Vitis rupestris B38 Confers Isolate-Specific Quantitative Resistance to Penetration by *Erysiphe necator*

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ABSTRACT

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Vitis rupestris B38 is a North American grapevine resistant to the major pathogen of cultivated grapevines, *Erysiphe necator*. Sources of powdery mildew resistance, like *V. rotundifolia*, are widely used in grape breeding but are already threatened, even before commercialization, by isolates that can reproduce on *Run1* and other rotundifolia-derived breeding lines. Thus, complementary sources of resistance are needed to improve resistance durability. The segregation of foliar powdery mildew severity in an F₁ family, derived from a cross of *V. rupestris* B38 × *V. vinifera* ‘Chardonnay’, was observed in the field over three growing seasons and in potted vines following single-isolate inoculation. A pattern

of continuous variation was observed in every instance. Mechanisms of resistance were analyzed on the resistant and susceptible parent by using microscopy to quantify the ability of the pathogen to penetrate and to form a colony on detached leaves. While ‘Chardonnay’ was susceptible in all tested conditions, *V. rupestris* B38 resistance was characterized by a reduction in pathogen penetration, with an effect of leaf position and significant differences among powdery mildew isolates. Segregation of the ability of the pathogen to penetrate and form a colony in F₁ individuals showed a pattern of quantitative penetration resistance with no delay or restriction on colony formation once penetration has been achieved. Moreover, *V. rupestris* B38 showed an enhanced penetration resistance to a powdery mildew isolate with the ability to overcome the *Run1* gene, making it an interesting resistance source to prolong the durability of this gene.

North American grapevines such as *Vitis rupestris* have co-existed with powdery mildew, a major pathogen of cultivated grapevines (*V. vinifera*), for hundreds of years. The powdery mildew fungus, *Erysiphe necator*, originated in the eastern United States and spread to Europe and other grape-producing regions (6), causing significant economic losses due to decreases in yield and quality, and increased fungicide applications. Despite our knowledge of the biology of the fungus and its interaction with *V. vinifera*, the durable mechanisms by which most wild North American grapevines tolerate powdery mildew have not been elucidated.

In the powdery mildew life cycle, a conidiospore germinates, forms an appressorium, and attempts to penetrate through the plant cuticle and cell wall. On a susceptible vine, it establishes a haustorium to obtain nutrients and to secrete effectors that inhibit the immune response. Plant tissues are colonized by extension and branching of hyphae, producing more appressoria and associated haustoria (26,30). In contrast, resistant plants have developed mechanisms to prevent the invasion of biotrophic pathogens. Both, preformed physical and chemical barriers (e.g., leaf surface wax and antimicrobial metabolites) and pathogen associated molecular patterns (PAMP)-triggered immunity (PTI)—also known as basal

defenses—play a relevant role in resistance against plant pathogens. In the vast majority of plant–pathogen interactions, the complex PTI response is able to prevent plant colonization. However, effector molecules released by fungal haustoria may interfere with PTI and modify the interaction toward compatibility (33). In response, plants have evolved a second layer of protection, effector-triggered immunity (ETI), characterized by a gene-for-gene interaction (21) mediated by R-genes that recognize specific effectors, triggering a cascade of reactions. As such, attacked cells undergo programmed cell death (PCD), thus limiting pathogen growth and survival by obstructing their nutrient uptake (33) and restoring resistance.

In general, the mechanisms of qualitative resistance (caused by a single gene) are better understood than quantitative resistance (due to the action of several loci). Hypotheses for quantitative disease resistance can be classified as (i) genes that regulate morphological or developmental phenotypes, (ii) alleles of genes involved in basal defense, (iii) chemical components of the plant–pathogen interaction, (iv) loci involved in defense signal transduction, (v) weak forms of R-genes, or (vi) unique forms of genes previously unidentified (41). R-genes conferring qualitative resistance against powdery mildews have been quickly overcome (10), and attempts to improve the durability by the stacking of multiple R-genes or multiple alleles of a single R-gene have only slightly extended the durability of resistance (7). In contrast, stacking of R-genes and quantitative resistance genes has been suggested to provide a more durable approach (8,39).

Genes responsible for qualitative resistance to powdery mildew have been identified, mapped, and characterized; the R-genes *Run1* (2,5,18,40), *Ren1* (11,31), and *Ren4* (37,42,44) are dominant genes that provide qualitative resistance, usually accompanied by PCD. Utilization and stacking of some of these major genes in grapevine breeding programs has been reported (15,34).

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*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

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Quantitative resistance to powdery mildew has been observed in several North American species, including *V. rupestris* (4). *V. rupestris* is a powdery mildew host exhibiting quantitative variation in foliar resistance among *V. rupestris* accessions (9,14,45), as well as within single accessions tested in different environments (9). Microscopic analysis of the resistance mechanism of one genotype of *V. rupestris* indicated quantitative resistance, characterized by quantitatively reduced penetration success rate when compared with *V. vinifera* and low levels of PCD (18). Major quantitative trait loci (QTLs) responsible for quantitative powdery mildew resistance have been identified from North American sources. These QTLs are *Ren2* from *V. cinerea* (12,13); *Run2.1*, *Run2.2*, and *Run5* from *V. rotundifolia* (3,44); and *Ren3* from the hybrid cultivar Regent, whose pedigree includes *V. aestivalis*, *V. berlandieri*, *V. cinerea*, *V. labrusca*, *V. lincedumii*, *V. riparia*, and *V. rupestris* (20). Finally, the moderate QTL *Sen1* was associated with alleles from 'Chardonnay' that increased powdery mildew severity (1).

In this work we sought to understand the genetics and mechanisms of powdery mildew resistance in *V. rupestris* B38 through a quantitative study of disease segregation in an F₁ family derived from the cross of *V. rupestris* B38 × *V. vinifera* 'Chardonnay'. Disease progression was studied using natural infections in the field and single isolate inoculations of potted vines. Furthermore, quantitative microscopy was used to compare mechanisms and powdery mildew isolate specificity of resistance in *V. rupestris* with the susceptible parent 'Chardonnay', and to study the response of progeny to single-isolate detached-leaf inoculations.

MATERIALS AND METHODS

Plant material. An F₁ family of 84 vines was obtained from the cross of *V. rupestris* B38 (resistant) and *V. vinifera* 'Chardonnay' (susceptible) in 2008. Seedlings were planted first to a nursery and then established in a vineyard and cultivated as described in Barba et al. (1). Captan 80WPG, which provides no powdery mildew control under vineyard conditions, was used to control downy mildew so that otherwise healthy leaf tissue could be rated for powdery mildew resistance. A subset of the family and parental vines were vegetatively propagated in 2010 as follows: dormant cuttings were taken from the vineyard in early December, stored at 4°C for 5 weeks, and potted in a mixture of perlite/soil (3:1) with bottom heat at 26°C until sufficient rooting took place. Vines were then grown in a greenhouse under a 16 h photoperiod at 27 to 30°C. Vines were pruned in order to assure uniform vegetative growth when needed.

Powdery mildew isolates. Isolates were obtained from the sources described in Table 1 and propagated weekly on 'Chardonnay' leaves as follows: leaves were surface-sterilized with 1% sodium hypochlorite for 5 min, rinsed twice with sterile distilled water, and placed in plates containing sterile 1% agar. A leaf infected with a single isolate was tapped over a newly plated leaf. Plates were sealed with parafilm and incubated at 25 ± 2°C with a 16 h photoperiod.

TABLE 1. Description of *Erysiphe necator* isolates used, including isolate name, genotype of source vine, resistance status of the source vine, and location

Isolate name	Vine genotype	Vine resistance	Location
LN YM	<i>V. vinifera</i> 'Merlot'	Susceptible	Lockport, NY ^a
NY19	<i>V. vinifera</i> 'Chardonnay'	Susceptible	Burdett, NY ^a
NY90	<i>V. vinifera</i> 'Chardonnay'	Susceptible	Burdett, NY ^a
RoACS	<i>V. vinifera</i> 'Cabernet Sauvignon'	Susceptible	Hurdle Mills, NC ^b
NY1-137	Interspecific hybrid	<i>Run1</i>	Geneva, NY ^c
RoAwms3	<i>V. rotundifolia</i> wild type	Presumed resistant	Hurdle Mills, NC ^b

^a L. Cadle-Davidson, unpublished data.

^b Frenkel et al. (22).

^c Cadle-Davidson et al. (10).

Quantification of powdery mildew penetration and colony formation on detached leaves. One hundred germinated conidia per sample (either whole leaf or leaf disc) were observed with a compound microscope and classified as having (i) a primary hypha leading to an appressorium, (ii) a single unbranched secondary hypha at least twice as long as the conidium, or (iii) multiple or branching secondary hyphae. Penetration success rate was quantified as the proportion of germinated conidia (categories i, ii, and iii) in categories ii and iii. Colony success rate was quantified as the percentage of penetrating conidia (categories ii and iii) in category iii.

Segregation of powdery mildew severity on vines. Powdery mildew infection was scored on F₁ vines subject to natural disease pressure for 3 years in the vineyard. The first field evaluation was conducted once in early October 2009 using a visual index tool that classified infected vines in four categories (Table 2). This system was replaced by the IPGRI scale established by the Organization Internationale de la Vigne et du Vin utilizing a disease progression scale ranging from 1 (no visible mycelium) to 9 (strong sporulation and abundant mycelium) as described by the International Union for the Protection of New Varieties of Plants (32). This change was made to provide a better classification of infected vines, where differences between genotypes were observed (see last row in Table 2), and to standardize field evaluations among international research groups (31,40). Field assessments conducted between July and September 2010 and 2011 used the IPGRI scale. In order to observe the progression of the disease during 2010, a total of four field assessments were conducted: 9, 20, and 30 August and 7 September, by which time susceptible checks were heavily infected. In 2011, field assessments were conducted on 12 July and 18 August. Pearson's correlation coefficients (*r*) between assessments were calculated.

Single isolate inoculation was performed on replicated potted plants of 37 F₁ genotypes propagated from dormant hardwood cuttings and grown in a screenhouse. Vines were infected with *E. necator* isolate NY90 (Table 1) using heavily sporulating colonies on 'Chardonnay' vines as a source of inoculum. Distribution of conidia was achieved by moving a box fan through each screenhouse for 2 min. Disease severity was recorded 7, 10, and 14 days postinoculation (dpi) as the proportion of leaf surface covered by powdery mildew. Vines were organized in two replicated screenhouses, with each being a completely randomized block. Least square means were determined by analysis of variance (ANOVA) using factorial combinations of predictors: vine genotype, block, and dpi.

Foliar ontogenic resistance of *V. rupestris* B38 and *V. vinifera* 'Chardonnay'. Leaf position was determined starting from the shoot tip with the first unfolded leaf designated as position one. Duplicate leaf samples from positions two to five were collected from greenhouse grown *V. rupestris* B38 and 'Chardonnay' vines. Leaves were prepared as described above. Inoculation was performed by spraying leaves with a suspension of

TABLE 2. Evaluation scale for powdery mildew on grapevine leaves in 2009

Score	Description	IPGRI comparison
0	Absent	1: Very low (tiny spots or no symptoms, neither visible sporulation nor mycelium)
1	Less than five small spots	3: Low (limited patches <2 cm in diameter, limited sporulation and mycelium)
2	Five to twenty spots, growing	5: Medium (patches usually limited with a diameter of 2 to 5 cm)
3	Widespread powdery mildew, dense sporulation	7: High (vast patches, some limited, strong sporulation and abundant mycelium) to 9: Very high (very vast unlimited patches or totally attacked leaf blades, strong sporulation and abundant mycelium)

10⁵ conidia/ml of 10-day-old LNYM in 0.001% Tween 20. Plates containing infected leaves were air-dried in a sterile hood before being sealed with parafilm and incubated at 25 ± 2°C for 2 days. Powdery mildew structures were stained using Coomassie dye (42). Quantification of powdery mildew penetration and colony formation was performed as described above.

The experiment was repeated twice. Least square means were determined by ANOVA using factorial combinations of predictors: vine genotype, experiment, and leaf position (nested within vine genotype). Mean differences were compared using Tukey's honest significant difference (HSD) test at $\alpha = 0.05$.

Isolate specificity on *V. rupestris* B38 and *V. vinifera* 'Chardonnay'. Fully expanded leaves at the fourth position from *V. rupestris* B38 and 'Chardonnay' were collected from noninfected greenhouse vines and surface sterilized as described above. For each powdery mildew isolate described in Table 1, two water agar Petri dishes containing four leaf discs (1 cm diameter) from each parent were infected by touching 10-day-old sporulating colonies to the adaxial leaf disc surface. Plates were incubated at 25 ± 2°C for 2 days. After trypan blue staining, fungal structures and host cell death under appressoria were evaluated (18). Quantification of powdery mildew penetration and colony success rate per plate were determined as described above.

The experiment was repeated twice with a completely balanced design. Least square means were determined by ANOVA using factorial combinations of predictors: vine genotype, powdery mildew isolate and experiment. Mean values were compared using Tukey's HSD test at $\alpha = 0.05$.

Segregation of powdery mildew severity on detached leaves. Leaves from a subset of 53 F₁ vines and parents were used to quantify the segregation of resistance to *E. necator* LNYM (Table 1) under controlled in vitro conditions. Leaves at the fifth position from two growing shoots per vine were labeled, detached, and placed in distilled water for transport. Surface sterilization, spray inoculation with *E. necator* LNYM, incubation, Coomassie staining, and quantification of powdery mildew penetration and colony success rate were performed as described above. The experiment was repeated twice with a completely balanced design.

Least square mean values were determined by ANOVA using predictors: vine genotype and experiment.

RESULTS

Segregation of powdery mildew severity on vines. Powdery mildew severity segregated with a pattern of continuous variation whether tested under natural disease pressure in the field during three seasons (Fig. 1A to C) or with single-isolate inoculations on potted vines (Fig. 1D). Under field conditions, *V. rupestris* had little to no powdery mildew severity throughout the growing season, while 'Chardonnay' became heavily infected early in the season. Pearson's correlation coefficients (*r*) were strongest among time points within an epidemic, such as vineyard severity at various time points within a year (Table 3). In addition, individual F₁ vines with low powdery mildew severity in August 2010 also had low severity in August 2011, even though their overall *r* values ranged from 0.30 to 0.42. At these late time points, vines 03, 18, 35, 39, 44, 50, 67, 70, and 74 had consistently lower severity than the progeny average (Supplementary Table S1). Quantitative segregation was also observed when measured as mean powdery mildew leaf coverage at 7, 10, and 14 dpi in the potted vine assay (Fig. 1D), with genotype being a significant predictor (*P* = 0.0001). These single-isolate data from potted vines had a moderate correlation with some natural powdery mildew severity ratings in the vineyard, such as 14 dpi versus field rating on 08.20.2010 (*r* = 0.56) or on 07.12.2011 (*r* = 0.39) (Table 3).

Foliar ontogenic resistance of *V. rupestris* B38 and *V. vinifera* 'Chardonnay'. Both leaf position and vine genotype were significant predictors of the penetration success rate (*P* < 0.0001). For both *V. rupestris* B38 and 'Chardonnay', penetration success rate remained stable from the second to fourth leaf, with a reduction at the fifth leaf that was only significant in *V. rupestris* (Fig. 2). Mean differences between *V. rupestris* B38 and *V. vinifera* 'Chardonnay' penetration at leaf positions two, three, and four were 45.5, 44.0, and 46.8%, respectively, while the mean difference at leaf position five increased to 60.3% (Fig. 2). Differences in colony success rate among leaf positions were not significant.

Fig. 1. Frequency distribution (violin plot) of powdery mildew disease progression on the progeny of *Vitis rupestris* B38 (♦) × *V. vinifera* 'Chardonnay' (⊗) measured as follows. **A**, Severity on naturally infected field vines in 2009 using a 0 to 3 severity index (Table 2). **B**, Severity on naturally infected field vines on four dates (month.day) during the 2010 growing season, using the IPGRI scale to track disease progression (32). **C**, Severity on naturally infected field vines on two dates during the 2011 growing season, using the IPGRI scale. **D**, Least square mean leaf coverage 7, 10, and 14 days postinoculation by isolate NY90 on potted vines, measured as a percentage. The black bar within each violin indicates the interquartile range, the white dot indicates the mean value, and the overlaid gray area indicates the probability density. Symbols ♦ and ⊗ indicate the value for parental genotypes *V. rupestris* B38 and *V. vinifera* 'Chardonnay', respectively.

Isolate specificity on *V. rupestris* B38 and *V. vinifera* ‘Chardonnay’. Vine genotype was the most significant predictor of penetration and colony success rates at the fourth leaf position ($P < 0.0001$), while powdery mildew isolate was a significant factor for penetration success rate ($P < 0.0001$), but not for colony success rate ($P = 0.1246$). On *V. vinifera* ‘Chardonnay’, penetration success rates did not differ significantly among the six isolates. Penetration success rates ranged from 77 to 93% on ‘Chardonnay’, and 95 to 99% of penetrating conidia formed a colony (Fig. 3). In contrast, the penetration success rate on *V. rupestris* B38 was significantly lower ($P < 0.001$) and varied among isolates ($P < 0.001$). *Erysiphe necator* isolates collected from *V. vinifera* ranged from 49 to 26% of the germinated conidia, while isolate RoAwmus3 (from *V. rotundifolia*) had a 16% penetration success rate. The least virulent *E. necator* isolate on *V. rupestris* B38 was NY1-137, collected from a hybrid vine with the *Run1* gene from *V. rotundifolia* (10), with a 7% penetration success rate (Fig. 3). Host cell death under appressoria was infrequent at 48 h postinoculation in ‘Chardonnay’ and *V. rupestris* B38 leaves (Fig. 4).

Segregation of powdery mildew severity on detached leaves. On the fifth fully expanded leaf, penetration success rate of *E. necator* LNYM segregated with a pattern of continuous distribution (Fig. 5). LNYM penetrated ‘Chardonnay’ with a 10-fold higher success rate than *V. rupestris* B38. Once penetration occurred, the colony success rate for the susceptible parent and F_1 progeny was close to 100%, and never lower than the resistant parent (80%) (Fig. 5). In both penetration and colony ratings, genotype was a significant predictor ($P < 0.0001$).

DISCUSSION

We characterized *V. rupestris* B38 resistance to powdery mildew and found continuous variation in disease severity, supporting previous findings of polygenic inheritance of powdery mildew resistance in *V. rupestris* (4). Factors involved in quantitative disease resistance were analyzed, showing evidence for enhanced penetration resistance and increased ontogenic resistance in *V. rupestris* B38 compared with ‘Chardonnay’, along with a differential response to pathogen genotypes, most notably including enhanced resistance to an isolate able to overcome *Run1* gene. Together, these findings allowed us to hypothesize that *V. rupestris* resistance is due to the action of several mechanisms, making it an interesting source of quantitative resistance to powdery mildew that may enhance the durability of *Run1* resistance.

Resistance in *V. rupestris* B38 $\times$ *V. vinifera* ‘Chardonnay’ progeny segregated in a quantitative manner. While *V. rupestris* B38 had little to no powdery mildew infection throughout the season, the mean infection of the progeny tended toward susceptibility by

the end of the season, but lagged behind the susceptibility of ‘Chardonnay’ (Fig. 1). Quantitative variation in the resistance response can be due to genetic and environmental effects. In this work, powdery mildew resistance was evaluated over 3 years, and a quantitative segregation pattern was observed in every instance, with a moderate correlation between field seasons (Table 3). Compared with other crops where replicated field trials are often used, cost and labor requirements restrain the number of vines placed in a vineyard, limiting the use of replicate vines in favor of planting more genotypes. In order to reduce environmental variance, a subset of the segregating family was screened with a single isolate in both replicated potted vine assays and detached leaf assays. Those results corroborated the continuous variation pattern found in field assessments, and ANOVA showed significance of vine genotype in the prediction of the observed phenotype for both the potted vine assay ($P < 0.0001$) and detached leaf assay ($P < 0.0001$).

Furthermore, in our experiments, *V. rupestris* resistance did not resemble the qualitative resistance from other *Vitis* sources. Our results are strikingly different from the observed phenotypes on *Ren4* or *Run1* vines, where no powdery mildew was observed on resistant genotypes, with test crosses segregating in a 1:1 ratio in either field or greenhouse evaluations (2,40,42). Moreover,

Fig. 2. Effect of leaf position on *Erysiphe necator* isolate LNYM penetration success rate on parent genotypes *Vitis vinifera* ‘Chardonnay’ and *V. rupestris* B38. The ability of powdery mildew to penetrate was calculated as the percentage of germinated conidiospores forming at least one secondary hypha by 2 days postinoculation. Error bars indicate 95% confidence limits, and letters above bars indicate statistically significant differences in penetration success rate (Tukey’s honest significant difference test at $P < 0.05$).

TABLE 3. Correlations between powdery mildew ratings^a

Type of inoculum	Assay condition	Observation	10.08.09	08.09.10	08.20.10	08.30.10	09.07.10	07.12.11	08.18.11	7 dpi	10 dpi	14 dpi	Penetration	Colony
Natural	Field	10.08.09	1.00											
		08.09.10	0.38	1.00										
		08.20.10	0.38	0.61	1.00									
		08.30.10	0.26	0.45	0.66	1.00								
		09.07.10	0.40	0.55	0.60	0.56	1.00							
		07.12.11	0.07	0.37	0.37	0.18	0.22	1.00						
		08.18.11	0.19	0.42	0.35	0.30	0.29	0.44	1.00					
Single isolate	Potted vines	7 dpi	0.14	0.02	0.25	0.19	0.36	0.06	0.36	1.00				
		10 dpi	-0.02	0.35	0.48	0.23	0.39	0.24	0.35	0.61	1.00			
		14 dpi	0.16	0.31	0.56	0.32	0.43	0.39	0.36	0.49	0.77	1.00		
		Penetration	0.03	0.10	0.19	0.17	-0.14	0.22	0.03	-0.09	0.27	0.10	1.00	
	Petri dish	Colony	0.00	0.10	0.07	0.02	-0.21	0.10	0.05	0.16	0.39	0.22	0.76	1.00
		Number of genotypes	82	84	84	84	84	72	72	37	37	37	53	53

^a Experimental conditions, number of individuals per observation, and Pearson’s correlations are indicated. Vineyard rating dates (month.day.year). Potted vine severity was rated 7, 10, and 14 days postinoculation (dpi) with *Erysiphe necator* isolate NY90. Detached leaves in Petri dishes were rated for penetration and colony success rate 48 h postinoculation with *E. necator* isolate LNYM.

microscopic characterization of *E. necator* interactions with vine genotypes bearing *Run1* or *Ren1* genes have shown the presence of PCD under appressoria associated with ETI responses triggered by R-genes (17,31). In contrast, we did not observe frequent host cell death with any isolates tested on *V. rupestris* B38. All these differences lead us to reject the hypothesis that the *V. rupestris* B38 mechanism of resistance to powdery mildew is controlled by a single R-gene, and raises the question of which mechanisms contribute to its quantitative resistance.

Penetration resistance. One mechanism of resistance of *V. rupestris* B38 was a reduction in penetration success rate of *E. necator* at 2 dpi on all leaf ages observed (Fig. 2). An increased number of conidia were arrested at the appressorium formation stage (Figs. 3 and 4B), compared with 'Chardonnay' where the vast majority of germinated conidia were able to penetrate and create a colony (Figs. 3 and 4A). In their progeny, the penetration success rate segregated in a quantitative pattern skewed toward susceptibility, which suggests that more than one allele and/or locus from *V. rupestris* B38 may be required for full resistance (Fig. 5), possibly combined with the action of susceptibility alleles from the *V. vinifera* parent (1). Here, we describe the *V. rupestris* B38 response as quantitative penetration resistance with no significant PCD observed. This agrees with previous findings following inoculation with another powdery mildew isolate onto a different *V. rupestris* genotype, which also had quantitative resistance, characterized by having 44% of germinated spores failing to penetrate, and low levels of PCD (18). In *V. rupestris* B38 we found significant differences in the ability to penetrate the epidermis and establish secondary hypha, accompanied by a high percentage of successful colonies once penetration had been achieved (Fig. 3). Also, the colony success rate was consistently high across segregating progeny (90 to 100%, Fig. 5), indicating that, unlike *Ren1* (31), *V. rupestris* B38 mechanisms of resistance do not slow colony formation. That is, once a haustorium is established and a secondary hypha is formed, there is minimal resistance to the formation of a colony.

Penetration resistance can be due to the effect of preformed barriers or of PTI. In *Vitis* species, the involvement of these mechanisms in the interaction with powdery mildew has been reported. At the transcriptome level, susceptible *V. vinifera* vines responded with defense oriented reprogramming that failed to prevent powdery mildew infection, while a resistant cultivar, the *V. aestivalis*-derived interspecific hybrid cultivar Norton, showed enhanced transcription of a few genes related to basal defense, which corresponded with a quantitatively increased penetration-resistance phenotype (24).

Other studies have focused on critical pathways for the nonhost response, such as actin cytoskeleton formation and vesicle trafficking. While actin cytoskeleton formation was shown to be partially required for nonhost and host resistance, vesicle trafficking was

Fig. 4. Interaction of *Erysiphe necator* isolate NY1-137 on *Vitis vinifera* 'Chardonnay' and *V. rupestris* B38. **A**, Compatible interaction of isolate NY1-137 on *V. vinifera* 'Chardonnay' showing appressorium (app ▼), haustorium (▲ hau), and multiple hyphae (▲ hy). **B**, Incompatible interaction (penetration resistance) of isolate NY1-137 on *V. rupestris* B38, characterized by a high frequency of germinated conidia arrested in appressorium formation (app ▼). Scale bars = 50 μ m.

Fig. 5. Frequency distribution (violin plot) of penetration success rate and colony success rate of single isolate LNYM on the progeny of *Vitis rupestris* B38 (♦) $\times$ *V. vinifera* 'Chardonnay' (⊗). Penetration was measured as the percentage of germinated conidiospores forming at least one secondary hypha, and colony success rate as the percentage of penetrating conidiospores generating multiple hyphae. A black bar within each violin indicates the interquartile range, white dot indicates the mean value of the sample, and the overlaid gray area indicates the probability density of the sample.

Fig. 3. Quantification of the response of *Vitis vinifera* 'Chardonnay' and *V. rupestris* B38 to *Erysiphe necator* isolates ROACS, NY90, NY19, LNYM, NY1-137, and RoAwmus3. Powdery mildew development at 2 days postinoculation was categorized as the percentage of conidia with appressorium only, a single secondary hypha, or a colony with multiple secondary hyphae. Nonoverlapping letters to the right of the bars indicate statistically significant differences in penetration success rate measured as the percentage of germinated conidiospores forming at least one secondary hypha (Tukey honest significant difference test at $P < 0.05$).

shown to be required by *E. necator* to establish a compatible interaction with susceptible grapevines (18). We favor a hypothesis that the specific mechanism involved in *V. rupestris* B38 enhanced penetration resistance could relate to one or more quantitative factors underlying basal defenses, with the effectors of some isolates (e.g., NY1-137) unable to overcome the basal defenses.

Isolate specificity. Variation in powdery mildew response to *V. rupestris* has been reported under different conditions. In Cadle-Davidson et al. (9,10) a detached leaf assay with *V. rupestris* B38, with a single powdery mildew isolate, showed no infection 21 dpi, but natural infection was observed at two field locations. In our experience, *V. rupestris* B38 showed no infection in the nursery (year 1) or in its first year of establishment in a vineyard (year 2), but low levels of infection in year 3 (Fig. 1). Seasonal variation can be due to maturity differences of the vines, variable inoculum pressure, environment, and canopy density. In addition, some of the observed variation may be explained by genotypic differences of pathogen populations over time. In our experiments, seedlings were maintained in a no-spray nursery with extreme levels of powdery mildew on some vines, and then planted to a permanent vineyard in which ‘Chardonnay’ vines with high levels of powdery mildew were interspersed (Fig. 1A to C). This local source of inoculum and capacity for recurrent adaptation and selection on vines carrying resistance alleles could explain the earlier appearance of symptoms observed each year (Fig. 1), the switch in relative resistance of some progeny over seasons and the lower correlation among years ($r < 0.42$, Table 3), compared with correlations within years ($r > 0.44$, Table 3). This, combined with the evidence for races within powdery mildew populations (43) and the high genetic diversity found among isolates from Geneva, NY (23), created an argument for studying the interaction of *V. rupestris* B38 with a panel of *E. necator* isolates of powdery mildew and a single-isolate assay of the progeny in order to reduce the influence powdery mildew genetic variation on resistance ratings (Fig. 1D).

Although we did not observe a classic qualitative gene-for-gene interaction of *V. rupestris* B38 with *E. necator*, at a microscopic level it was possible to detect significant differences in penetration success rate among powdery mildew isolates, without frequent cell death under the appressorium in all cases. Isolates coming from *V. vinifera* sources showed significantly higher penetration success rates on *V. rupestris* B38 than those isolated from resistant vines, including *V. rotundifolia* or interspecific hybrid vines with the *Run1* gene introgressed from *V. rotundifolia* (Fig. 3, Table 1). Consistent with previous findings (22,23,25), isolates showed no significant differences in growth on *V. vinifera*. Thus, the continuous variation observed in the virulence of isolates on *V. rupestris* B38 reflects a differential response of each isolate to resistance loci rather than a fitness cost. This continuous variation could be due to variation among isolates in effectors of small additive consequence involved in the suppression of basal defenses.

These results contrast with studies in transgenic vines carrying the *Run1* locus, where powdery mildew isolates from *V. rotundifolia* were able to overcome the *Run1* gene, and powdery mildew isolates from *V. vinifera* sources were stopped by a hypersensitive response characterized by PCD (17). Our findings suggest that *V. rupestris* B38 powdery mildew resistance may complement the effect of *Run1*, helping prolong the durability of this resistance gene.

In *V. vinifera* ‘Chardonnay’, all isolates showed similar high levels of penetration (Fig. 3). The ease of infection on ‘Chardonnay’ leaves regardless of the isolate may indicate the presence of susceptibility factors that allow *E. necator* to infect ‘Chardonnay’ more efficiently than *V. rupestris* B38. This observation correlates with recent findings of a susceptible QTL in ‘Chardonnay’ (1).

Developmental stage. In other crops like barley or wheat, the genetics of adult-plant resistance are characterized by slowing powdery mildew infection and extending the latent period, thereby providing incomplete but durable resistance (35,36,46). In grapevines, a direct relationship between developmental stages and

powdery mildew resistance has been reported; from broad observations on field assessments (16,28,47) to detailed characterization of penetration resistance on berries (9,19,27,29), and macroscopic resistance on grapevine leaves (14,38).

Here we showed that the ability of *E. necator* to penetrate and establish a colony was also influenced by leaf maturity. We observe both an overall differential penetration success rate between *V. rupestris* B38 and ‘Chardonnay’ and significant differences among leaf positions within each vine genotype (Fig. 2). Maximum severity of powdery mildew penetration was observed at the third leaf for both *V. rupestris* B38 and ‘Chardonnay’ (50.5 and 94.5%, respectively), which has been correlated with the carbohydrate sink-to-source transition (38). We observed that *V. rupestris* B38 developed resistance faster than ‘Chardonnay’, showing a further reduction of penetration success rate from 50.5% in the third leaf to 10.3% in the fifth leaf, compared with a reduction from 94.5% in the third leaf to 70.7% in the fifth leaf of ‘Chardonnay’. Thus, in addition to enhanced penetration resistance, *V. rupestris* B38 vines deploy a less susceptible canopy compared with ‘Chardonnay’.

Isolate-specific segregation. Here we presented a quantitative method to assess the response of the progeny against a single isolate. Using this approach, we observed a quantitative segregation pattern for penetration success rate at 2 dpi in progeny from *V. rupestris* B38 and ‘Chardonnay’ and uniformly compatible responses after penetration of isolate LNYM. Given the control of environmental and pathogen variability at a precise leaf age, this approach is a simplification of the conditions of a vine in the field. While detached leaf assays enable controlled screening for the dissection of resistance mechanisms and potentially the underlying quantitative trait loci, detached leaf assays likely do not capture the complete basis of field resistance. Thus, we recommend the continued use of field trials as a complementary tool for resistance evaluation and selection.

In this work, we showed that *V. rupestris* B38 resistance to powdery mildew segregates in a quantitative manner under field as well as controlled conditions. The components of resistance include: isolate-specific penetration resistance that is enhanced on older leaves, with neither prominent PCD nor colony resistance. Moreover, we identified *V. rupestris* B38 as highly resistant to penetration for powdery mildew isolates able to overcome *V. rotundifolia*-derived resistance. Based on this observation, we suggest the use of *V. rupestris* B38 as a genetic source of quantitative resistance to powdery mildew, which when combined with other sources of resistance such as *Run1*, may provide improved and extended resistance against powdery mildew.

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