS Institute, Cary, N.C.). A plot of the residuals against predicted values was used to visually assess the residuals. To achieve normal distribution, the data points of cells per milliliter concentration were natural log-transformed, and eight outliers out of 2,080 data points (0.4%) were removed. Ten additional outliers out of 2,080 data points (0.5%) were removed from the phenotypic datasets. Visual assessment of the residuals after removal of the outliers showed no dramatic deviation from normality. Genotype and block had 525 and four levels, respectively. To compare the means of each genotype, we evaluated the datasets by treating the variables of genotype and block as fixed factors and then performed a mean separation using Tukey-Kramer's Honestly Significant Difference (HSD). The three mean phenotypic disease scores for each genotype were plotted onto corresponding natural log-transformed mean Xf populations in stem tissues, and the strength of the relationship was measured by the Pearson correlation coefficient (R) using the SAS CORR procedure.

To estimate variance components and respective standard errors, we removed the negative control and parental genotypes from the datasets, and the population was analyzed via the restricted maximum likelihood (REML) method of the SAS MIXED procedure. REML analysis is now the method of choice for estimating heritability and variance components as it does not require a balanced design (Falconer and Mackay 1996). Variance components for broad-sense heritability estimates were made under a random model by treating the variables genotype and block as random factors. Due to the unbalanced nature of the design, the Satterwaith method was used for estimating degrees of freedom for all analyses. Broad-sense heritability (H^2) estimates were calculated on a single-plant basis via the equation $H^2_{(\text{plant basis})} = \sigma^2_g/.(\sigma^2_g + \sigma^2_e)$ (Fehr 1991) where the genetic variance (σ^2_g) and experimental error variance (σ^2_e) were listed under the 'Covariance Parameter Estimates' heading. Broad-sense heritability estimates were calculated on a genotype mean (entry mean) basis via the equation $H^2_{(\text{mean basis})} = \sigma^2_g/.(\sigma^2_g + (\sigma^2_e/r))$ (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 (SE) of the H^2 estimates were calculated using $\text{SE}(H^2_{(\text{plant basis})}) = \text{SE}(\sigma^2_g)/.(\sigma^2_g + \sigma^2_e)$ and $\text{SE}(H^2_{(\text{mean basis})}) = \text{SE}(\sigma^2_g)/.(\sigma^2_g + (\sigma^2_e/r))$, respectively, (Hallauer and Miranda 1988; Nyquist 1991) where the term $\text{SE}(\sigma^2_g)$ refers to the square root of the variance of the genetic variance estimate and was calculated using the COVTEST option of the MIXED procedure.

Variance components for narrow-sense heritability estimates were analyzed under a random model within the MIXED procedure by treating the variables male, female, block and the male × female interaction as random factors. Female, male and block each had four levels. Narrow-sense heritability estimates were calculated on a genotype mean basis via the equation $h^2_{(\text{mean basis})} = \sigma^2_{\text{add}}/.(\sigma^2_{\text{add}} + \sigma^2_{\text{dom}} + (\sigma^2_e/r))$ where $\sigma^2_{\text{add}} = 4((\sigma^2_{\text{males}} + \sigma^2_{\text{females}})/2)$ and $\sigma^2_{\text{dom}} = 4(\sigma^2_{\text{males}\times\text{females}})$ (Hallauer and Miranda 1988). Narrow-sense heritability (h^2) estimates were calculated on a single-plant basis via the equation $h^2_{(\text{plant basis})} = \sigma^2_{\text{add}}/(\sigma^2_{\text{add}} + \sigma^2_{\text{dom}} + \sigma^2_e)$. The term σ^2_e again refers to the experimental error variance, but unlike with the broad-sense heritability formulas, σ^2_e is the experimental error excluding the additive and dominance genetic variances. Approximate standard error (SE) of the h^2 estimates were calculated using the formula $\text{SE}(h^2_{(\text{mean basis})}) = 4(\text{SE}(\sigma^2_{(\text{males})}/.(\sigma^2_{\text{add}} + \sigma^2_{\text{dom}} + (\sigma^2_e/r))$ (Hallauer and Miranda 1988), where the term $\text{SE}(\sigma^2_{\text{males}})$ refers to the square root of the variance of the genetic variance estimate of the males.

Prior to analyzing the segregation patterns of the different families using a standard goodness-of-fit method, we employed another distinct procedure,

termed CSA. The CSA is a statistical method using regressive models to assess the possible segregation of a major Mendelian locus in a background of polygenic variation (Bonney 1984). The analysis can be used to estimate the magnitude of a major gene in addition to the magnitude of the remaining minor genes and environmental variation (Jarvik 1998). Various genetic models are fit to pedigree information and phenotype measurements, and a likelihood is computed for each model, permitting hypothesis tests via a likelihood ratio statistic. Elston et al. (1975) provides a discussion of the criteria, which must be satisfied before declaration of the presence of a segregating major locus can be asserted with the intent to reduce the probability of a false declaration of a major locus. For a comprehensive review of CSA, see Lynch and Walsh (1998).

The CSA in this study utilized quantitative measurements of PD resistance based on mean Xf populations in stem tissues of the mating-design genotypes. All the necessary models were fit using the computer program (SAGE 2002 ver. 4.2; Statistical Solutions, Cork, Ireland). However, having been designed to analyze data from pedigrees of humans, the SAGE software does not permit (due to computational limitations) the multiple mating strategy of a Design II diallel. Accordingly, the one inter-connected "family" of the mating design was broken into four distinct, non-overlapping families built around each of the four male lines of the cross. The proportion of the total genetic variance due to a possible major gene was estimated. Posterior probabilities were calculated for estimated parental major gene genotypes.

To conduct a standard goodness-of-fit analysis on segregation patterns, the quantitative measurements of PD resistance based on mean Xf populations were converted to qualitative classifications. Genotypes were classified as resistant, moderately resistant, moderately susceptible or susceptible after visual assessment of the frequency distributions. Breaks or separations between the primary modes within the frequency distributions were used to empirically define each classification's cutoff threshold. The same classification thresholds were determined by and used across all 16 families. Standard chi-square analyses of each of the full-sib families, of the cumulative group of R × R crosses and of the cumulative group of R × S and S × R crosses tested the probability that the differences between observed and expected segregation ratios were due to chance. The null hypotheses were a single dominant gene model and two-gene model with and without complementary gene action.

Results

Disease evaluation of parental genotypes

Combined screen data on the parents of the mating design indicate that the *V. arizonica* hybrid parents (females D8909-15, J8909-02, F8909-16 and males F8909-01, F8909-08 and F8909-26) are resistant to PD. The two *V. rupestris* × *V. rupestris* parental genotypes (female C8909-07 and male C8909-19) are both susceptible to the disease under the screening conditions of this experiment. Results from the screens are presented in Tables 2, 3 and 4 and are similar to those obtained in a previous evaluation of all genotypes in the 8909 populations (data not shown). Each of the six parental *V. arizonica* hybrid genotypes had low stem bacterial populations of 1×10^5 cells/ml [<11.5 natural log(cells/ml)] or lower for tissue samples in grinding buffer (Table 2). In contrast, the two *V. rupestris* × *V. rupestris* parental genotypes had significantly higher ($P<0.0001$) stem Xf populations of above 5×10^6 cells/ml [15.5 natural log(cells/ml)]. The Xf inoculations had a moderate to low effect on cane maturation of the *V. arizonica* hybrid genotypes (CMI scores under 2.5), however one of these genotypes, F8909-16, had a moderate mean CMI score of 3.5 (Table 3). The two *V. rupestris* × *V. rupestris* parental genotypes had significantly higher ($P<0.05$) CMI scores of greater than 5.0. Leaf scorch symptoms were not as effective at distinguishing the parental genotypes as none were found to be significantly different. All of the *V. arizonica* hybrid parental genotypes had high mean leaf scorch values, at 65–96% relative to 100%, for the *V. rupestris* parental genotypes (Table 4).

Correlation among disease evaluations

A positive correlation was identified between each phenotypic disease score and stem Xf populations when evaluated cross the entire mating design population of 525 genotypes. The CMI scores had a moderately high correlation as measured by the correlation coefficient ($R=0.73$, $P<0.0001$) (Fig. 1a). Modification of the index by adding shoot stunting scores (CMSSI) increased the correlation to $R=0.78$ ($P<0.0001$) (Fig. 1b). Mean leaf scorch values had a considerably lower correlation ($R=0.40$, $P<0.0001$) with Xf populations due to a large number of genotypes with high leaf scorch values but low stem Xf populations (Fig. 1c).

Quantitative analysis and heritability

Broad-sense heritability estimates for PD resistance, a direct measure of how easily the trait is distorted by environmental effects, were calculated from measurements obtained using the four disease evaluation methods (Table 5). Heritability was measured on a genotype mean (entry mean) basis and on a single-plant basis. Broad-sense heritability for PD resistance on a genotype mean basis was high for each of the PD evaluation methods (0.97–0.77) with the measurement of Xf populations producing the highest heritability. A broad-sense heritability of 0.97 indicates that approximately 97% of the phenotypic variability is accounted for by genotype. Broad-sense heritability on a single-plant basis was lower for each of the disease

Table 2 Mean Xf populations in stem tissue extracts of the parental genotypes and the susceptible grandparent A de Serres, as determined by ELISA, 16 weeks after inoculation with Xf

Genotype	Mean no. of cells/ml	Mean natural log (cells/ml)	SE	Repeat no.	Minimum natural log (cells/ml)	Maximum natural log (cells/ml)	Progeny mean natural log (cells/ml)
C8909-07[a,b]	8,250,663 a [d]	15.92	0.06	4	15.83	16.08	13.98
A de Serres [a]	6,428,692 a	15.58	0.27	4	14.81	16.03	
C8909-19[a,c]	5,927,276 a	15.58	0.12	3	15.34	15.71	12.90
F8909-16 [b]	104,315 b	10.74	0.78	4	9.10	12.63	11.56
F8909-08 [c]	64,921 b	10.65	0.55	4	9.32	12.01	11.63
F8909-01[c]	27,999 b	9.75	0.52	4	9.15	11.30	11.56
F8909-26[c]	25,237 b	10.07	0.21	4	9.55	10.53	11.64
D8909-15[b]	24,856 b	9.86	0.39	4	9.29	10.99	11.61
J8909-02[b]	24,536 b	9.96	0.34	4	9.04	10.49	11.20

[a]Susceptible genotype
[b]Female parent
[c]Male parent
[d]Means followed by different letters differ significantly from each other ($P < 0.05$ Tukey-Kramer's HSD)

Table 3 Mean CMI score (scale: 0–6) for the parental genotypes and the susceptible grandparent A de Serres 16 weeks after inoculation with Xf

Genotype	Mean CMI score	SE	Repeat no.	Minimum CMI score	Maximum CMI score	Progeny mean CMI score
C8909-19[a,b]	5.67 a [d]	0.33	3	5.00	6.00	4.55
C8909-07[a,b]	5.50 a	0.29	4	5.00	6.00	4.67
A de Serres[a]	5.25 a,b	0.25	4	5.00	6.00	
F8909-16[b]	3.50 b,c	0.29	4	3.00	4.00	3.49
J8909-02[b]	2.25 c	0.75	4	1.00	4.00	3.05
F8909-08[c]	2.00 c	0.71	4	1.00	4.00	3.02
D8909-15[b]	0.75 d	0.25	4	0.00	1.00	2.35
F8909-01[c]	0.75 d	0.25	4	0.00	1.00	2.71
F8909-26[c]	0.75 d	0.25	4	0.00	1.00	2.89

[a]Susceptible genotype
[b]Female parent
[c]Male parent
[d]Means followed by different letters differ significantly from each other ($P < 0.05$ Tukey-Kramer's HSD)

Table 4 Mean percentage leaf scorch for the parental genotypes and the susceptible grandparent A de Serres 16 weeks after inoculation with Xf

Genotype	Mean % leaf scorch	SE	Repeat no.	Minimum % leaf scorch	Maximum % leaf scorch	Progeny mean % leaf scorch
A de Serres[a]	100 a [d]	0	4	100	100	
C8909-07[a,b]	100 a	0	4	100	100	93
C8909-19[a,c]	100 a	0	3	100	100	95
F8909-16[b]	96 a	4	4	85	100	89
J8909-02[b]	94 a	6	4	75	100	83
D8909-15[b]	86 a	9	4	60	100	79
F8909-08[c]	85 a	9	4	65	100	82
F8909-01[c]	76 a	17	4	30	100	79
F8909-26[c]	65 a	14	4	35	100	84

[a]Susceptible genotype
[b]Female parent
[c]Male parent
[d]Means followed by different letters differ significantly from each other ($P < 0.05$ Tukey-Kramer's HSD)

evaluation methods (0.89–0.48), indicating the importance of using replication to reduce environmental variation.

Estimates of narrow-sense heritability, a measure of how easily a given phenotype is passed on from parent to progeny, were calculated for the four disease measurements (Table 5). Narrow-sense heritability for PD resistance on a genotype mean basis was moderate for each of the PD evaluation methods (0.63–0.37). In contrast, narrow-sense heritability estimates when measured on a single-plant basis were reduced by roughly half (0.34–0.13), indicating that, as with broad-sense heritability estimates, narrow-sense heritability estimates are improved with replication.

Complex segregation analysis

Likelihood-ratio (LR) statistics were used to determine if the distribution of progeny resistance levels based on stem Xf populations were consistent with the segregation of a major gene in a background of polygenic variation. A two-mean model (variance due the action of a major gene with a dominant allele and polygenic inheritance) was compared to a one-mean model (all variance due to polygenic inheritance). The two-mean model gave a significant improvement ($P < 0.00001$, LR = 372.4 with 2 df) in the LR statistic relative to a one-mean model, thus indicating the existence of a major gene acting with a dominant resistance allele. The proportion of the total genetic variance due to the major gene was 91%. Posterior probabilities were calculated for estimated parental major gene genotypes and are listed in Table 6: each had associated probabilities of 1.0. The estimated posterior probabilities were 0.0 for estimated genotypes other than those listed.

The majority of the resistance segregating in the mating design is derived from the grandparent b43-17, a *V. arizonica*/*candicans* type from Monterrey Mexico. To determine the effect of the origin of the major gene on the segregation of resistance, the four families with resistance derived from Y14-122 (a *V. arizonica* × *V. vulpina* hybrid with the *V. arizonica* originating from southern Arizona) and the four families with resistance derived from b42-26 (a Baja California *V. arizonica*) were evaluated in separate analyses. The CSA of the four families sharing the Y14-122 grandparent resulted in the same conclusions as those drawn with the comprehensive analysis, with a two-mean model giving a significant improvement over the null one-mean model. However, the CSA of the four families sharing the b42-26 grandparent resulted in a three-mean model that was significantly better than either a one-mean or two-mean model. This result indicates that the resistance derived from b42-26 likely has a different mode of action than the other sources of resistance in the mating design.

Qualitative segregation analysis

In order to conduct chi-square analyses on the segregation of PD resistance in the mating design, we converted the quantitative measurements of mean Xf populations to the qualitative classifications of resistant (R), moderately resistant, moderately susceptible or susceptible (S) based on the cutoff thresholds listed in Table 7 and defined above. All four classifications were used for testing the two-gene hypothesis without complementary gene action. When testing the single-gene hypothesis and the two-gene hypothesis with complementary gene action, resistant and moderately resistant classifications were grouped together, and the moderately susceptible and susceptible genotypes were grouped together.

Chi-square analyses were conducted on the segregation of the progeny in the individual families. The ob-

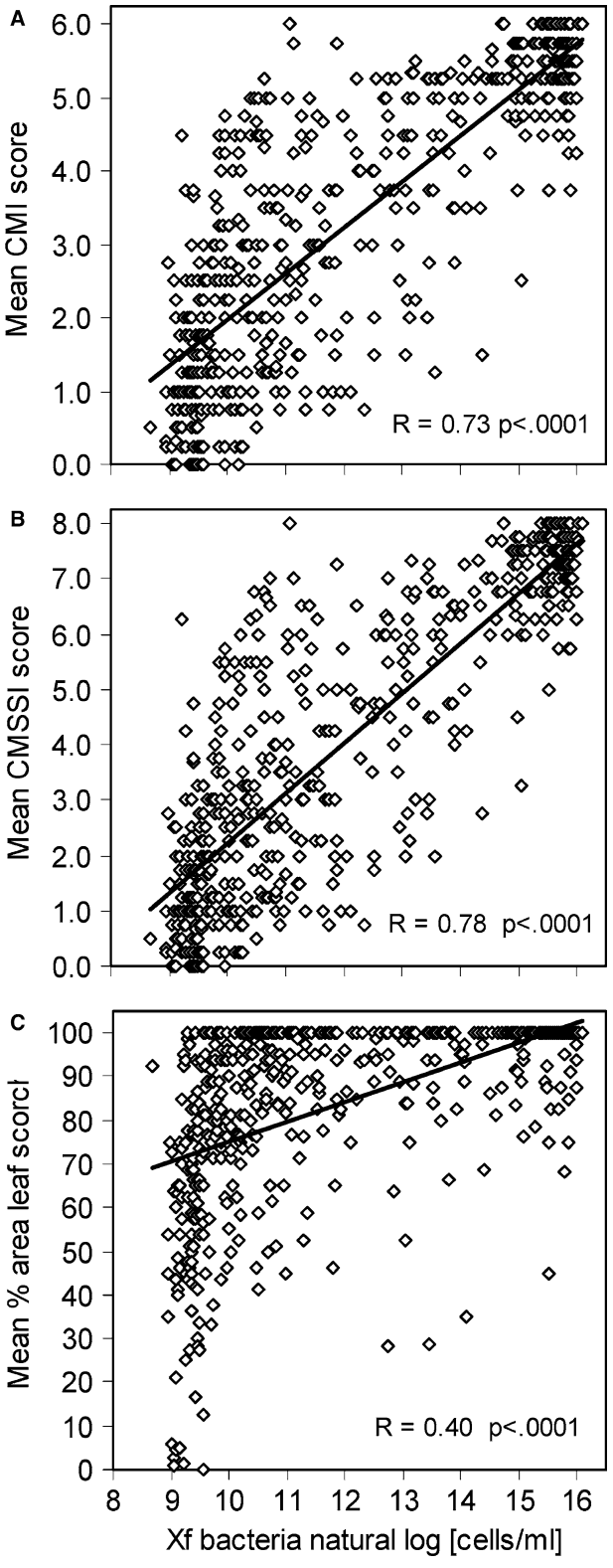

Fig. 1 Correlation of PD phenotypic symptoms to mean Xf populations of each genotype in the Design II population. Bacteria numbers were determined by ELISA, and correlations to CMI scores (**a**), CMSSI scores (**b**) and percentage leaf scorch (**c**) were measured with the Pearson correlation coefficient (*R*)

Table 5 Heritability of PD resistance in the broad-sense (H^2) and narrow-sense (h^2) based on CMI scores, CMSSI scores, leaf scorch values and Xf populations in stem-tissue extracts

Scoring method	H^2 Genotype mean basis	H^2 Single-plant basis	h^2 Genotype mean basis	h^2 Single-plant basis
Xf populations	0.97 ± 0.06	0.89 ± 0.06	0.52 ± 0.22	0.23 ± 0.10
CMI scores	0.91 ± 0.06	0.74 ± 0.05	0.60 ± 0.13	0.32 ± 0.07
CMSSI scores	0.93 ± 0.06	0.78 ± 0.05	0.63 ± 0.11	0.34 ± 0.06
Leaf scorch	0.77 ± 0.06	0.48 ± 0.04	0.37 ± 0.16	0.13 ± 0.06

Table 6 Estimated parental major gene genotypes

Parent	Estimated major gene genotype	Probability
D8909-15	Rr	1.0[a]
J8909-02	Rr	1.0[a]
F8909-16	Rr	1.0[a]
C8909-07	rr	1.0[a]
F8909-01	Rr	1.0
F8909-08	Rr	1.0
F8909-26	Rr	1.0
C8909-19	rr	1.0

[a]The posterior probability number was made with four estimates derived from four distinct, non-overlapping families built around each of the four male genotypes

Table 7 PD resistance thresholds for qualitative segregation analysis utilizing natural log-transformed Xf concentrations in stem-tissue extracts

Threshold natural log (cells/ml)	Classification
< 11.0 (< 60,000)	Resistant
> 11.0 < 12.5 (> 60,000, < 300,000)	Moderately resistant
> 12.5 < 14.0 (> 300,000, < 1,200,000)	Moderately susceptible
> 14.0 (> 1,200,000)	Susceptible

served segregation ratios and probability estimates for the chi-square analyses are listed in Tables 8 and 9. Representative segregation patterns for R × R crosses and R × S crosses are shown in Fig. 2a, b, respectively. The S × S cross (Fig. 2c) had 0 resistant and 21 susceptible progeny, which fit the expected ratio of 0:1 under a one-gene or a two-gene (with or without complementary gene action) hypothesis. Only one family (C8909-07 × F8909-01) out of the 16 full-sib families could be excluded ($P = 0.03$) from the single-gene hypothesis. In contrast, 9 out of 16 families could be excluded from the two-gene without complementary gene action hypothesis and 4 out of 16 families could be excluded from the two-gene with complementary gene action hypothesis. The presence of susceptible progeny in the R × R crosses and the absence of resistant progeny in the S × S cross confirm that this resistance is controlled by a dominant allele.

Additional chi-square analyses were conducted on the segregation of the genotypes in the cumulative R × R population (325 genotypes) and in the cumulative R × S and S × R population (179 genotypes), and the single-gene hypothesis could not be excluded in either

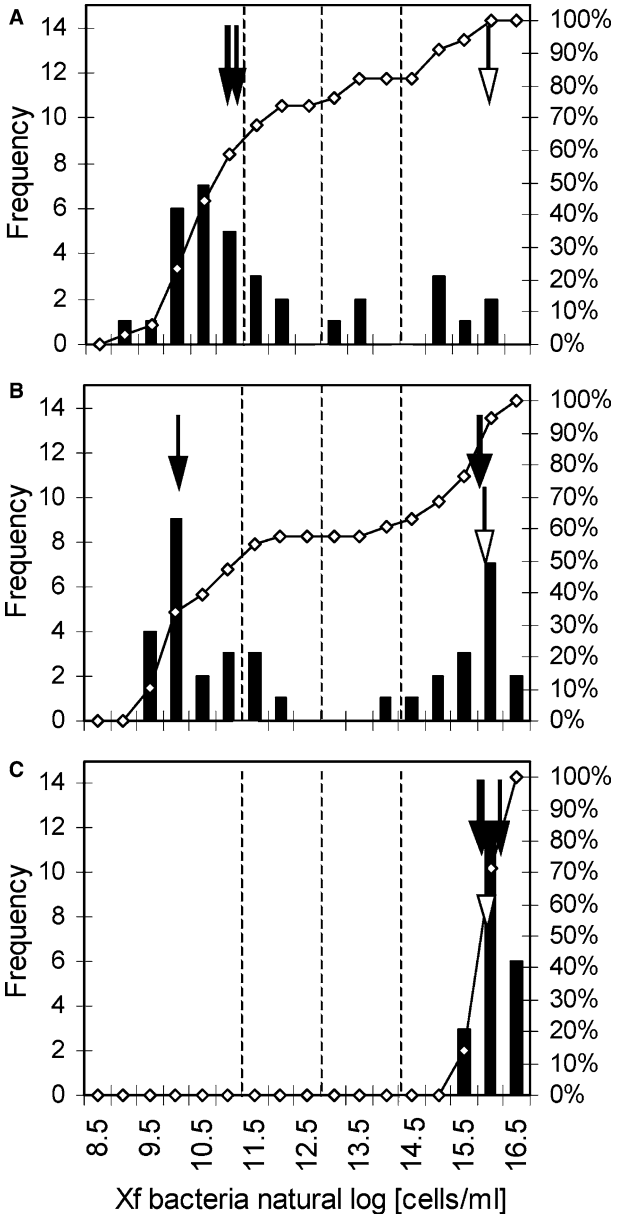

Fig. 2 Frequency distributions for mean Xf concentrations, as determined by ELISA, in stem-tissue extract of the progeny from the R × R cross of F8909-16 × F8909-08 (**a**), the R × S cross J8909-02 × C8909-19 (**b**) and the S × S cross C8909-07 × C8909-19 (**c**). *Black arrows* indicate mean Xf populations in the parental genotypes, the *open arrow* indicates mean Xf populations in the PD-susceptible grandparent, *dashed lines* indicate resistance thresholds for designating genotypes resistant, moderately resistant, moderately susceptible or susceptible, *plotted line* indicates cumulative percentage (*right vertical axis*) of the genotypes in the population

Table 8 Chi-square tests of the segregation ratios[a] from the nine families derived from resistant by resistant crosses

Cross	Observed ratios:		Probability under expected ratios:		
	Four classes	Two classes	9:3:3:1	9:7	3:1
J8909-02 × F8909-01	29:1:4:3	30:7	0.02*	0.03*	0.87
J8909-02 × F8909-08	21:6:2:9	27:11	< 0.01*	0.34	0.96
J8909-02 × F8909-26	24:4:4:4	28:8	0.23	0.08	0.99
D8909-15 × F8909-01	19:8:7:2	27:9	0.95	0.16	1.00
D8909-15 × F8909-08	29:2:4:2	31:6	0.05	0.01*	0.68
D8909-15 × F8909-26	15:8:8:7	23:15	0.23	0.96	0.64
F8909-16 × F8909-01	19:4:3:6	23:9	0.02*	0.37	0.98
F8909-16 × F8909-08	20:5:3:6	25:9	0.03*	0.25	1.00
F8909-16 × F8909-26	22:6:0:9	28:9	< 0.01*	0.13	1.00
Cumulative 9 crosses	198:44:35:48	242:83	< 0.01*	< 0.01*	1.00

*Indicates that observed ratio is significantly distorted from expected at $P < 0.05$

[a]Expected ratio of 9:3:3:1 is under a two-gene without complementary gene action hypothesis; expected ratio of 9:7 is under a two-gene with complementary action hypothesis; expected ratio of 3:1 is under a single dominant gene hypothesis

Table 9 Chi-square tests of the segregation ratios[a] from the six families derived from resistant-by-susceptible or susceptible-by-resistant crosses

Cross	Observed ratios:		Probability under expected ratios:		
	Four classes	Two classes	1:1:1:1	1:3	1:1
J8909-02 × C8909-19	18:4:1:5	22:16	< 0.01*	< 0.01*	0.81
C8909-07 × F8909-01	5:0:1:19	5:20	< 0.01*	0.95	0.03*
C8909-07 × F8909-08	5:8:3:16	13:19	0.01*	0.24	0.77
C8909-07 × F8909-26	5:1:5:8	6:13	0.16	0.93	0.46
D8909-15 × C8909-19	8:4:9:15	12:14	0.08	0.72	0.26
F8909-16 × F8909-19	12:2:3:12	14:15	0.01*	0.04*	1.00
Cumulative 6 crosses	55:19:22:85	72:107	< 0.01*	< 0.01*	0.08

*Indicates observe ratio is significantly different from expected $P < 0.05$

[a]Expected ratio of 1:1:1:1 is under a two-gene without complementary gene action hypothesis; expected ratio of 1:3 is under a two-gene with complementary action hypothesis; expected ratio of 1:1 is under a single dominant gene hypothesis

(Tables 8 and 9, respectively). In contrast, chi-square analyses of the cumulative R × R population excluded both of the two-gene hypotheses (Table 8) and the two-gene without complimentary gene action hypothesis for the cumulative R × S and S × R population (Table 9).

Discussion

In this study we have characterized the inheritance of resistance to *Xylella fastidiosa* within a *V. rupestris* × *V. arizonica* hybrid population. Complex segregation analysis supports the existence of a major gene significantly affecting the expression of PD resistance and accounting for 91% of the total genetic variance. Chi-square analysis demonstrated that 15 of the 16 families segregated in accordance with that expected from a single gene with a dominant allele controlling resistance to PD. The dominance of resistance over susceptibility is in agreement with the Mortensen study (1968); however, the conclusion of a major gene controlling PD resistance is not in agreement with the complementary gene action model proposed by Mortensen. Heritability of a trait is dependent on various factors: the reference population; the way in which the phenotype is measured; the environmental conditions in which genotypes are screened; the experimental unit (single plant, replicated genotype mean or family mean) upon which selection is made. Any of these factors alone or in combination may explain the different results from the two studies.

While the heritability of a trait tends to be similar in different populations (Falconer and Mackay 1996), it is possible that the resistance studied by Mortensen and derived from *V. aestivalis*, *V. simpsonii* and *V. shutttleworthii* has a different mode of inheritance from that derived from our *V. arizonica* accessions. It is also possible that the heritability estimates measured in this study may be influenced by the interspecific nature of the parental genotypes. If either of these proves true, it would not be appropriate to extrapolate these results to populations with different genetic backgrounds. Finally, while the overall conclusions of the mating design in this study point toward a single-gene model as the simplest explanation for the genetics of PD resistance inherited from *V. arizonica*, one distinct source of resistance tested here may also have a different mode of inheritance.

The results of the four crosses utilizing the susceptible female C8909-07 are not entirely consistent with the segregation patterns of the cumulative population. One of these crosses (C8909-07 × F8909-01) can be statistically excluded from a single-gene hypothesis, and none of the four crosses can be excluded from a two-gene hypothesis with complementary gene action. However, the hypothesis of complementary gene action within the crosses with C8909-07 does not adhere to the results from the remaining 12 crosses in the mating design. An additional hypothesis that could explain all of the results is that a susceptibility locus with dominant suppression of the resistance gene is segregating within the C8909-07 genotype. This could explain the 1:3 ratios in the S × R cross of C8909-07 × F8909-01 in this study and the results from the Mortensen study. Such examples of dominant inhibitor alleles at a secondary epistatic locus (i.e. susceptibility locus) have previously been proposed to explain results in the inheritance of resistance to root-knot nematode (Cousins and Walker 2002). Further studies will need to be conducted to confirm or reject this hypothesis.

The segregation of the four families sharing D8909-15 as the female parent with resistance derived from b42-26 was also not entirely consistent with the overall segregation analyses. While none of the four families could be excluded from a single-gene hypothesis, the crosses also

could not be excluded from a two-gene without complementary gene action hypothesis. In addition, three out of the four crosses with D8909-15 could not be excluded from the two-gene with complementary action hypothesis. Results from the CSA of these families sharing the b42-26 grandparent resulted in a three-mean model that was significantly better than a one-mean or two-mean model. These results suggest that this resistance has a different mode of action from the other sources of resistance in the mating design. The geographically independent origin of the *V. arizonica* grandparent b42-26, collected in Baja California, Mexico, lends credence to this hypothesis.

Another factor that can influence heritability estimates is the way in which the phenotype is measured. In this study, four different measures of disease were used to evaluate the inheritance of resistance, including one direct measurement of Xf populations within stem tissues and three disease symptom indices. The measurement of stem Xf populations under greenhouse conditions has been shown to correlate well with field PD resistance (Krivanek and Walker 2005). In this study, we also found a high correlation of CMI and CMSSI scores with Xf populations in the stem tissue ($R = 0.73$ and 0.78, respectively). In contrast, the symptom of leaf scorching correlated poorly ($R = 0.40$) with Xf populations in stem tissue. This poor correlation was primarily due to the fact that a large number of genotypes with low Xf populations also had high levels of leaf scorching. These results are in agreement with previous studies finding high levels of leaf scorch symptoms in field-resistant genotypes (Hopkins et al. 1974; Milholland et al. 1981; Hopkins and Thompson 1984; Lu and Cousins 2003; Krivanek et al. 2005).

A third factor that can influence heritability estimates is the unit of selection. Heritability estimates here were made both on a single-plant basis and on a mean of the clonal replications of each genotype in order to determine the effects of the experimental unit on the heritability of PD resistance. Broad-sense heritability estimates were high for each of the PD screening methods and indicated that approximately 77–97% of the phenotypic variability is accounted for by the genotype, depending on the resistance screen used. When evaluations were conducted on a single-plant basis the percentage of phenotypic variance accounted for by genetic variance dropped to 48–89%, confirming the importance of using replications to reduce the effects of environmental variation in any of the PD resistance evaluations even when conducted under greenhouse conditions.

The high broad-sense heritability values when calculated on a genotype mean basis confirm that most of the measurable variation in the crosses of this study was due to genes rather than environmental influences. The limited variation in the S × S cross (C8909-07 × C8909-19) in which each progeny had consistently high levels of Xf in stem tissues [15.25–16.10 natural log (cells/ml)] also supports this conclusion. In order to quantitatively measure how easily the resistance gene(s) can be passed from parents to progeny, calculations of heritability in the narrow-sense were made.

Narrow-sense heritability for PD resistance on a genotype mean basis was moderate for each of the PD evaluation methods but was reduced by roughly half when measured on a single-plant basis, indicating that (as with broad-sense heritability) estimates are improved with experimental replication. The primary value of a narrow-sense heritability estimate is that it allows a plant breeder to predict and quantify the amount of progress that can be expected when selection is conducted on a given trait (Hanson 1963). Results from this study indicate that considerable genetic variation exists for PD resistance in this population. The moderate heritability for PD resistance when measured by CMSSI scores or Xf populations on a genotype mean basis should also allow relatively rapid advancement in breeding resistant genotypes when screened under the screening conditions described here.

Crosses of the resistant genotypes in this study, along with others in the 8909 population, are being made to advanced selections of *V. vinifera* in the interest of breeding PD-resistant wine, table and raisin cultivars. While the PD resistance in these genotypes is likely controlled by a single resistance locus, the possibility that it is governed by more complex epistatic interactions still exits. Expression of the resistance within a *V. vinifera* genetic background may be different than that observed in this study. Future heritability measurements and quantitative trait locus studies of PD resistance in domesticated grape backgrounds are in progress.

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