A Single Dominant Locus, *Ren4*, Confers Rapid Non-Race-Specific Resistance to Grapevine Powdery Mildew

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ABSTRACT

Ramming, D. W., Gabler, F., Smilanick, J., Cadle-Davidson, M., Barba, P., Mahanil, S., and Cadle-Davidson, L. 2011. A single dominant locus, *Ren4*, confers rapid non-race-specific resistance to grapevine powdery mildew. Phytopathology 101:502-508.

In the present study we screened the progeny of *Vitis vinifera* × *V. romanetii* populations segregating for resistance to powdery mildew and determined the presence of a single, dominant locus, *Ren4*, conferring rapid and extreme resistance to the grapevine powdery mildew fungus *Erysiphe necator*. In each of nine *Ren4* pseudo-backcross 2 (pBC_2) and pBC_3 populations (1,030 progeny), resistance fit a 1:1 segregation ratio and overall segregated as 543 resistant progeny to 487 susceptible. In full-sib progeny, microscopic observations revealed the reduction of penetration success rate (as indicated by the emergence of secondary hyphae) from 86% in susceptible progeny to below 10% in resistant progeny. Similarly, extreme differences were seen macroscopically. Ratings for *Ren4* pBC_2 population 03-3004 screened using natural infection in a California vineyard and greenhouse and using artificial inoculation of an aggressive New York isolate were fully consistent among all three pathogen sources and environments. From 2006 to 2010, *Ren4* pBC_2 and pBC_3 vines were continuously screened in California and New York (in the center of diversity for *E. necator*), and no sporulating colonies were observed. For population 03-3004, severity ratings on leaves, shoots, berries, and rachises were highly correlated (R^2 = 0.875 to 0.996) in the vineyard. Together, these data document a powdery mildew resistance mechanism not previously described in the Vitaceae or elsewhere, in which a dominantly inherited resistance prevents hyphal emergence and is non-race-specific and tissue-independent. In addition to its role in breeding for durable resistance, *Ren4* may provide mechanistic insights into the early events that enable powdery mildew infection.

Additional keywords: *Mlg*, PAMP-triggered immunity, *Uncinula necator*.

Powdery mildews are epiphytic pathogens of nearly all crop plants. Conidia landing on susceptible host tissues will germinate, form an appressorium for penetrating the cuticle and cell wall, and then form a primary haustorium inside a plant epidermal cell. If successful in evading host defenses, a secondary hypha will grow superficially, forming additional appressoria and haustoria as it extends and branches to colonize the plant surface. Being a multicyclic disease, this colonization can rapidly lead to an epiphytotic of massive proportions. Since all widely planted *Vitis vinifera* grape cultivars are susceptible to the powdery mildew fungus *Erysiphe necator* (syn. *Uncinula necator*), grape growers in the United States routinely apply 30 million pounds of sulfur every year in addition to chemicals with greater specificity to manage powdery mildew (30). Thus, grape cultivars with powdery mildew resistance would represent a significant financial and environmental improvement over their widely planted susceptible counterparts.

The lifecycle of powdery mildews can be disrupted by physical barriers as well as active strategies known as PAMP-triggered immunity (PTI) and effecter-triggered immunity (ETI) mounted by nonhost or host plants, respectively (19,23). PTI follows host recognition of broadly conserved pathogen associated molecular patterns (PAMPs), which are microbial products, such as chitin. Against powdery mildews, PTI reduces the incidence of primary haustorium formation and is exemplified by nonhost resistance, in which host-specific powdery mildews on nonhost plants can only germinate to form an appressorium and rarely develop further. ETI encompasses the resistance responses typical of most single, dominant resistance genes to-date in that it relies on the perception of a pathogen effector by a host receptor, typically a nucleotide binding leucine-rich repeat (NB-LRR) *R*-gene. As a result, ETI acts after formation of the haustorial feeding structure and secretion of effector proteins into the host epidermal cell, enabling some degree of hyphal growth, ranging from a single cell to a colony with trailing host necrosis (19). ETI is often overcome by the deletion or mutation of a single effector.

The inheritance, durability, and mechanisms of resistance to powdery mildew have been widely studied in barley, and the resultant data support PTI and ETI hypotheses (26,29). The recessively inherited resistance gene *mlo* confers a prehaustorial, non-race-specific resistance and is one of the primary models for PTI and nonhost resistance. *mlo* resistance is used in over 50% of barley acreage and has remained durable since first reported in 1942—exceptional in powdery mildew resistance (20). *Mlg* is a dominant resistance gene that also confers a prehaustorial penetration resistance but is race-specific (15). This suggests either that some effectors are secreted prior to haustorial formation, as in ETI resistance, or that isolates of the pathogen *Blumeria graminis* f. sp. *hordeii* are differential in the production of PAMPs

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that are recognized in *Mlg* PTI resistance. Other resistance genes in barley include *Mla* genes typical of posthaustorial gene-for-gene resistance (28) and partial resistance genes that reduce penetration success in older leaves (5).

Genetic characterization and breeding for fungal resistance in *Vitis* lag behind those in barley due to negative fruit quality characteristics associated with resistance sources and long selection cycles necessary for grape breeding (7,12,31). While not as data-rich as the barley pathosystems, resistance to powdery mildew in grapevine is being addressed through the identification of novel resistances, their mechanisms, and evaluation of their potential durability in the field. The *Run1* gene confers an ETI hypersensitive resistance phenotype that has been introgressed from the muscadine grape species *V. rotundifolia* into *V. vinifera* in a large (≈1 Mb) chromatin block with suppressed recombination spanning two NB-LRR multigene families (2). While this locus shows promise for typical dominant *R*-genes, its incorporation into grape cultivars using traditional methods may be difficult because of linkage drag associated with the recombination-suppressed block of muscadine chromatin surrounding the resistance gene(s).

Although minor quantitative variation in the degree of susceptibility of *V. vinifera* cultivars has been documented (22), the species had been considered to be universally susceptible to powdery mildew. However, the first case of qualitative resistance (*Ren1*) was recently reported in the Central Asian *V. vinifera* cultivar Kishmish vatkana and manifests as a lack of macroscopic symptoms. Microscopically, however, a cell-death response occurs on a much slower timeframe that *Run1*-mediated HR and hyphal growth is merely slowed, not stopped in resistant individuals (16). Characterized as a single, dominant resistance gene, *Ren1* has been localized to a region of the genome containing NB-LRR sequences, which is in keeping with its post-haustorial ETI resistance phenotype.

Evidence exists for the presence of grape powdery mildew races (13) and this being the case, resistance genes exhibiting ETI are likely to be overcome within a few years of deployment (14,19). Consequently, both *Ren1* and *Run1* may be of short-lived utility given their probable modes of action. While several *Mlo* orthologues have been identified in *V. vinifera* and may provide durable resistance, natural *mlo*-based resistance has not been identified in grapevine, and engineered cultivars may be many years away. Quantitative trait loci resistances such as *Ren2* and *Ren3* (7,33) found in North American (NA) germplasm may hold promise for durability of at least partial resistance. However, interspecific hybrids are associated with potentially negative fruit quality characters, and none other than *Run1* has been cloned to date, thus keeping negative linkage disruption reliant on traditional breeding methods, large population sizes, and years of evaluation.

Given these challenges to effective powdery mildew resistance using known resistance sources, a relatively untapped Asian germplasm pool may offer some promise. Powdery mildew resistance has been identified in thirteen different Asian *Vitis* species (31,32). In general, no Asian species proved to be as highly resistant as NA species, yet there was significant variation within species. These studies reported slightly differing resistance findings due to differences in germplasm screened; however, both concluded that *V. romanetii* and the closely related *V. davidii* should be of considerable value for the introgression of powdery mildew resistance into *V. vinifera* (31,32).

In the present study we screened the progeny of *V. vinifera* × *V. romanetii* pseudo- (or modified-) backcross (pBC) populations segregating for resistance to powdery mildew and determined the presence of a single, dominant locus conferring extreme resistance that prevents emergence of secondary hyphae. Both the resistance source and the resistance phenotype identified here are novel in the Vitaceae and thus we name this resistance locus resistance to *Erysiphe necator 4* (*Ren4*).

MATERIALS AND METHODS

Germplasm. *V. romanetii* 'C166-026' was obtained from the USDA-ARS repository in Davis, CA. A segregating pBC_2 population 03-3004 was generated (n = 57), as described in Tables 1 and 2, and was established in the greenhouse. For each segregant, 14 dormant cuttings were taken with the goal of obtaining seven healthy plants: three plants for greenhouse evaluation; two for microscopy and laboratory evaluation; and two for field planting. Cuttings were rooted by dipping in Hormex No. 8 rooting powder (Brooker Corp., Chatsworth, CA) and sticking them in sand over bottom heat of 26°C. When roots were 2 to 5 cm long, plants were potted, staked, and trained. As part of the breeding program, seven additional pBC_2 populations and one pBC_3 population were developed by cross-hybridizing related, resistant progeny to *V. vinifera* breeding lines (Table 2).

Greenhouse disease evaluation. Selections were screened for resistance to powdery mildew as young vines in greenhouses maintained at the USDA-ARS San Joaquin Valley Agricultural Sciences Center (Parlier, CA). Disease incidence at this age can predict the future incidence and severity of mildew infections on mature vines in the vineyard (22). Dormant grapevine cuttings taken in January 2006 were rooted and established in the greenhouse in February. Greenhouse vines that were several months to 1 year old were grown in 6 cm square × 18 cm tall Anderson pots (Anderson Die and Manufacturing, Portland, OR). Two powdery mildew susceptible *V. vinifera* 'Ruby Seedless' vines were placed in the middle of each tray containing 13 test vines to provide natural inoculum source for the test vines. Greenhouse powdery mildew assessments were conducted on two vine plots and evaluated for foliar disease incidence (percentage of leaves that exhibited powdery mildew symptoms) and disease severity, or coverage (percent leaf area infected). Symptoms were evaluated when 70% of susceptible control 'Ruby Seedless' leaves exhibited sporulating colonies. After the first evaluation in September 2006, the epidemic was allowed to progress further for a second evaluation in November 2006. The presence of mycelia was confirmed by microscopy. Incidence and severity scores were averaged and

TABLE 1. Pedigree of parents developed in this study of *Ren4* resistance to *Erysiphe necator*[a]

Generation[b]	Female	Male	Resistant progeny
F1	***C166-026***	*V. vinifera*	***B36-44**** and ***B36-45****
pBC1	Raisin de Palestine	***B36-45****	***C87-14*** and ***C87-41****
pBC1	Rangspray	***B36-45****	***C87-106****
pBC1	B53-106*	***B36-44****	***B88-69****
pBC2	C70-76*	***C87-106****	***Y313-137****

[a] Resistant individuals are denoted in bold italics and seedless individuals are denoted with an asterisk (*). Resistant progeny listed here were selected as parents (here or Table 2) based on lack of powdery mildew symptoms in the field and other positive traits under selection in the breeding program, including seedlessness and fruit quality traits.

[b] The F1 cross was made by D. Cain with a *Vitis vinifera* breeding selection while at Sun World (Bakersfield, CA). pBC = pseudo-backcross to a *V. vinifera* genotype.

categorized as follows: resistant (R) 0 to 20%; moderate (M) 21 to 40%; and susceptible (S) 41 to 100%.

A similar approach was used to screen for resistance on young vines in greenhouses at the USDA-ARS Grape Genetics Research Unit (Geneva, NY). Seed of population 07-3553 were germinated in December 2007 and maintained in 8 cm square × 8 cm tall pots (T.O. Plastics, Clearwater, MN). Ratings were recorded for foliar disease incidence and disease severity when more than 70% of leaves on susceptible 'Chardonnay' seedlings exhibited sporulating colonies. After the first evaluation in August 2008, the epidemic was allowed to progress further for a second evaluation in September 2008. Due to the extreme phenotype of resistance, vines with any sporulating powdery mildew were rated as susceptible.

Vineyard disease evaluation. Plants were grown at USDA, ARS San Joaquin Valley Agricultural Sciences Center (latitude 36°81′N: longitude 119°72′W). The soil is a fine sandy loam and the vines were drip-irrigated. Vines were grown on their own roots at 4 m × 0.5 m spacing, using a single T-trellis with a 0.75 m cross-arm on a 2 m stake and were cane pruned. No fungicides were applied. 'Ruby Seedless' plants were interplanted every 15th vine as an inoculum source and to check for the amount of natural powdery mildew infection. Mildew assessments were performed between July and October for 3 years after the plants started fruiting in their third leaf. Disease severity was evaluated on leaf, shoot, rachis, and berry in order to determine tissue specificity of the resistance. Ratings on each tissue were recorded separately based on visual observation (1 = no infection [R]; 2 = very few small colonies; 3 = <50% coverage; 4 = >50% coverage [S]).

Laboratory disease evaluation. Detached leaves were collected from disease-free potted vines on 12 September 2006, from the USDA, Parlier, CA greenhouse. Up to eight leaves per genotype were collected: the fourth fully expanded leaf and a mature leaf, from two replicate shoots per vine and from two replicate vines per genotype. The leaves were stacked in a standardized order, stored in sealed bags at 4°C, and shipped on ice overnight to Geneva, NY. Upon receipt, leaves from a single vine were placed into a labeled, flexible plastic compact disk (CD) sleeve that had nine holes punched into it to facilitate wetting during subsequent leaf sterilization and washing. Leaves in CD sleeves were surface sterilized by submersion into calcium hypochlorite (0.88 g/liter) for 2 min with agitation and then washed three times in sterile distilled water for 5 min each. The leaves were removed from each CD sleeve and plated adaxial side up onto petri dishes (100 × 15 mm) containing 18 ml of 1% water agar amended with 0.01 g/liter natamycin (Haorui Pharma-Chem, Edison, NJ) to prevent growth of fungal contaminants on the agar. Residual water was evaporated by removing the petri dish cover in a sterile laminar flow hood. After preparation of a complete batch, inoculation was conducted using *E. necator* isolate 10-18-1 collected from a 'Chardonnay' plant in Dresden, NY in 2003 (4). Spore suspensions were made by shaking conidiating leaves in 40 ml of distilled water with 0.001% Tween 20 (Sigma-Aldrich, St. Louis, MO), and the concentration was adjusted to 5×10^4 conidia/ml using a hemacytometer. Leaves were inoculated with approximately 0.5 ml of the spore suspension using a Preval paint sprayer (Coal City, IL) and then placed into a 20 ± 2°C growth chamber with 12 h photoperiod. Coverage was rated at 21 days post-inoculation as the percentage of leaf area with powdery mildew mycelia. Due to the extreme phenotype of resistance, any colonies observed were interpreted as indicative of susceptibility.

Coomassie staining. Two susceptible genotypes (*V. vinifera* 'Riesling' and susceptible breeding progeny Y553-50) and two resistant breeding progeny (Y553-20 and Y553-27) were used. Y553 progeny are full siblings from population 07-3553. The third and fourth youngest leaves were detached, sterilized as described above, and placed in 1% agar (Acros, Geel, Belgium) for all assays. Leaves with actively sporulating colonies were used for inoculation by touching sporulating colonies directly to the leaf to be inoculated. Plates containing inoculated leaves were incubated at 20°C for 3 days. Four disks were collected from each leaf for staining with Coomassie blue (adapted from Doster and Schnathorst [8]), and the experiment was repeated twice. Leaf disks (1 cm^2) were collected using a cork borer and placed in a 24-well plate for clearing in 3:1, vol/vol, ethanol: acetic acid, changing the solution three to four times until the tissue was completely bleached and then transferred to 50% ethanol for long-term storage. The solution was briefly replaced with Coomassie stain (Coomassie Brilliant Blue R-250 [0.12 g/liter] [Sigma-Aldrich, St. Louis, MO] in 50% [vol/vol] methanol, and 10% [vol/vol] glacial acetic acid) to stain the mycelium, rinsed with several water changes, and mounted for viewing on a microscope slide in 50% glycerol.

Categorization was determined as follows: random samples of at least 50 spores per leaf disk were observed with a compound light microscope and categorized as (i) germinated spore with an appressorium, (ii) germinated spore with a secondary hypha, or (iii) germinated spore with multiple or branching secondary hypha. Penetration was defined as the proportion of spores within categories (ii) and (iii), whereas microcolony formation was defined as the proportion of spores within category (iii). Proportion and confidence limits (95%) of penetration and microcolony formation were determined using the method of Wilson (34).

Resistance gene characterization. Segregation ratios within each population were determined by pooling any classes with powdery mildew infection to give only two phenotypic groups because only 0.05% of the progeny were rated intermediate. Since powdery mildew resistance is rare in *V. vinifera* (fewer than 0.1%

TABLE 2. Powdery mildew resistance segregation ratios for *Ren4* crosses screened in Parlier, CA or Geneva, NY greenhouses

Population	Generation[a]	Female[b]	Male[b]	Severity ratings[c]	
				R	S
03-3004	pBC2	***C87-41****	B70-57*	38	19
07-3007	pBC2	***C87-41****	C58-37*	113	114
07-3008	pBC2	***C87-41****	A85-40*	99	71
07-3051	pBC2	***C87-106****	B82-43*	32	18
07-3052	pBC2	***C87-106****	A50-33*	45	46
07-3053	pBC3	B82-43*	***Y313-137****	57	55
07-3054	pBC2	B82-43*	***C87-106****	28	29
07-3056	pBC2	B82-43*	***B88-69****	40	41
07-3553	pBC2	***C87-14***	B82-43*	91	94
			Total	543	487

[a] pBC = pseudo-backcross to a *Vitis vinifera* genotype.
[b] Resistant individuals are denoted in bold italics and seedless individuals are denoted with an asterisk (*).
[c] The number of resistant (R) and susceptible (S) progeny are shown for each population. Due to the extreme phenotype of *Ren4* resistance, progeny with any sporulating powdery mildew were rated as susceptible.

of known cultivars), susceptible parents are assumed to be homozygous recessive (rr) at resistance loci. Taking into account the high heterozygosity of *Vitis* spp. and that resistance segregated in all crosses, the resistant parent in each cross is assumed to be heterozygous (Rr) for at least one resistance locus. To determine the number of genes segregating in a given population, phenotypic classes were tested with a χ^2 goodness of fit test against predicted ratios (i.e., 1R:1S single gene; 3R:1S two genes; etc.).

RESULTS

Progeny of the segregating population 03-3004 were either highly resistant or highly susceptible to powdery mildew, a result that was consistent between laboratory, greenhouse, and field disease screens (Table 3). Leaves of all resistant progeny had no macroscopically visible colonies, whereas leaves of sensitive progeny were completely covered with sporulating colonies (Fig. 1). Based on the above assumptions for the parental genotypes

TABLE 3. Disease ratings for progeny from *Vitis vinifera* × *V. romanetii* pseudo-BC_2 population 03-3004 challenged with three independent pathogen sources, under greenhouse, field, or laboratory conditions

	CA greenhouse		CA vineyard					NY detached leaf	
Segregant	Leaf index (%)[a]	Rating	Leaf index[b]	Rating	Shoot index	Rachis index	Berry index	Leaf coverage (%)[c]	Rating
Y315-55	0	R	1	R	1	1	1	0	R
Y315-56	0	R	1	R	1	1	1	0	R
Y315-57	0	R	1	R	1	–	–	0	R
Y315-58	0	R	1	R	1	1	1	0	R
Y315-61	0	R	1	R	1	–	–	0	R
Y315-62	0	R	1	R	1	–	–	0	R
Y315-63	0	R	1	R	1	1	1	0	R
Y315-64	0	R	1	R	1	1	1	0	R
Y315-67	0	R	1	R	1	–	–	0	R
Y315-70	0	R	1	R	1	1	1	0	R
Y315-71	0	R	1	R	1	–	–	0	R
Y315-72	0	R	1	R	1	1	1	0	R
Y315-73	0	R	1	R	1	–	–	0	R
Y315-74	0	R	1	R	1	–	–	0	R
Y315-76	0	R	1	R	1	–	–	0	R
Y315-77	0	R	1	R	1	–	–	0	R
Y315-78	0	R	1	R	1	–	–	0	R
Y315-79	0	R	1	R	1	–	–	0	R
Y315-80	0	R	1	R	1	–	–	0	R
Y315-81	0	R	1	R	1	–	–	0	R
Y315-82	0	R	1	R	1	–	–	0	R
Y315-83	0	R	1	R	1	1	1	0	R
Y315-84	0	R	1	R	1	–	–	0	R
Y315-85	0	R	1	R	1	1	1	0	R
Y315-86	0	R	1	R	1	–	–	0	R
Y315-87	0	R	1	R	1	1	1	0	R
Y315-88	0	R	1	R	1	–	–	0	R
Y315-89	0	R	1	R	1	–	–	0	R
Y315-90	0	R	1	R	1	1	1	0	R
Y315-91	0	R	1	R	1	–	–	0	R
Y315-92	0	R	1	R	1	–	–	0	R
Y315-94	0	R	1	R	1	–	–	0	R
Y315-97	0	R	1	R	1	–	–	0	R
Y315-98	0	R	1	R	1	–	–	0	R
Y315-99	0	R	1	R	1	1	1	0	R
Y315-93	0	R	1	R	1	1	1	*	*
Y315-95	0	R	1	R	1	2	1	*	*
Y315-96	0	R	1	R	1	1	1	*	*
Y315-65	95	S	4	S	4	4	2	100	S
Y315-66	92	S	4	S	4	–	–	90	S
Y315-68	98	S	4	S	4	4	3	100	S
Y315-69	98	S	4	S	4	–	–	90	S
Y315-75	50	S	4	S	4	–	–	90	S
Y315-100	95	S	4	S	3	4	2	60	S
Y315-101	96	S	4	S	4	–	–	50	S
Y315-102	64	S	4	S	4	–	–	20	S
Y315-109	97	S	4	S	4	–	–	20	S
Y315-103	98	S	4	S	4	4	2	*	*
Y315-105	97	S	4	S	4	4	4	*	*
Y315-106	96	S	4	S	4	–	–	*	*
Y315-107	96	S	4	S	4	4	4	*	*
Y315-108	97	S	4	S	4	4	4	*	*
Y315-110	96	S	4	S	4	4	3	*	*
Y315-111	97	S	4	S	4	–	–	*	*
Y315-113	97	S	4	S	4	–	–	*	*
Y315-112	97	S	4	S	4	–	–	nd	nd
Y315-114	98	S	4	S	4	–	–	nd	nd

[a] Mean disease rating (average of incidence and severity) in September 2006: 0 to 20 = resistant (R), 41 to 100 = susceptible (S).
[b] Vineyard rating: 1 = no visible powdery mildew (R), 4 = more than 50% coverage (S).
[c] Maximum coverage for any replicate leaf in 10% increments. Any amount of powdery mildew = S; no powdery mildew = R; nd = no data; * denotes three or fewer replicates survived as detached leaves.

used to generate these populations (heterozygous resistant × homozygous susceptible), the lack of nonparental phenotypes among the progeny suggested the action of a single dominant resistance gene, a hypothesis not rejected by a χ^2 goodness of fit test ($P = 0.103$). The hypothesis of a single dominant locus was independently supported by analysis across all nine related populations screened in CA and NY greenhouses (Table 2; $P = 0.596$). Disease reactions among the different tissue types evaluated (leaf, shoot, rachis, and berry) were consistent (Table 3). Correlation coefficients between tissue type responses were at least 0.875 (berry versus rachis) and showed significant correlation ($P < 0.0001$) (Table 4). During vineyard, greenhouse, and laboratory evaluations from 2006 to 2010, no sporulating colonies of *E. necator* were observed on resistant progeny.

As observed by light microscopy, resistant genotypes showed a marked reduction in powdery mildew penetration. A small proportion of germinated spores were able to penetrate leaf tissue and form secondary hypha (microcolonies)—9.7% for Y553-20 and 1.2% for Y553-27—compared with 86.4% penetration success in the susceptible full sib Y553-50 (Fig. 2). This difference is clearly seen by Coomassie staining in Figure 3, which also shows that few host cells reacted visibly at the infection site at 3 days postinoculation.

Fig. 1. Progeny of *Vitis vinifera* × *V. romanetii* pseudo-BC_2 population 03-3004 showing phenotypes of extreme susceptibility (left) or extreme resistance (right) characteristic of *Ren4* resistance to *Erysiphe necator*.

TABLE 4. Correlation coefficients for powdery mildew disease ratings by tissue type in the *Vitis vinifera* × *V. romanetii* pseudo-BC_2 population 03-3004 rated in a Parlier, CA vineyard

Correlations	Leaf	Shoot	Rachis	Berry
Leaf	...	0.996	0.990	0.883
Shoot	...	...	0.980	0.903
Rachis	...	...	...	0.875
Berry	...	...	...	...

DISCUSSION

Our data demonstrate the presence of powdery mildew resistance in *V. romanetii* that is phenotypically novel both among *Vitis* spp. described thus far (9,16) and among dominant powdery mildew resistance genes described in other plants (5,15,28). The efficacy of resistance against different inoculum sources in CA field, CA greenhouse, NY detached leaf, and particularly NY greenhouse screens—in the center of origin for *E. necator* (3)—over the past 5 years strongly suggest that this resistance is nonrace-specific (Table 3). Segregation analysis of our data across 1,030 progeny in nine independent populations clearly and repeatedly indicated the presence of a single dominant locus controlling this resistance. Both the resistance source and the resistance phenotype identified here are novel in the Vitaceae, and thus we name this resistance locus resistance to *Erysiphe necator 4* (*Ren4*). Justification for naming the locus is further supported by studies mapping the locus to chromosome 18 (S. Mahanil, D. W. Ramming, and L. Cadle-Davidson, *unpublished data*), which has not previously been associated with powdery mildew resistance. The dominant, extreme, and rapid resistance seen in *Ren4* (Figs. 2 and 3) appears to be unique among powdery mildew pathosystems and may be an example of nonhost resistance newly available for application and fundamental research.

Based on previous mapping studies in *Vitis*, at least four different powdery mildew resistance loci are known, having sources in several North American *Vitis* species and in central Asian *V. vinifera* (1,6,7,12,25,33). Generally, the phenotypic data were collected based on a macroscopic rating scale that does not detail

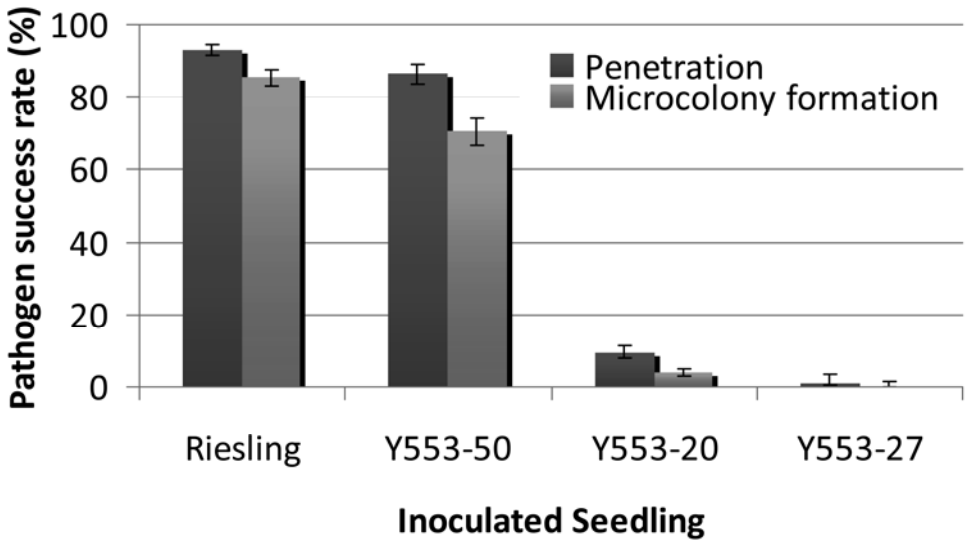

Fig. 2. Histogram showing percentage of successful penetration and microcolony formation by germinated conidia of *Erysiphe necator*. *Vitis vinifera* 'Riesling' and Y553-50 (a progeny from the *V. vinifera* × *V. romanetii* pseudo-BC_2 population 07-3553) are susceptible controls. Y553-20 and Y553-27 are resistant progeny from 07-3553, therefore full sibs of Y553-50. The numbers of conidia counted to quantify penetration and microcolony success rate were: 1014 conidia for Riesling; 568 for Y553-50; 1053 for Y553-20; and 251 for Y553-27. Error bars represent 95% confidence intervals.

the tissue- or cell-level host responses to pathogen challenge. The few cases where microscopy data are available help to emphasize that the type of resistance we report here is novel. *Ren1* resistance allows powdery mildew penetration by *E. necator* but slows subsequent hyphal growth (16). Similarly, *Run1* resistance allows penetration and formation of secondary hyphae; however, programmed cell death of the penetrated epidermal cell rapidly halts elongation of secondary hyphae (9).

In the present study, successful penetration and secondary hyphal emergence on resistant genotypes was exceedingly rare, and this resistance response was not HR-dependent (Figs. 2 and 3), though infrequent host cell death was observed. Similarly, host cell death in *mlo* resistance may occur 30 h after penetration resistance successfully halts infection (26). Programmed cell death (PCD), whether a true *R*-gene-mediated, ETI response or not (29), typically requires penetration and haustorium formation by the fungus (19) and allows some secondary hyphal growth (2,16). We did not directly observe a lack of haustoria, however extremely low incidence of growth beyond appressorium formation on resistant genotypes strongly suggests an absence of or nonfunctional haustoria formation (Figs. 2 and 3; Table 3). Thus, the resistance reported here appears to be due to a preformed barrier or PTI rather than ETI resistance. PTI resistance is the result of basal defenses recognizing PAMPs from nonadapted pathogens rather than the specific effectors of adapted pathogens (19,23). The response includes the release of antimicrobial compounds, toxic aglycones, a build-up of cell wall components, or some combination of the three in the vicinity of an appressorium (attempted penetration) and results in failed penetration by the fungus (23).

Several characterized resistance genes from other species are known to confer PTI, but none display a dominant genetic action along with distinct resistant versus susceptible allelic phenotypes. The *PEN1*/*ROR2*, *PEN2*, and *PEN3* alleles confer penetration resistance in *Arabidopsis*; however, their recessive alleles play active roles in ETI and PCD-mediated nonhost interactions, resulting in an intermediate resistance phenotype (26,36). *pmr6, pmr4/gsl5*, and *cev1* independently confer broad-spectrum powdery mildew resistance in *Arabidopsis* due to mutations in pectate lyase, cellulose synthase, and cellulose synthase isoform A3, respectively (10,18,24). *Mlo* resistance results from mutations in a plant-specific seven transmembrane domain protein present in multigene families and orthologues of which have been shown to be conserved and confer resistance in many different plant species (11,17,26,35). All of these genes confer complete or partial penetration resistance as recessive alleles and are associated with nonhost resistance.

Ren4 from *V. romanetii* is dominant yet seemingly provides nearly complete, non-race-specific resistance reminiscent of the nonhost resistance genes *pmr6*, *pmr4/gsl5*, *cev1*, and *mlo*. Evaluations of *V. romanetii* and closely related *V. davidii* germplasm in China reveal an astonishing level of resistance to powdery mildew (31,32) for species that did not coevolve with *E. necator* (21,27). Were it not for the dominant action of the gene identified here, these data taken together would support classification of *Ren4* as a nonhost resistance gene. However, because not all accessions of *V. romanetii* are resistant to *E. necator* (32), by definition, *Ren4* should not be considered a nonhost resistance gene (23). Further, without evidence of a specific PAMP receptor involved, we hesitate to categorize *Ren4* resistance as PTI. As a gene without perfect analogy, *Ren4* must for now be defined simply as conferring rapid non-race-specific resistance.

Only two preliminary screening studies have been performed in *V. romanetii* (31,32) both of which have identified powdery mildew resistant accessions. There has been no report of allelism tests between resistant accessions, and considering the range of phenotypes reported, multiple resistance genes may be present (32). Wan and colleagues (31) identified downy mildew resistance in this species, which invites further resistance screening with additional pathogens.

The first pBC_2 table and raisin grape selections with *Ren4* resistance to powdery mildew were planted in production trials at USDA/ARS Parlier, CA in 2009 and 2010. The table grape selection in the trial has 12/16 in. diameter berries that average 5.4 g with small aborted seeds the size of 'Thompson Seedless'. The five raisin selections in the trial have fruit quality rated as high as the best natural dry-on-the-vine selection with aborted seeds the size of Thompson Seedless. The source of powdery

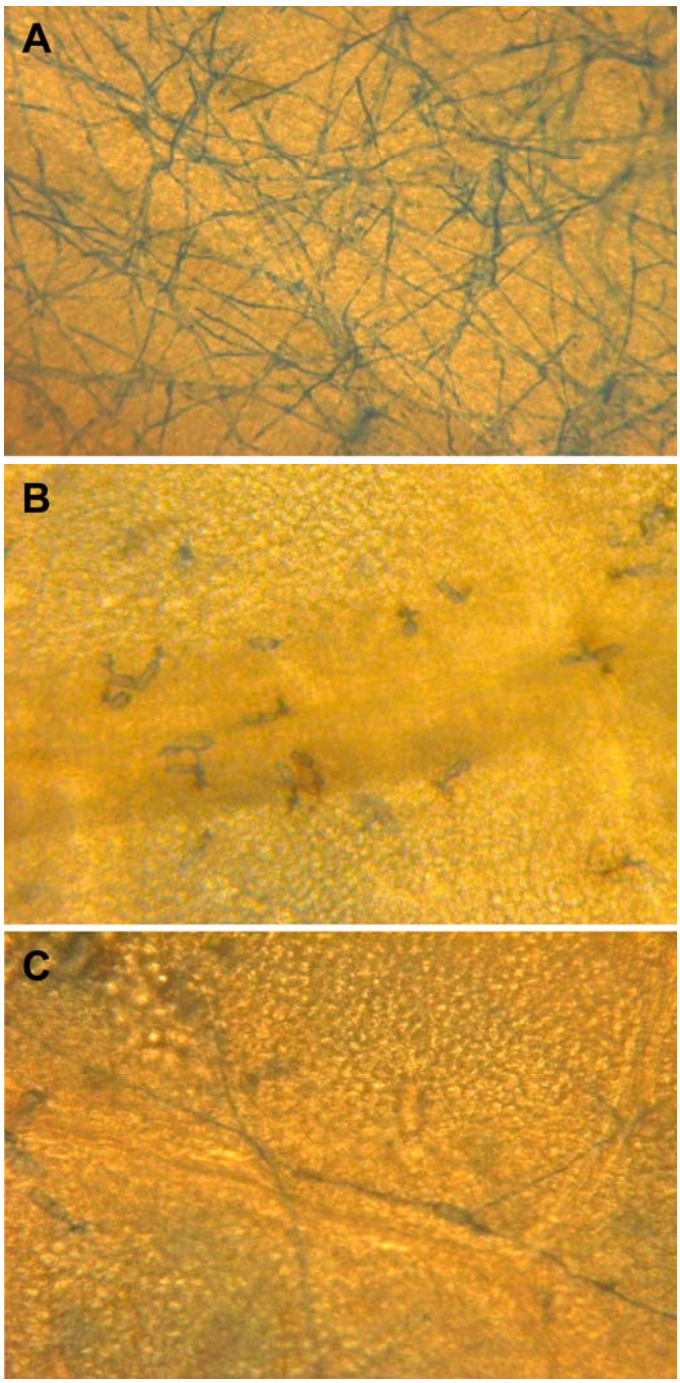

Fig. 3. *Erysiphe necator* development on leaves of a characteristic progeny (Y553-20) with *Ren4* resistance from the *Vitis vinifera* × *V. romanetii* pseudo-BC_2 population 07-3553. Coomassie blue staining of *E. necator* on cleared leaves shows **A,** complete susceptibility of *V. vinifera* 'Riesling' demonstrated by unrestricted hyphal growth and overlapping microcolonies; **B,** no penetration on Y553-20 as indicated by lack of secondary hyphal growth; and **C,** rare instance of successful penetration and microcolony formation on otherwise resistant Y553-20.

mildew resistance, C166-026, has small seeded berries, 8/16 in. diameter with black fruit, so significant improvement in fruit quality has already been achieved among these pBC_2 progeny.

Resistance gene durability is critical for woody perennial crops such as grapevine. Unlike annual crops, grapevines are expected to be productive for at least 20 years—plenty of time for a pathogen to overcome ETI. This fact and the long generation time (3 to 5 years from seed to seed) underscore the need for broad and durable resistance when developing new grape cultivars. In this regard *Ren4* should be of importance in breeding for powdery mildew resistance in grapes, but at the same time the spectrum of resistance, potential durability of resistance, and protective management strategies deserve further investigation.

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